Effect of industrial emissions on haematological and biochemical parameters of *Channa striata* fresh water fish

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Received: October 2023 Accepted: February 2024

Abstract

Fishes are largely used for the assessment of aquatic environment quality and are accepted as bio indicators of environmental pollution. Researches of biochemical response against to spontaneous elements furnish worthwhile information on the influence of the exterior environment on the internal physiology of fish. For monitoring fish health haematological parameters have been perceived as one of beneficial measure. In the current research the fresh water fish, *Channa striata,* haematological profile was studied. Fishes were categorized into four groups and each solitary group of fish subjected to 10 ppm concentrations of SO_2, NO_2 and H_2S after every 10 days interval for the span of 30 days**.** One group of fish was maintained as control without any treatment. After experimental period specimen were allowed for collection of blood to test the haematological profile. In haematological profile, stipulated blood parameters influence is discerned on RBC count, WBC count, Hb, and PCV furthermore on Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC). The industrial emissions in particular SO_2, NO_2 and H_2S considerably declined the value of RBCs, Hb, PCV, MCH, MCV, MCHC, while on the contrary WBC count elevated discretely with H_2S treatment when compared with other gaseous pollutants employed in present experiment. To conclude, all the industrial emissions engendered haemotoxicity in fish. The fishes exposed to SO_2, NO_2 and H_2S also allowed to check biochemical parameters i.e. moisture, total ash, rehydration ratio, total sugars, proteins, total carbohydrates, crude fiber and fat content in fresh and dried biomass of respective treatments against to control treatment. The obtained results concluding that the emissions such as SO_2, NO_2 and H_2S not only shown impact on haematological profile it also decreased biochemical parameters of fresh water fish *Channa striata.*

Keywords: *Channa striata*, Biochemical profile, Haematological profile, Industrial emissions, Sulpher dioxide (SO_2), Nitrogen dioxide (NO_2) and H_2S

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Introduction

The water and soil quality of aquatic ecosystem play prominent role in growth morphology and biochemical response of an aquatic organisms (Priyamvada *et al.,* 2012; Priyamvada *et al.,* 2012; Priyamvada *et al.,* 2013). Considering last few decades elevation in population density, excessive industrialization and agricultural activities gave rise to enormous wastes run through in fresh water resources. Throughout last few decades, contamination of fresh water with a diverse range of pollutants has become a matter of apprehension (Vutukuru, 2005). Natural aquatic system massively may get contaminated by pollutants let out from domestic, industrial and other man made activities. Considerable metropolitan cities in India i.e. Delhi, Hyderabad, Mumbai and Kolkata were delineated to be contained with urban sewage, industrial emissions and liquid flows off from agricultural and industrial activities (Gandhi *et al.,* 2013; Gandhi *et al.,* 2014). Antecedent studies described various rivers in India which has been polluted with pesticides and fertilizer remnants from superfluous-application of agricultural chemicals and heavy metals from domestic waste of industries (Leong *et al.,* 2007; Abbas Alkarkhi *et al.,* 2008; Yap and Pang, 2011; Gandhi *et al.,* 2016; Gandhi *et al.,* 2018). Ganga, Yamuna, Damodara, Gomati and Musi rivers are instance of the utmost polluted rivers in India owing to the expeditious evolution of the economy besides urbanization. Oxygen is most important source for surviving of all living organisms including aquatic animals. Due to change in water quality with discharge of industrial and agricultural waste the oxygen levels were depleting continuously and improved levels of SOx, NOx and other gaseous pollutants levels are increasing (Sirisha *et al.,* 2015).

Industrial emissions consist several gaseous pollutants in a higher concentration which changes the quality of fresh air and causes air pollution and make its way into rivers, lakes, ponds, streams and other surface water resources. Among the various emissions of industries SO_2, NO_2 and H_2S dissolves in water vapors easily and makes water into more acidic and such conditions are tough environment for some fish and other aquatic animals to survive and reproduce within. Taking this factor into consideration the present study was investigated the biochemical and haematological response of a fresh water fish *Channa striata* under various industrial emissions such as SO_2, NO_2 and H_2S.

Materials and methods

The fish, *Channa striata* commonly known as mud fish. It is a state fish of Telangana and locally called as korameenu (Fig. 1) were collected with the help of local fisherman from lake located in the region of Ibrahimpatnam, Rangareddy, Telangana state. These fishes were carried to laboratory for the haematological tests and biochemical parameters in a polythene bags with pre aerated water. In the experiment all the active specimens, both sexes of *Channa*

striata (15.40±0.12 cm in length and 28.69±1.030 gm in weight) were employed. In order to elude ant dermal infection fish were treated with 0.02% $KMnO_4$ for 2 minutes. The fishes were divided into 4 different experimental groups and stocked at random into 4 different were assigned. The hereinafter experimental groups were retained in a plastic container with 60 liters of water for the spell of 30 days.

Group 1: The first group was perpetuate in fresh water (exempt from air and water pollutants) and water quality parameters specified in Table 1.

Figure 1: Morphological description of *Channa striata*.

Table 1: Water quality criteria used for present investigation.

pH	7.2 - 7.6
Temperature	27 ± 1°C
Dissolved oxygen	7.1 – 7.3 ppm
Salinity	0.38 – 0.45 ppt
Alkalinity	270 mg/L as $CaCO_3$
Hardness	330 mg/L $CaCO_3$
Total dissolved solids	0.13 – 0.15 ppm
Biological oxygen demand	38 – 40 mg/L

Group 2: Fish exposed to 10 ppm concentration of SO_2 gas for every 10 days time interval for a period of 30 days through air pump in a plastic container with 60 liters of fresh water.

Group 3: Fish exposed to 10 ppm concentration of NO_2 gas for every 10 days time interval for a period of 30 days through air pump in a plastic container with 60 liters of fresh water.

Group 4: Fish exposed to 10 ppm concentration of H_2S gas for every 10 days time interval for a period of 30 days through air pump in a plastic container with 60 liters of fresh water.

