Original Research Paper

Effect of Slum Water on Physiology of *Heteropneustes fossilis* Collected from Silchar, Assam

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Abstract: Poverty and overpopulation are not just prevailing in big cities, but have also become a matter of concern in small towns. Safe drinking water is considered a basic human need, yet millions of people do not have access to an adequate and safe water supply. The present study is focused on the quality assessment of water bodies of slum areas of Silchar, Assam, India and to evaluate the physiological stress on *Heteropneustes fossilis* from household water pollution. Water samples from six different sites were collected and their physical parameters were tested. Fishes (n=35) of approximately similar size (15.8±0.84 cm) and weight (30.0±1.2 gm) were exposed to each water samples for a period of 10 days and their morphological and behavioural changes were observed. The RBC and WBC counts of the fishes were also determined. Physical properties of the water samples revealed that the pH ranges from 4.79-6.10; conductivity 0.07-0.46 mS/cm; total dissolved solids 0.03-0.29 ppm; free CO_2 13-26 ppm and dissolved oxygen 2.2-3.5 ppm. An abundance of bacteria was observed in the water samples and the predominant isolates were identified as *Escherichia coli*, *Klebsiella* sp., *Bacillus* sp. and *Pseudomonas* sp. Fishes exposed to pond water were initially active with no morphological and behavioural changes. However, from day-5 onwards, their swimming had become idle and lethargic, their dark black skin had gradually attained a pale texture and the mucus content of skin had gradually reduced. Despite providing food and air regularly, the fishes had gradually stopped eating, with swimming pattern changed from dorsal to ventral side upward and decreased opercular activity. Hematological studies showed decreased RBC count and increased WBC count in pond water treated fishes, inferring blood balance disturbance and infection in fishes. Adequate measures should be taken by the respective residents of the selected areas to minimise the degree of water contamination and use of proper disinfectants, or else the hazardous condition of the water will deteriorate the aquatic biodiversity and create serious health problems in humans.

Keywords: Slum Water, *Heteropneustes fossilis*, Haematological Indices, Coliform Bacteria

Introduction

Water is the vital asset for the entire living world. Every civilization begins nearby a water body. Human beings not only consume water but are also dependent on bathing, washing and all other kinds of household activities. Increase in population, lack of space, poverty and immigration has led to the flourishment of slum areas. Industrial wastes, unhygienic domestic activities and sanitation have a significant contribution in contaminating the water bodies (Bardach *et al.*, 1965; Shiddamallayya and Pratima, 2008). The household sewage and harmful chemicals having inadequate treatment facilities may degrade the quality of the water bodies and make them unsuitable for human use. The aquatic organisms are adversely affected by water

pollution. Fishes are not only the most diverse group of organism inhabiting the aquatic ecosystem but also the major contributor to the human diet (Khalili Tilami and Sampels, 2018). Though some fishes have the capability of surviving in the adverse situations, the high level of pollution in recent times has been giving a tough competition to the survivability of the fishes.

Heteropneustes fossilis (Asian stinging catfish) is a species of air sac catfish locally known as Shingi or Shing. *H. fossilis* is native to India, Bangladesh, Pakistan, Nepal, Sri Lanka, Burma and Thailand (Talwar, 1991). This species breeds in confined waters during the monsoon months but can breed in ponds, derelict ponds and ditches when sufficient rainwater accumulates (Puvaneswari *et al.*, 2009). *H. fossilis* is of high economic importance and high demand because of its medicinal value (Talwar, 1991). It is considered to be highly nourishing and well preferred because of its less spine, less fat and high digestibility in many parts of the Indian subcontinent (Khan *et al.*, 2003). The unhygienic practices and lack of knowledge in the concerned slum areas have led to the deterioration of the quality of nearby water bodies concerning the physical properties as well as the microbial diversity. As a result, diversity, number and survivability of the fishes have been significantly affected and the water has become non-consumable and non-preferable for human use (Haseena *et al.*, 2017). The contaminated water not only makes the fishes prone to pathogenic attack but may also lead to several water-borne diseases in human. The present study focuses on the assessment of water qualities, collected from six different sites of Silchar, Assam, India. The study also investigates the effect of water qualities on morphological and hematological indices of *H. fossilis*.

