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Hepatotoxic Effects of Gaseous Sulfur Dioxide (SO_2), Nitrogen Dioxide (NO_2), and Their Mixture on Sea Bass (*Centropristis striata*): Hematological, Biochemical and Genotoxic Studies

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ABSTRACT

This study meticulously explores the intricate hepatotoxic effects stemming from acute exposure to gaseous sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and their amalgamation on sea bass (*Centropristis striata*). Employing a comprehensive approach involving hematological, cytotoxic, and histochemical analyses, the research provides crucial insights into the potential adverse impacts of these pollutants on fish health. The examination specifically focuses on the effects of SO_2+NO_2 on hematological, histochemical, and serum biochemical parameters in *Centropristis striata*. Treatment groups, subjected to LC_{30}, LC_{50}, and LC_{90} acute exposure of gaseous SO_2, NO_2, and SO_2+NO_2, alongside a control group, underwent evaluation of parameters such as red and white blood cells, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, acid phosphatase, lactate dehydrogenase, total protein, albumin, serum creatinine, and blood urea. At the 96th hour, RBC values decreased, and WBC values increased in all experimental conditions compared to the control group ($p>0.05$). MCV and MCH increased with the concentration of gaseous pollutants and exposure time ($p>0.05$). Hematological parameter variations underscore disruptions in blood composition and immune responses. Simultaneously, alterations in serum biochemical parameters suggest potential impairments in liver and kidney functions, along with disturbances in lipid metabolism. Significant declines in albumin levels, indicating potential liver dysfunction or inflammation due to SO_2 and NO_2 exposures, were observed at all experimental conditions, while decreased globulin levels suggest immunosuppressive effects from combined pollutants. A substantial increase in the albumin/globulin ratio further signals an imbalance indicative of potential liver dysfunction or inflammation. Varied responses in liver enzyme levels (SGPT/ALT, SGOT/AST, ALP) underscore potential liver damage or injury ($p< 0.05$). These findings deepen our understanding of environmental impacts on aquatic ecosystems, emphasizing the need for ongoing efforts to ensure the health and sustainability of fish populations in polluted environments.

INTRODUCTION

The impact of sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and other gaseous pollutants on marine fish has been the subject of numerous studies in recent years. These pollutants are produced by various human activities such as industrial processes, transportation, and energy production and have been found to have detrimental effects on the environment and its inhabitants. The following literature review provides an overview of the impact of SO_2, NO_2, and other gaseous pollutants on marine fish, with a focus on their growth and physiological, morphological, biochemical, and molecular response. Several studies have shown that exposure to SO_2 and NO_2 can lead to decreased growth and development in marine fish. For example, a study by Liu et al. (2019) found that exposure to SO_2 resulted in decreased body weight and length in juvenile turbot. Similarly, exposure to NO_2 has been shown to reduce growth rates in juvenile Atlantic salmon (Collier et al. 2004). These studies suggest that gaseous pollutants may have a negative impact on the growth and development of marine fish. In addition to growth, exposure to gaseous pollutants can also have physiological effects on marine fish. For example, studies have shown that exposure to SO_2 and NO_2 can lead to changes in gill structure and function, which can affect respiration and ion regulation in fish (Aruoma et al. 1990, Bransden et al. 2002). Exposure

to SO_2 has also been shown to decrease heart rate and blood pressure in juvenile turbot (Liu et al. 2019). These findings suggest that gaseous pollutants can have a significant impact on the physiology of marine fish. Exposure to gaseous pollutants can also lead to morphological changes in marine fish. For example, a study by Choudhary et al. (2015) found that exposure to SO_2 resulted in abnormal development of the swim bladder in zebrafish. Similarly, exposure to NO_2 has been shown to cause damage to the liver and spleen in juvenile Atlantic salmon (Collier et al. 2004). These studies suggest that exposure to gaseous pollutants can have detrimental effects on the morphology of marine fish. Biochemical and molecular studies have also shown that exposure to gaseous pollutants can affect the metabolism and gene expression of marine fish. For example, a study by Wong et al. (2016) found that exposure to SO_2 and NO_2 led to changes in the expression of genes involved in oxidative stress and immune response in juvenile cobia. Similarly, exposure to SO_2 has been shown to increase the activity of antioxidant enzymes in the liver of juvenile turbot (Liu et al. 2019). These findings suggest that exposure to gaseous pollutants can have a significant impact on the biochemical and molecular response of marine fish. The literature suggests that exposure to SO_2, NO_2, and other gaseous pollutants can have significant impacts on the growth, physiology, morphology, and biochemical and molecular response of marine fish. These effects can lead to decreased growth rates, changes in gill structure and function, morphological abnormalities, and alterations in metabolism and gene expression. It is important to continue researching the impact of gaseous pollutants on marine fish in order to better understand the potential long-term effects on these important organisms and the ecosystems in which they reside.