All the experiments performed in triplicates. During experimental period, fish were fed with *ad libitum* diet of rice bran and oil cake. The excrement and other waste siphoned off diurnal to deplete ammonia content in water (Fig. 2).

Haematological studies

Collection of blood

Samples of blood were collected from the experimental fishes and control with the assistance of 24 gauge needle and stowed in heparinized glass tube. Dacie and Lewis (1984) method was employed to evaluate the haematological parameters viz., total Red Blood Cells (RBC), White Blood Cells (WBC), Haemoglobin (Hb), Haematocrit (Ht), Mean Cell Haemoglobin (MCH) and

Mean Cell Haemoglobin Concentration (MCHC).

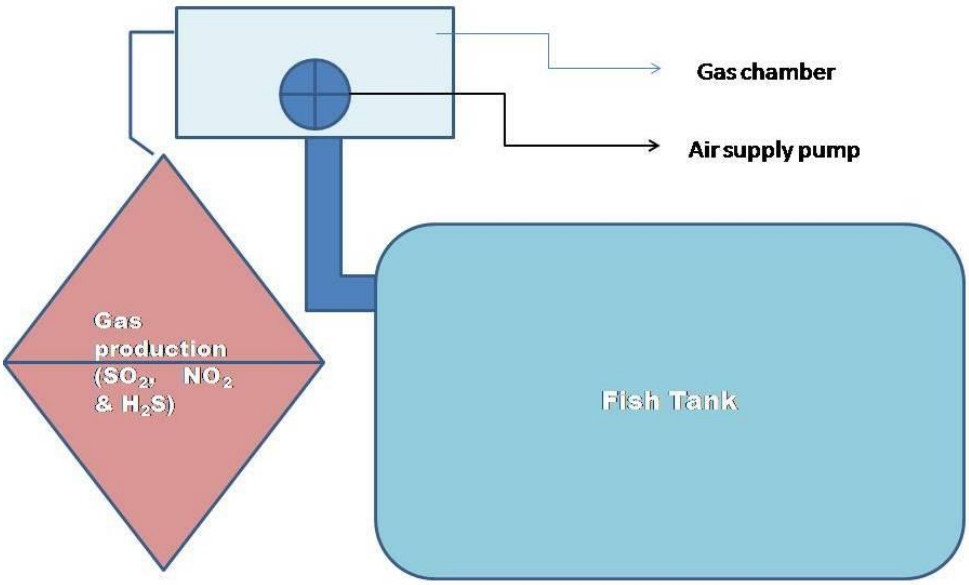

Figure 2: Experimental design to evaluate industrial emissions on *Channa striata.*

Enumeration of red blood corpuscles (RBC)

Haemocytometer pipette is employed to gently suck up the blood samples until the marking 0.5 is attained (marked 0.5; 1.0 and 101). Subsequently the diluting fluid was drawn up to the mark 101. This furnished a dilution of 1 in 200. In due course, to start the mixing the pipette was gradually rotated. The pipettes was unswervingly seized by its ends amid the forefinger and thumb and quivered rigorously for about one minute. The finger was later detached from the pipette and the diluting fluid in the capillary tube was expelled. Following a few drops of the diluted blood have panned out, a small drop was passed on to the counting slide. At the minimum five sets of sixteen squares were taken into consideration for computation of red blood cells. The squares in each set should be scrutinized invariably in horizontal rows of four at a time. Barely the indicated ones on the upper and on the left – hand lines were computed. The below given formula followed to calculate total RBC count:

$$\text{Number of } {}^{\text{RBC}}/_{\text{Cu mm}} = \frac{\text{Total no, of Carpuscles counted}}{\text{Total no, of small squares counted}} \text{ X dilution X } 10$$

Enumeration of white blood corpuscles (WBC)

Haemocytometer's Neubauer Counting Chamber as utilized to count total WBC. From the control and treated fish WBC was enumerated. The samples of blood were sucked until the 0.5 level in WBC pipette and diluted till the level 11 with diluting fluid (Turk's fluid=Gention violet, glacial acetic acid 3 mL and distilled water 97 mL). This yielded a dilution of 1 in 20. Rest of the protocol

remains same as RBC counting. For computation of leucocytes four sets of sixteen squares were counted out of nine squares. Alternatively, examining the squares in rows of four, whole set of a sixteen can easily be enumerated at once:

$$\text{Number of } {}^{\text{WBC}}/_{\text{Cu mm}} = \frac{\text{Total no, of leucocytes counted}}{\text{Total no, of large squares counted}} \text{ X dilution X 10}$$

Estimation of haemoglobin (Hb) content
Sahli's Haemometer (Superior, Germany) with permanent glass comparison standards was employed to determine the Haemoglobin content in the blood and articulated in gm Hb/100 mL blood.

Determination of Haematocrit value (Ht or Packed Cell Volume)
Evaluation of Haematocrit value was by centrifuging the blood in heparinzied haematocrit tubes (Germany) at 7000 rpm/min for 30 minutes. Computation of haematocrit per cent was from the volume of blood absorbed and packed cell volume after centrifugation.

Mean corpuscular volume (MCV)
Determination of Mean Corpuscular Volume (MCV) content was from the values of packed cell volume and erythrocyte count with the succour of the formula and articulated in femtolitre:

$$\text{MCV} = \frac{\text{PCV X 10}}{\text{Erythrocyte count } ({}^{\text{million cells}}/_{\text{Cu mm blood}})}$$

Mean corpuscular haemoglobin (MCH)
Estimation of Mean Corpuscular Haemoglobin (MCH) content was from the values of haemoglobin content and erythrocyte count with the aid of the formula and articulated in picograms:

$$\text{MCH} = \frac{\text{Hb } ({}^{\text{gm}}/_{\text{100 ml}}) \text{ X 10}}{\text{Erythrocyte count } ({}^{\text{million cells}}/_{\text{Cu mm blood}})}$$

Estimation of mean cell haemoglobin concentration (MCHC)
Determination of Mean Cell Haemoglobin Concentration (MCHC) was from the values of haemoglobin and the haematocrit percentage with the help of the formula and articulated in percentage.

$$\text{MCHC} = \frac{\text{Hb } ({}^{\text{gm}}/_{\text{100 mL}})}{\text{Haematocrit percentage}}$$

Biochemical studies

The fish, *Channa striata* after blood collection made into small pieces to calculate biochemical parameters in the fresh and dry biomass of control and treatment specimen.