Materials and Methods

Collection of Water Samples

Water samples were collected from 6 different ponds of Cachar district, Assam, India. A preliminary survey was conducted by interacting with local residents to access the condition and use of water for the domestic purpose (Table 1). Water samples were collected in sterile bottles, properly packed, labelled and immediately bought to the laboratory for determining physical properties and bacterial diversity.

Analysis of Physical Parameters

Measurement of conductivity, Total Dissolved Solids (TDS) and pH were performed by Water and Soil Analysis Kit (Deluxe, Model LT- 60; ISO 9001: 2008). Free carbon dioxide (CO_2) and Dissolved Oxygen (DO) were determined by Winkler's method of performing titrations (APHA 1989). For determining dissolved oxygen, samples were fixed with $MnSO_4$ and KI solutions and immediately brought to the laboratory. Water samples were also collected from Public Health Engineering (PHE), Silchar and were found to be in the permissible range, suitable for life forms. The water collected from PHE, Silchar act as a control for performing later experiment.

Determination of Bacterial Diversity in Water

About 1 mL of the five-fold serial dilution of water samples were inoculated in Mac Conkey (Kaper *et al.*, 1978) and incubated at 37°C for 24 hours. The total bacterial colonies were counted in microprocessor colony counter and the distinct individual colonies were subcultured by streak plate method. The isolates were identified by Gram staining and biochemical tests (Indole, MR, VP, Citrate, Catalase and Oxidase test) (Cappuccino and Sherman, 1996).

Toxic Effect of Effluents on Fish Growth

Thirty-five *H. fossilis* of equal size (15.8±0.84 cm) and equal weight (30.0±1.2 gm) were collected from local fisherman. Fishes were distributed in seven containers (46×40×13 cm^3), containing water from six selected ponds. Fishes were also kept in a control container filled with water, collected from PHE, Silchar.

Table 1: Topography, location and use of pond water by the local residents

Location	Latitude/ Longitude	Use of water for household purposes	Defecation	Domestic waste disposal	Use of water for drinking	Use of disinfectant	Availability of Fishes
Malini Beel	24°83'36.7" N/ 92°78'09.6" E	Yes	Yes	Yes	Yes	No	Small to medium size
Ashram Road	24°81'12.4" N/ 92°79'84.3" E	Yes	No	Yes	No	Yes	Small sized fish
Deb Para	24°80'87.8" N/ 92°79'46.5" E	Yes	Yes	Yes	Yes	No	Small sized fish
Near Shishu Mandir School	24°82'19.3" N/ 92°78'04.3" E	Yes	Yes	Yes	Yes	No	Small sized fish
Tapaban Nagar	24°81'79.6" N/ 92°78'26.0" E	Yes	Yes	Yes	No	Yes	Small to medium sized
Chirukandi	24°83'11.7" N/ 92°77'31.5" E	Yes	No	Yes	No	Yes	Small to medium sized

In each container, five fishes were exposed and observed for ten days. The fishes in each sample were provided artificial food and oxygen with the help of an aerator. After every 2-3 days, the water of each sample was changed by the stock water of the respective sample. Throughout the study period, the skin texture, swimming pattern and operculum movement and variations in the behaviour of *Heteropneustes fossilis* were noted. The RBC and WBC count of the fishes were determined.

Determination of RBC and WBC Count

RBC and WBC count of the fishes were determined using haemocytometer, collected on day 0, day 5 and day 10. Blood was obtained from the tail region of *Heteropneustes fossilis*, sucked into both the RBC and WBC pipettes up to 0.5 marks. In the RBC pipette, RBC diluting fluid was sucked up to 101 marks, shaken thoroughly to mix diluting fluid and blood and the final dilution becomes 200 times. While in the WBC pipette, WBC diluting fluid was sucked up to 11 marks, shaken thoroughly and the final dilution becomes 20 times. The mixtures were put to Neubauer's chamber one after another and covered with a coverslip maintaining a distance of 0.1 mm between the coverslip and the chamber. The slides were then visualized under microscope and RBCs and WBCs were counted (Poddar *et al.*, 2002).