Moisture

The moisture content was estimated by drying the weighed sample up to a constant weight in hot air oven at 70±2^{o}C. The dried sample was then cooled to room temperature in desiccator prior to weighing. Moisture in terms of percentage was calculated as:

$$Moisture\ (\%) = \frac{Weight\ of\ fresh\ sample - Weight\ of\ dried\ sample}{Weight\ of\ fresh\ sample}\ X\ 100$$

Total ash

Silica dishes were weighed first and then 5-10 g of the dried samples were weighed into each. Samples were ignited on a Bunsen burner. Material was kept at 525^{o}C for 4-6 h in a muffle furnace to get ash. Dishes were then allowed to cool and weighed. The difference in weight gives the total ash content and is expressed as percentage (Ranganna, 2007).

Determination of rehydration ratio

Rehydration ratio was evaluated with the aid of weighted samples of dehydrated specimen which were immersed in a beaker comprising 100 mL distilled water enclosed with watch glass and was supposed to boil for few minutes. The water was drained out and on filter paper the samples were air dried and weighed again. The rehydration ratio was articulated as ratio of the weight of rehydrated product to the weight of dried product (Ranganna, 1986).

Fat determination

Pearson (1999) method of solvent extraction in a Soxhlet reflux apparatus was employed for fat determination. Precisely 2g of the sample was encased in a porous material (Whatman filter paper) and positioned in the reflux flask. Accurately 2g samples was afresh quantified into one more paper and encased in another soxhlet flask to devise replicate. The soxhlet flasks were ascended on weighed oil extraction flask comprising 200 ml of petroleum ether. All the parts of the soxhlet apparatus were integrated and heat employed via the electro-thermal heating mantle, the heated solvent vaporized and condensed in the reflux flask comprising the sample was excerpted from the sample until the flask was detached from individual set up after 4 hours and dried for 3 minutes in the oven at 60^{o}C.

Crude fiber determination

For the estimation of Crude Fiber, Weende method elucidated by Pearson (1996) was deployed. Precisely 5g samples were quantified, each sample encased in two fold muslin cloth and

boiled in 200 mL of 1.25% H_2SO_4 for 30 minutes under reflux. Each cloth was washed thoroughly with boiling water. The cloths each was transferred back to boiling flasks containing 1.25% NaOH solution. Boiling was done for 3 minutes under reflux. The clothes were washed and transferred to an already weighed porcelain crucible 1 (w) dried in the oven constant weight 2 (w). Then the samples were taken to the furnace and reduced to ashed at 550ºC they were cooled in a desiccators and the weight (w) noted.

Total carbohydrates determination

Into a clean test tube 1 mL of homogenous extract was taken and the total volume was made up to 2 mL with distilled water. Then 4 mL of antherone reagent was added and incubated at room temperature for 10 minutes. The intensity of color developed was read at 620 nm in spectrophotometer against with blank adjusting at zero.

Total protein determination

The biuret method was followed to estimate total protein content in fishes used for current study. Into a clean test tube 1 mL of homogenous extract was taken and the total volume was made up to 2 mL with distilled water. Then 3 mL of biuret reagent was added and incubated at room temperature for 10 minutes. The intensity of color developed was read at 540 nm in spectrophotometer against with blank adjusting at zero.

Results

Haematological parameters of RBC

The haematological parameters of RBC contents were observed in the control to be 3.92±0.45 mg/g wet weight after 30 days of experimental period. The RBC contents were significantly decreased when fishes exposed to 10 ppm of SO_2 (2.86±0.24) NO_2 (3.06±0.35) and H_2S (3.72±0.89). The decrease in the haematological parameters of RBC was more in fishes exposed to SO_2 compare to the RBC count of fishes exposed to NO_2 and H_2S (Fig. 3).

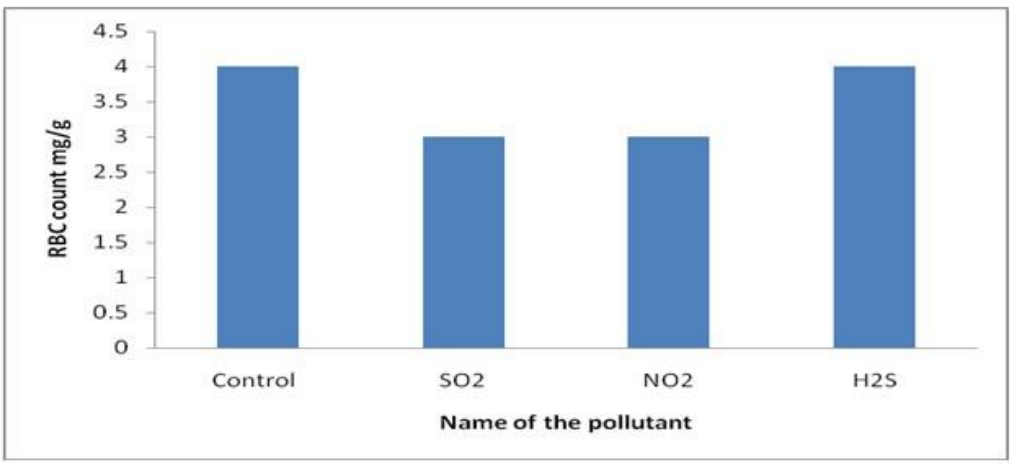

Figure 3: RBC change in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Haematological parameters of WBC

The haematological parameters of WBC contents were observed in the control to be 14.96±1.33 mg/g wet weight after 30 days of experimental period. The WBC contents were significantly increased when fishes exposed to 10 ppm of SO_2 (18.41±0.33) NO_2 (17.24±2.43) and H_2S (19.49±0.90). The increase in the haematological parameters of WBC was more in fishes exposed to H_2S compare to the WBC count of fishes exposed to NO_2 and SO_2 (Fig. 4).

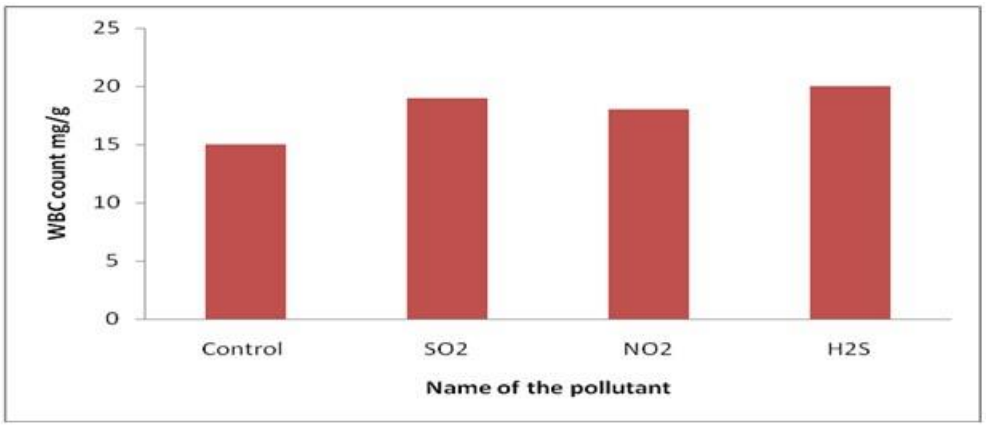

Figure 4: WBC change in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Haematological parameters of Haemoglobin

The haematological parameters of haemoglobin contents were observed in the control to be 12.22±1.22 mg/g wet weight after 30 days of experimental period. The haemoglobin contents were significantly decreased when fishes exposed to 10 ppm of SO_2 (9.04±0.82) NO_2 (9.02±0.62) and H_2S (11.64±0.26). The decrease in the haematological parameters of haemoglobin was more in fishes exposed to SO_2 and NO_2 compare to the haemoglobin percent of fishes exposed to H_2S (Fig. 5).

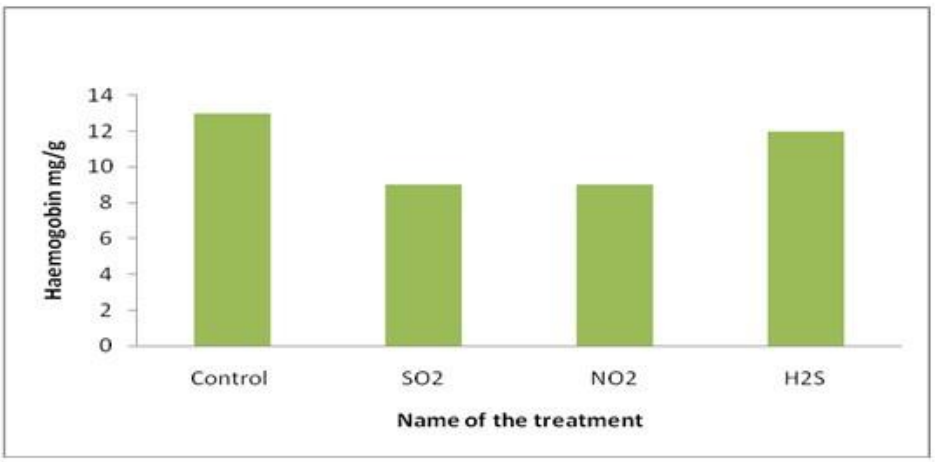

Figure 5: Haemoglobin (Hb) change (mg/g) in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Haematological parameters of PCV

The haematological parameters of PCV contents were observed in the control to be 38.96±2.18 mg/g wet weight after 30 days of experimental period. The PCV contents were significantly decreased when fishes exposed to 10 ppm of SO_2 (24.68±1.28) NO_2 (22.06±2.46) and H_2S (26.48±1.45). The decrease in the haematological parameters of PCV was more in fishes exposed to NO_2 compare to the PCV count of fishes exposed to SO_2 and H_2S (Fig. 6).

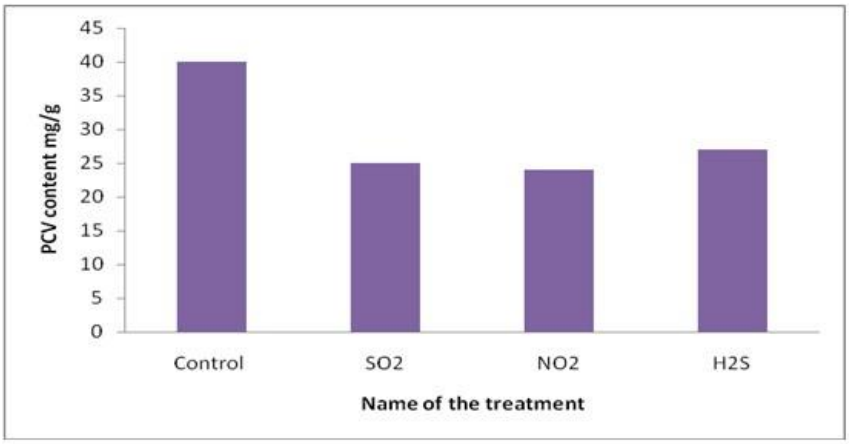

Figure 6: PCV content change (mg/g) in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Haematological parameters of MCV

The haematological parameters of MCV contents were observed in the control to be 97.50±6.22 mg/g wet weight after 30 days of experimental period. The MCV contents were significantly decreased when fishes exposed to 10 ppm of SO_2 (83.3±2.15) NO_2 (73.33±1.42) and H_2S (71.18±1.29). The decrease in the haematological parameters of MCV was more in fishes exposed to H_2S compare to the MCV count of fishes exposed to SO_2 and NO_2 (Fig. 7).