Haematological parameters were calculated by taking mean of two replicates from each group and is expressed as mean ± standard deviation. However, on day 5 and day 10, some containers showed total mortality and thus results cannot be obtained.

Results and Discussion

The survey conducted with the residents of the selected sites provides the information that water of six selected sites is used for all kinds of household purposes and disposal of wastes, four sites are used for human defecation purpose and three have never been disinfected. The survey also showed the availability of different types of fish from small to medium size. However, the number of fishes in each site has been decreasing gradually, according to the survey (Table 1). The normal pH range of pond water should be within 6.5-9.0 (Prajapati *et al.*, 2018). It has been observed that the pH level of the water samples ranged from 4.79-6.10 (Table 2), which can provide stress to the survival and growth of fish as well as aquatic flora (Kawamura *et al.*, 2015).

The degree of pollution of a water body can be assessed from the total amount of solids (ions) dissolved in it, which in turn determines its ability to conduct electricity. However, higher values of TDS and conductivity indicate a higher degree of pollution, while considerably lower values resemble an unsuitable environment for fish growth and survival. Electrical

conductivity ranges from 0.07-0.46 mS/cm, inferring that the water samples are in acceptable condition for the survival of aquatic fauna. Moreover, studies have revealed that the TDS in freshwater is usually 0.65 times the conductivity (Thirumalini and Joseph, 2009) to support diverse aquatic life. The water quality test of the selected ponds showed that the TDS was in the range of 0.03-0.29 ppm (Table 2).

Carbon-dioxide released by the aquatic fauna is used up by the phytoplankton for photosynthesis. Higher the amount of free CO_2 in the water body, higher the survival rate of aquatic flora, making the environment highly toxic to the fishes (Khan *et al.*, 2018). Free CO_2 can be directly related to the oxygen dissolved in water. Usually water body containing a higher amount of DO will have a lesser amount of free CO_2 (Wurts and Durborow, 1992). The present study subsequently reported a low level of DO and free CO_2, thus contradicting the general correlation and indicating an ecological imbalance in the water. The free CO_2 level of the studied ponds ranges from 13-26 ppm, with DO level ranging between 2.2-3.5 ppm (Table 2), indicating a high degree of pollution and unsustainable condition for fish life. Researchers have shown that catfish can tolerate CO_2 level from 20-30 ppm provided the DO concentration is above 5 ppm (Wurts and Durborow, 1992).

Presence of faecal coliform bacteria in the water samples indicates disposal of human and other animal faeces and also indicates the presence of other pathogens in the water (Ksoll *et al.*, 2007). However, for the maintenance of ecological balance, the presence of diverse types of beneficial bacteria is necessary, but within a reasonable range. According to WHO guidelines, pond water used for human consumption and household purposes should not contain more than 50 coliform bacteria per 100 mL of water (Culpepper *et al.*, 2016). Total bacterial count ranges from 3.14×10^5 to 9.96×10^5 per 100 mL of water, indicating a high degree of faecal contamination and high chances of human beings and fishes being prone to water-borne diseases. The results of Gram staining and biochemical tests revealed the presence of *Escherichia coli*, *Klebsiella* sp., *Bacillus* sp. and *Pseudomonas* sp., (Table 3). This abundance of such bacteria indicates the deterioration of the aquatic ecosystem (Chakraborty and Bhadury, 2015; Köchling *et al.*, 2017) and also the possibility of getting prone to the water-borne diseases (Cabral, 2010).