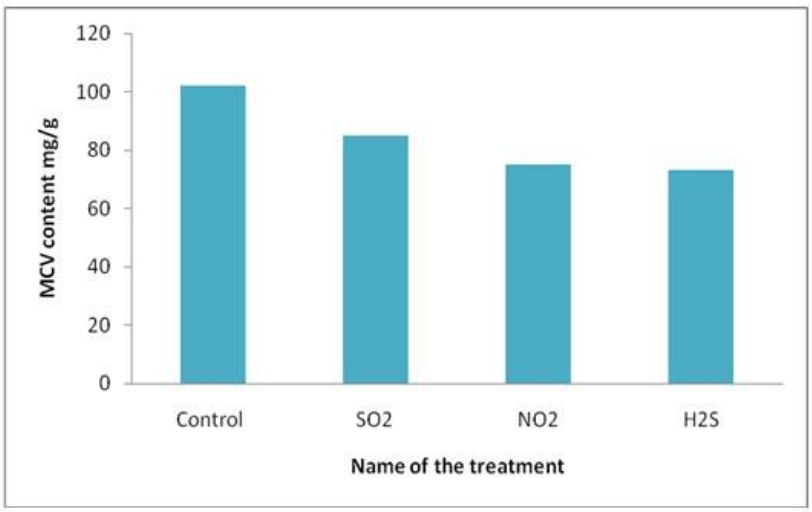

Figure 7: MCV content change (mg/g) in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Haematological parameters of MCH

The haematological parameters of MCH contents were observed in the control to be 30.41±2.64 mg/g wet weight after 30 days of experimental period. The MCH contents were significantly decreased when fishes exposed to 10 ppm of SO_2 (30.13±0.46) NO_2 (30.00±0.56) and H_2S (23.28±2.92). The decrease in the haematological parameters of MCH was more in fishes exposed to H_2S compare to the MCH count of fishes exposed to SO_2 and NO_2 (Fig. 8).

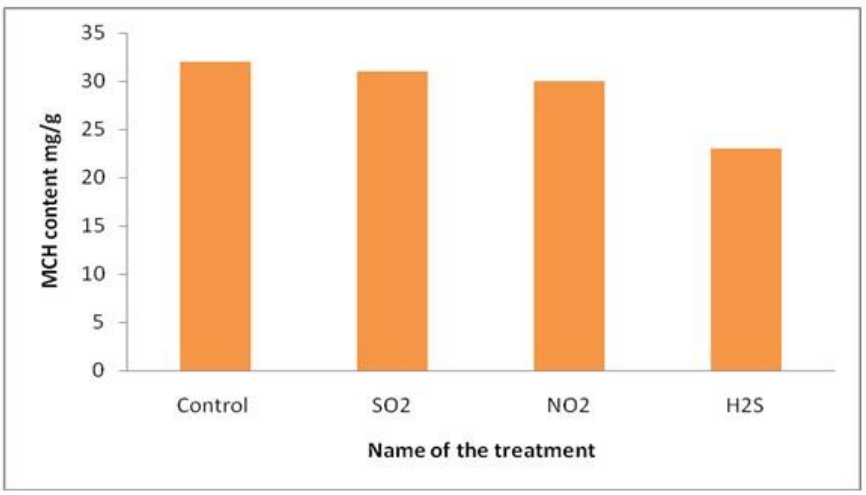

Figure 8: MCH content change (mg/g) in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Haematological parameters of MCHC

The haematological parameters of MCHC contents were observed in the control to be 35.76±2.35 mg/g wet weight after 30 days of experimental period. The MCHC contents were significantly decreased when fishes exposed to 10 ppm of SO_2 (34.61±1.80) NO_2 (33.83±1.24) and H_2S (31.11±2.92). The decrease in the haematological parameters of MCHC was more in fishes exposed to H_2S compare to the MCHC count of fishes exposed to SO_2 and NO_2 (Fig. 9).

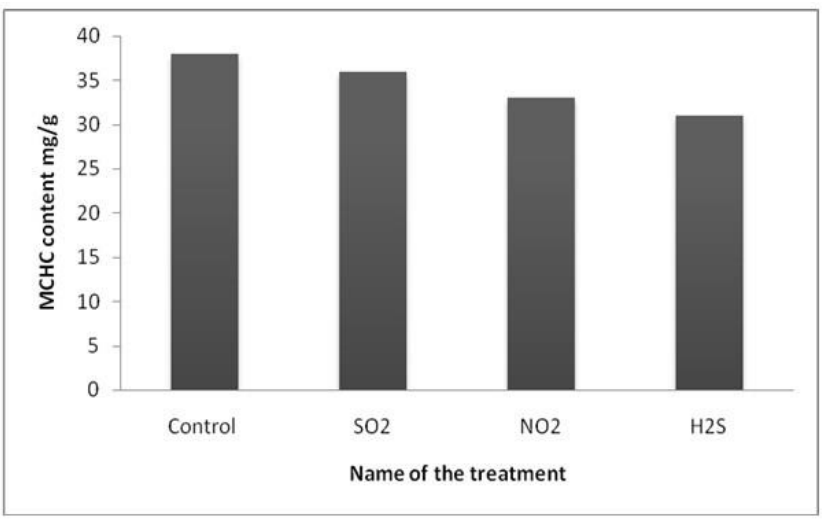

Figure 9: MCHC content change (mg/g) in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Biochemical attributes

Moisture

From Table 2, it was observed that the moisture content has decreased at all treatments. The content of moisture was less in fish samples treated with respective air pollutants compares to control treatment which indicating the industrial emissions decreases the water content of cell tissues very rapidly. Due to the reduction in moisture content, it was observed that the crude protein, crude lipid and ash all shown significant change when compare to control treatment. This low moisture content is an indication that the dried fish samples have a tendency to be very stable. The present study reveals that among the tested industrial emissions SO_2 gas shown adverse effect on moisture content followed by H_2S and NO_2.

Table 2: Moisture content (%) in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Treatment/test sample	Initial fresh weight (g)	Final dry weight (g)	% moisture
Control	11.7	7.63	68
SO_2 treated fish	11.7	4.39	39
NO_2 treated fish	11.7	4.72	43
H_2S treated fish	11.7	4.65	42

Total ash

The ash content all most similar in all fish samples treated against to industrial emissions. There is no significant change was observed statistically among the treatments. There is a significant reduction in ash content compare to control treatment. The ash content of control was 1.86±0.03, for SO_2 treatment 1.21±0.04, for NO_2 treatment 1.16±0.01 and 1.19±0.03 for fish treated with H_2S gas.

Rehydration ratio

The rehydration values of different industrial emission treated fish samples after complete dry were calculated and tested for one way ANNOVA. From the results it is observed that rehydration values were decreased compare with test samples. There is no significant change in rehydration ratio of SO_2, NO_2 and H_2S treated fish samples.

Total fat

Control of acute test: Fat content in different body parts of the fish *Channa striata* was in the order of liver > gill > muscle. Changes in the total fat content in gill, liver and muscle of *Channa striata* exposed to SO_2, NO_2 and H_2S gases were shown in Table 3. Exposure of fish to SO_2 H_2S and NO_2 showed significant (p<0.05) decrease in lipid content of liver followed by gill and muscle.

Total Protein

Control of acute test: The protein content in different body parts of *Channa striata* was in the order of liver > muscle > gill (Table 4).

As compared to control, the protein content in all the organs decreased due to acute exposure of air pollutants 10 ppm concentration for every 10 days interval for duration of 30 days. The depletion was more significant (p<0.05) in liver,

followed by muscle and gill in all treatments compare to control treatment.

Table 3: Fat content (mg/g) in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Organ	Control	SO_2 treatment	NO_2 treatment	H_2S treatment
Gill	3.632 ± 0.05	0.284 ± 0.07	0.272 ± 1.24	0.338 ± 1.19
Liver	0.893 ± 0.02	0.614 ± 0.10	0.645 ± 0.28	0.803 ± 0.10
Muscle	0.116 ± 0.11	0.097 ± 0.00	0.099 ± 0.01	0.097 ± 0.00

Table 4: Total protein content (mg/g) in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Organ	Control	SO_2 treatment	NO_2 treatment	H_2S treatment
Gill	3.388 ± 0.54	2.987 ± 0.94	2.784 ± 0.34	3.247 ± 0.37
Liver	7.542 ± 0.89	5.741 ± 0.96	4.987 ± 0.95	5.624 ± 0.74
Muscle	4.552 ± 9.12	3.982 ± 0.54	3.458 ± 0.41	3.312 ± 0.29

Total Carbohydrates

Control of acute test: The total carbohydrates levels in different organs of the fish *Channa striata* were in the order of gill > muscle. Changes in the total carbohydrates in gill and muscle of fish *Channa striata* exposed to SO_2, NO_2 and H_2S gases are shown in Table 5. The carbohydrate content in all the organs decreased considerably upon exposure to SO_2, NO_2 and H_2S gases when compare to control treatment. The percent depletion was more significant in gill and muscle of SO_2 and NO_2 treatments compare to H_2S treatment.

Table 5: Total carbohydrate content (mg/g) in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Organ	Control	SO_2 treatment	NO_2 treatment	H_2S treatment
Gill	0.298 ± 0.01	0.212 ± 0.02	0.175 ± 0.02	0.141 ± 0.03
Muscle	0.130 ± 0.14	0.062 ± 0.01	0.033 ± 0.01	0.079 ± 0.15

Crude fiber

Control of acute test: The crude fiber levels in different organs of the fish *Channa striata* were in the order of muscle>gill>liver (Table 6).

Table 6: Total crude fiber (mg/g) in *Channa striata* exposed to gaseous toxicants SO_2, NO_2 and H_2S.

Organ	Control	SO_2 treatment	NO_2 treatment	H_2S treatment
Gill	1.325 ± 0.03	0.994 ± 0.23	0.979 ± 0.32	1.024 ± 0.04
Liver	0.974 ± 0.91	0.617 ± 0.06	0.659 ± 0.03	0.846 ± 0.01
Muscle	2.915 ± 0.04	2.624 ± 0.05	2.568 ± 0.06	2.725 ± 0.02

Changes in the crude fiber in gill, liver and muscle of fish *Channa striata* exposed to SO_2, NO_2 and H_2S gases are shown in Table 4. The fiber content in all the organs decreased considerably upon exposure to SO_2, NO_2 and H_2S gases when compare to control treatment. The percent depletion was more significant in gill and muscle of SO_2 and NO_2 treatments compare to H_2S treatment.

Discussion

In the physiological and physio-pathological evaluation of animals, the

study of different biochemical and cellular constituents in blood is of fundamental importance, because pollutants and other environmental factors can induce morphological and quantitative variations in blood parameters (Juneja and Mahajan, 1983; Ranzani-Paiva *et al.,* 1997; Ishikawa *et al.,* 2007). Hematological parameters can be used as fish health indicators have been proposed by Hesser (1960).

Hematology is used as an index of fish health status which detect physiological changes caused by different stress conditions like exposure to pollutants, metals, diseases, hypoxia, etc. (Blaxhall, 1972; Duthie and Tort, 1985). Therefore, the most common method to determine the sub-lethal effects of the pollutants are hematological techniques (Larsson *et al.,* 1985; Alwan *et al.,* 2009). In the diagnosis of stress, environmental pollution, mutagenesis and also the abiotic fish diseases, study on whole blood count, biochemical changes and micronuclei induction in fish serves as a significant tool, as variations in blood appears first before the onset of any morphological or degenerative changes (Javed *et al.,* 2016; Sharma *et al.,* 2023).