A healthy environment is an asset to the healthy morphology and behaviour of the aquatic organisms. The observations made within the ten days study revealed that all the fishes which were actively swimming had become idle and lethargic, decrease in body weight, attaining a pale texture and the mucus content of skin had gradually reduced. Despite providing food and air regularly, the fishes had gradually stopped eating, with swimming pattern changed from dorsal to ventral side upward and decline in opercular activity (Table 4).

Table 2: Physical parameters of the water samples

Location	pH	Conductivity (mS/cm)	Total Dissolved Solids (ppm)	Free Carbon dioxide (ppm)	Dissolved Oxygen (ppm)
Malini Beel	5.80	0.46	0.29	26	3.50
Ashram Road	6.10	0.32	0.21	13	2.50
Deb para	4.79	0.31	0.23	15	3.20
Shishumandir School	5.60	0.16	0.12	20	3.27
Tapaban Nagar	5.30	0.07	0.03	13	2.20
Chirukandi	4.86	0.39	0.26	13	2.80
PHE water	7.20	0.50	0.30	14	4.60

Table 3: Identification of predominant bacteria, present in the water sample

Location	Gram Staining	Oxidase test	Catalase test	Indole test	Methylred test	Voges Proskauer test	Citrate test	Suspect organism
Malini Beel	-	-	+	+	+	-	-	*Escherichia coli*
Ashram Road	-	-	+	-	-	+	+	*Klebsiella* sp.
Deb Para	-	-	+	+	+	-	-	*Escherichia coli*
Shishu Mandir School	-	-	+	+	+	-	-	*Escherichia coli*
Tapaban Nagar	+	-	+	-	-	+	-	*Bacillus* sp.
Chirukandi	-	+	+	-	-	-	+	*Pseudomonas* sp.

+ indicates positive tests; - indicates negative tests

Table 4: Observations of fish behavioural pattern in pond water treated fish

Characteristic	Day 0	Day 3	Day 6	Day 9
Skin texture	Dark black, and slimy skin	Some fishes started becoming pale white	Most of the fishes have become pale, and skin mucus content has reduced	All the fishes became completely pale, and skin with no mucus
Swimming pattern	Dorsal surface upward	The speed of swimming has reduced surface upward	The speed of swimming has slowed down, with ventral at the bottom of the container	Swimming has completely stopped, and fishes settled
Feeding pattern	Frequent visit to the water surface is supplied	Frequent visit to the water surface when food is supplied	Feeding has slowed down. A lesser number of visits when food to the water surface even when food is supplied	Feeding has completely stopped
Operculum movement	Normal	Vigorous	Slower	Slower
Activeness	Very active, and noisy at night	Some fishes have become less active	Very less	Completely stopped

Table 5: Mortality rate of the fishes

Location	Number of fishes alive (Day 0)	Number of fishes alive (Day 3)	Number of fishes alive (Day 6)	Number of fishes alive (Day 9)
Malini Beel	5	4	2	1
Ashram Road	5	2	0	0
Deb Para	5	3	2	1
Shishu Mandir School	5	4	3	1
Tapaban Nagar	5	2	1	0
Chirukandi	5	0	0	0
PHE water	5	5	4	3

The hostile environment of the pond water ultimately led to their abnormal mortality rate (Austin, 1998) (Table 5). However, the fishes kept in PHE water container also showed the changes mentioned above, but much later than those kept in the pond water samples.

RBC and WBC count of all the fishes were performed on Day 0 and were found to be in normal range. However, on day 10, a marked decline in RBC count was observed in pond water treated fishes, which was found to be 0.95 to 0.97×10^6 mm^{-3} (Table 6). The control fishes also showed a marginal decline in RBC count from $1.41 \pm 0.03 \times 10^6$ (Day 0) mm^{-3} to $1.26 \pm 0.02 \times 10^6$ mm^{-3} (Day 10), probably due to

competitive pressure and container effect (Doyle and Talbot, 1986). RBC count declined at a greater degree in pond water treated fishes indicates a high degree of pollution. Several studies on different fish species such as *Amblyceps mangois* (Nath, 2016), *Salvalinus fontinalis* (Holcombe *et al.*, 1976), *Colisa fasciatus* (Srivastava and Mishra, 1979) also revealed a decline in RBC count due to water contamination. WBC count in all the containers treated with pond water showed a drastic increase on Day 10 which was found to be 13.55×10^3 mm^{-3} to 13.87×10^3 mm^{-3} (Table 6). This may due to increased antibody production and recovery from infections (Velmurugan *et al.*, 2016).