In the whole body blood parameters are regarded as pathophysiological indicators and therefore when fish subjected to toxicants it become requisite in diagnosing the structural and functional status (Adhikari *et al.,* 2004). Numerous haematological indices such as White blood cells (WBC), Red blood cells (RBC), haemoglobin (HB), haematocrit (PCV), oxygen carrying capacity were employed as indicators of gaseous pollution in the aquatic environment and to assess the functional status of the fish (Shah and Altindag, 2004; Maheswaran *et al.,* 2008).

In general assessment of animal's health, total red blood cells (RBC), haemoglobin (Hb) and white blood cells (WBC) were often considered. Variation in these indices from reference gives warning of disease. Decline in red blood cells mostly leads to low packed cell volume (PCV) and Hb levels. Milligan and Wood (1982) reported that mean cell hemoglobin concentration measure was utilized to determine the amount of red blood cell swelling (decreased MCHC) or shrinkage (increased MCHC). Shaheen and Akhtar (2012) distinguished considerable decline in Hb content and RBC counts of fish *Cyprinus carpio* when exposed to Cr (VI). Fall-off in TEC, Hb and PCV indicates anemic condition of the animal. This anemic condition perhaps due to the inhibition of erythropoiesis or impair of haemopoietic tissue and transferring disfunction. MCV, MCH and MCHC are the components of full blood count and they are also known as red cell indices. It signifies that they indicate the size and haemoglobin content of erythrocytes. MCV represent the size of the erythrocytes, where high MCV suggests macrocytic condition and low as microcytic condition (Javed *et al.,* 2016). Singh (1995) reported descend in RBC count, Hb and PCV in the fish, *Channa punctatus* upon treatment with both copper and chromium. *Oreochromis niloticus* subjected to

cadmium exhibited consequential depletion in RBC, Hb and HCT (Kaoud *et al.,* 2011; Ibrahim and Elsayed, 2023).

Due to toxicant exposure there is reduction in RBC level has been reported by Chrowdhury *et al.,* (2004). Erythrocyte production can be reduced even with a low level of industrial effluent exposed for span of time (Abernatty *et al.,* 2003). It has been stated that the fall off in RBC give rise to haemolytic crisis and consequence in severe anemia in fish exposed to copper pollutant (Khangarot and Tripathi, 1990). According to Wedmeyer *et al.,* (1984) impaired osmoregulation over the gill epithelium induce haemodilution which might lead to the decline in erythrocyte.

During oxidation of ammonium to nitrate, an intermediate formed is nitrite. In case of fresh water fish upraise in ambient nitrite concentration is a serious problem because nitrate is actively absorbed over the gills against chloride. Nitrite is well known toxicant for fish and also for aquatic environment. The toxicity component is the nitrite ion (NO_2) which is presumed to infiltrate the blood via branchial chloride/bicarbonate exchange and fish such as Salmonids with high chloride ingestion rates are highly vulnerable than those with low chloride intake rates for example Carp fish. Chloride firmly relieves nitrite toxicity while estimating the toxicity concentration ratio of these ions is of great prominence.

Leucocytes necessitate in the immunological response (Santhakumar, 1999). The depletion of haemoglobin modifies the oxygen binding capacity (Wendelar-Bonga, 1997) and it furthermore indicates anemic condition in fish which owing to the stress related haemolysis (Adeyemo, 2007; Amte and Trupti, 2013). Correspondingly, decline in haemoglobin is due to heavy metal stress (Goel *et al.,* 1985). Reduction in haemoglobin was remarked in fish exposed to nickel in laboratory condition and it is because of the disintegration of erythrocytes (Ololade and Oginni, 2010).

The diminution in haematocrit values is a manifestation of anemia or oligohaemia (Wepner *et al.,* 1992). Analogous reduction in haematocrit value was perceived in *Clarias gariepinus* exposed to effluent from metal finishing company (Adakole, 2012). *Sarothedon melanotheron* exposed to industrial effluent also showed decreased PCV value. Lower MCV and MCH might be due to the high percentage of immature red blood cells in circulation. Whereas MCHC value showed only slight fluctuation (Nte *et al.,* 2011). Various researchers delineated that the cells discharged from the spleen, the erythropoietic organ perchance decreased MCV value (Ololade and Oginni, 2010; Singh *et al.,* 2010).

Haematological variables employed constantly when clinical diagnosis of fish physiology was applied to estimate the sublethal concentrations of pollutants (Wedemeyer and Yasutake, 1977). Various workers have described that the utilization of haematological parameters as criterion to certain

pollutant toxicity can dispense information on the physiological response of fish due to the intimate inter-relationship of the circulatory system with the external environment (Wepener *et al.*, 1992). In fishery management and disease investigation, fish blood is being interpreted progressively in toxicological research and environmental monitoring as a feasible indicator of physiological and pathological diversities (Mulcahy, 1975).

The haematological abnormalities under toxic stress furthermore emulated in other physiological activities like metabolism and oxygen consumption that culminate in death (Aruna and Gopal, 1987). Evaluation of haematological parameters could be worthwhile in analysing fish health (Blaxhall and Daisley, 1973) but the dissimilarity in fish haematologic parameters has been a concern for researches and health specialists. Hilmy *et al.* (1987) reported that when freshwater fish exposed to nitrate, profound changes ensue in haematological parameters and blood respiratory properties. Fish diseases could be diagnosed by considering blood of fish as an indicator. Consequently, haematological investigations are much indispensable in monitoring the fish health.

Exposure to cadmium might enhance a wide range of additional subtle influence on haematology and osmoregulation (Remyla *et al.*, 2008; Maundera *et al.*, 2011). In the present investigation on freshwater fish *Channa striatus* when subjected to 10 ppm concentrations of SO_2, NO_2 and H_2S after every 10 days interval for the span of 30 days ensued a declined value of RBC, Hb, PCV and MCHC however, WBC levels amplified in the blood. The present work corresponds with Kaoud *et al.* (2011) who reported that the RBC, Hb and haematocrit values were diminished significantly in fish, *Oreochromis niloticus* subjected to sub lethal concentration of cadmium. In the African catfish *Clarias gariepinus* exposed to mercuric oxide and carbohydrate mixed diet the haemoglobin levels were decreased (Zaki *et al.*, 2011). Danion *et al.* (2011) reported that reduction in haematocrit values and red blood cells in contaminated sea bass fish when correlated with control fish blood. Fish *Channa punctatus* exposed to cadmium chloride and mercuric chloride revealed significant decline in RBC and Hb and a rise in WBC count (Patil and Dhande, 2000).