Table 6: Result of the haematological study of fish

Location	RBC count ($\times 10^6$ mm^{-3})			WBC count ($\times 10^3$ mm^{-3})		
	Day 0	Day 5	Day 10	Day 0	Day 5	Day 10
Malini Beel	1.26±0.02	1.14±0.02	0.95[*]	7.8±0.03	9.23±0.05	13.87[*]
Ashram Road	1.41±0.02	1.12±0.01	-	8.1±0.02	9.36±0.02	-
Deb Para	1.38±0.02	1.13±0.02	0.95[*]	7.6±0.02	9.19±0.02	13.76[*]
Shishu Mandir School	1.33±0.02	1.16±0.02	0.97[*]	7.8±0.02	8.96±0.01	13.55[*]
Tapaban Nagar	1.30±0.01	1.13±0.01	-	8.2±0.01	9.38±0.02	-
Chirukandi	1.31±0.02	-	-	7.8±0.02	-	-
PHE water	1.41±0.03	1.38±0.01	1.26±0.02	7.9±0.01	8.32±0.01	9.18±0.01

Values are mean ± standard deviation of 2 replicates; '*' indicates single variable due to mortality of fishes; '-' indicates non-availability of fishes due to total mortality in that group.

The control fishes, however, showed a marginal increase from $7.9\pm0.01\times10^3$ mm^{-3} (Day 0) to $8.32\pm0.01\times10^3$ mm^{-3} (Day 5) and finally $9.18\pm0.01\times10^3$ mm^{-3} (Day 10). Many researchers also reported hematological changes in *Oreochromis aureus* (Allen 1994), *Anguilla anguilla* (Sahan *et al*., 2007), *Heteropneustes fossilis* (Srivastav and Roy, 2015) and *Anabas testudineus* (Velmurugan *et al*., 2016), which were exposed to industrial effluents, toxic contaminants and heavy metals.

Conclusion

The present study reveals the degree of pollution in slum areas of Silchar, India, indicating an unhealthy environment for the growth and survival of aquatic organisms. Physical parameters of water samples were not in accordance with the standard values. The abundance of coliform bacteria and less diversity of aquatic microflora indicates the use of the sites for defecation, making the water non-preferable for drinking and household activities. The gradual changes in the physiological behaviour, weight loss and the abnormal mortality rate of fishes during study indicate that the water from the selected sites is not suitable for the survival and growth of fishes. The decline in RBC count and rise in WBC count in the experimental fishes indicates an attack by germs and a high degree of pollution in the selected water bodies. Adequate measures should be taken by the respective residents of the selected areas to minimise the degree of water contamination and use of proper disinfectants, or else the hazardous condition of the water will deteriorate the aquatic biodiversity and create serious health problems in humans.

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Authors' Contributions

RR, BB and SN conceived and designed the experiments. RR did the preliminary survey works. RR performed the experiments. SB guided the microbiological experiments. RR and SN analysed the data. RR wrote the first draft of the manuscript. BB, SB and SN corrected the draft copy. All authors agreed to the final state of the manuscript.

Competing Interests

The authors declare that they have no competing interests.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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Abbreviations

H. fossilis: *Heteropneustes fossilis*; PHE: Public Health Engineering; $MnSO_4$; Manganese Sulfate; KI: Potassium Iodide; MR: Methyl Red; VP: Voges Proskauer; TDS: Total Dissolved Solids; DO: Dissolved Oxygen; RBC: Red Blood Corpuscle; WBC: White Blood Cell