The haematological parameters might have affected significantly from nutritional status of fish (Osuigwe *et al.*, 2005). The WBC possessed the greatest sensitivity to alteration in the environment (Mahajan and Dheer, 1979). The MCV, MCH and MCHC are corpuscular indices that have certain prominence in several animals in delineating anemia and could be employed in diagnosis and therapy (Coles, 1986). The MCHC is better indicator of red blood cell swelling; the inadequate PCV would imply anemia or oligohaemia (Wepener *et al.*, 1992). The

PCV observations are promising in estimating the consequence of stressors on the health of fish and are additionally utilized to evaluate the oxygen carrying capacity of blood (Larsson *et al*., 1985). The higher values of MCV and MCH may precise a condition of macrocytic anemia (Bomford *et al.,* 1975). Bhagwant and Bhikajee (2000) have reported that the fluctuation perceived in both MCV and MCHC in aluminium-exposed fish *Oreochromis mossambicus* hybrid for divergent periods evolved in a microcytic hypochromic anemia which proceeds to a macrocytic hypochromic type after 8 weeks. Indistinguishable pattern has been revealed in *Labeo umbratus* after subjection to disparate pollutants (Van vuren, 1986).

The main pollutants responsible for acid deposition (acid rains) are sulfur dioxide (SO_2) and nitrogen oxides (NOx). The acid rain (acid deposition) influences the mainly the pH of fresh water. Nitrogen and sulfuric emissions comes from industrial emissions mix with water vapour at unusual proportions to cause acid deposition with a pH of 4.2 to 4.7. The acidification of freshwater in an area is dependent on the quantity of calcium carbonate (limestone) in the soil. Limestone buffer (neutralize) the acidification of freshwater.

Joshi *et al.* (2002) have stated that heavy metal exposure results in an impaired intestinal absorption of iron leads to decline in RBC, Hb, PCV and MCHC. Variation in PCV, MCH and MCHC in the current investigation acknowledge with the prior findings of Banerjee and Banerjee (1988) and Nanda (1997). Air breathing fish, *Channa punctatus* subjected to cadmium showed considerable fall off in total haemoglobin content, erythrocyte count, MCHC, PCV (Karuppasamy, 1990). When *Channa gachra* exposed to mercuric chloride resulted in significant diminution in haemoglobin content and RBC (Patil and Dhande, 2000). Panigrahi and Misra (1978) reported that the fish *Anabas scandens* exposed with mercury showed low red blood cell (RBC) count and haemoglobin percentage. Fish *Tinca tinca* subjected to lead and mercuric chloride exhibited decline in RBC count, haemoglobin and Hct (Shah and Altindag, 2004). RBC values and anemia were delineated in fish such as *Salvalinus fontinalis* (Holcombe *et al.,* 1976), *Colisa fasciatus* (Srivastava and Mishra, 1979), rainbow trout, *Salmo gairdneri* (Haux and Larsson, 1982), *Barbus conchonius* (Tewari *et al.,* 1987) while exposure to various heavy metals.

Nwani *et al*. (2015) suggested that magnitude of RBC, PCV, Hb were diminished whereas extend of WBC was higher in paraquat treated blood of African catfish *Clarias gariepinus*. While Mahseer subjected to cypermethrin amount of RBC declined and WBC levels elevated in the blood (Ullah *et al.,* 2015). Abedi *et al.* (2013) observed that the blood of *Cyprinus carpio* has lower extent of Hb, RBC and PCV when treated with trivalent chromium. These earlier literatures furnishing substantial information concerning heavy metal toxicity in fresh and marine water fishes. The current

investigation may assist researchers to approach the physiology and haematological disquisition on *Channa striatus* under air pollution stress.

Toxic effects of pollutants are due to disturbance of the normal physiological functions of the organisms. Changes in biochemical constituents in the tissues due to exposure of gaseous pollutants stress has definite pattern. Metabolic activity of an organism reflects utilization of biochemical energy to counteract the toxic stress. In every individual, carbohydrates, protein, fiber and lipid/fat act as sources of energy for carrying out various activities. Due to gaseous pollutants toxicity in current investigation, the prime source of energy is affected severely and retard various processes in exposed individuals.

The results of the study reveal significant effect of gaseous pollutants on carbohydrate metabolism. Carbohydrate content in all the tissues under study was found to decrease continuously throughout the exposure period. Depletion in glucose in the present study may be due to its rapid utilization to meet the demands under toxic manifestation.

In the present study, the protein level decreased in all the organs exposed to gaseous pollutants as compared to control. The drop in the protein content may be on account of reduced protein synthesis during toxicity. According to Farkas (1975) lead treatment would reduce the binding of phenyl alanyl and lysyl tRNA to ribosome leading to protein depletion. The depletion of protein also suggests increased proteolysis and possible utilization of the product of their degradation for metabolic processes. They may be fed into TCA cycle through amino transferase system to cope up with excess demand of energy during toxic conditions (Chandravathy and Reddy, 1994). Syversen (1981) opined that the heavy metals, in general, interfere with protein synthesis.

Conclusion

Considerable changes were observed in Heamotological and Biochemical parameters of *Channa striata* exposed to SO_2, NO_2 and H_2S gases. The changes took place due to accumulation of *Channa striata* exposed to SO_2, NO_2 and H_2S gases in various organs shown their adverse effect on various metabolisms. These variations represent disturbances in the metabolic processes of the organism. Measurement of these Heamotological and Biochemical parameters indicates the effects of stress and abnormality in *Channa striata* which confirms the increasing concentration of gaseous industrial emissions (individually or mixture of gas) threat to the aquatic biota.

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