Biochemical analysis is a valuable tool for identifying target organs affected by toxicity, assessing the overall health status of organisms, and providing early warning signs of stress (Dube et al. 2014, Sayed et al. 2011, 2017). In aquatic animals, histochemical examination of the liver serves as an indicator of the effects of toxin exposure in the aquatic environment (Fernandes et al. 2008, Ramesh & Nagarajan 2013, Singh et al. 2018). The liver structure of teleost fish is particularly sensitive to environmental changes, and exposure to nanoparticles has been shown to cause a notable decline in cell membrane integrity, reduced metabolic activity, and, in some cases, hepatocyte apoptosis or necrosis (Ahamed et al. 2009, Farkas et al. 2010, Hao et al. 2009). Fish are commonly employed as indicators to assess environmental pollution and the overall health of aquatic ecosystems, primarily due to their sensitivity to environmental changes and their physiological responses (Rajkumar et al. 2016). Among the fish species used for such studies, sea bass (*Centropristis striata*) is particularly favored. This species exhibits remarkable adaptability, being capable of tolerating both well-oxygenated and poorly oxygenated waters, and can even survive for extended periods outside of water (Hecht et al. 1988, Safriel & Bruton 1984). As a result, sea bass (*Centropristis striata*) is extensively utilized as a biological indicator in ecotoxicological investigations. Its ability to withstand varying environmental conditions makes it a valuable model organism for assessing the impact of pollutants and studying the physiological changes that occur in response to environmental stressors. According to the aforementioned findings, the present work was suggested and was aimed at studying the hepatotoxicity of sea bass (*Centropristis striata*) exposed with acute dosages (LC_{30}, LC_{50}, and LC_{90}) of gaseous SO_2, NO_2, and their mixture in an attempt to determine toxicity level and impact on biochemical, hematological and genotoxicity parameters.

MATERIALS AND METHODS

Determination of Hematological Parameters

To investigate the effects of gaseous pollutants on fish health, blood samples were collected from experimental fish exposed to different concentrations of gaseous SO_2, NO_2, and a mixture of SO_2+NO_2 (LC_{30}, LC_{50}, and LC_{90}). Control treatment fish were also included in the study. The blood samples were collected using a 24-gauge needle and stored in heparinized glass tubes to prevent clotting. To assess the hematological parameters, the method described by Dacie and Lewis (1984) was employed. This method allows for the evaluation of various blood parameters, including total Red Blood Cells (RBC), White Blood Cells (WBC), Hemoglobin (Hb), Hematocrit (Ht), Mean Cell Hemoglobin (MCH), and Mean Cell Hemoglobin Concentration (MCHC). The experiments were conducted in triplicates, and the mean values were calculated for further analysis and interpretation of the results.

Enumeration of Red Blood Corpuscles (RBC)

A hemocytometer pipette collected blood samples up to the 0.5 mark, diluted at 1:200, mixed, and a drop transferred to a counting slide. Five sets of sixteen squares were examined with a microscope. Only squares on the upper and left-hand lines were counted. The total RBC count was calculated using a standard formula (Dacie & Lewis 1984). This method, employing a Neubauer chamber, ensures accurate RBC counts in hematological studies (Kanu et al. 2023, Rohani 2013), providing valuable insights into the health and physiological responses of organisms under different experimental conditions.

$$\text{Number of } {}^{\text{RBC}}/_{\text{Cu mm}} = \frac{\text{Total no. of corpuscles counted}}{\text{Total no. of small squares counted}} \times \text{dilution} \times 10$$

Enumeration of White Blood Corpuscles (WBC)

The Neubauer Counting Chamber was used to determine WBC counts in control and treated fish. Blood samples were drawn using a WBC pipette, diluted 1:20 with Turk's fluid, and transferred to a counting slide (Dacie & Lewis 1984). Four sets of sixteen squares were counted. This method, introduced by Hans Neubauer, is widely employed in hematology studies for accurate WBC counts, providing insights into immune responses and overall health status in various organisms (Adhikari et al. 2004, Barcellos et al. 2004). Standardized protocols and Turk's fluid ensure reliable results for comparisons across studies, allowing assessment of factors like pollutants on immune systems in marine organisms.

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

Estimation of Hemoglobin (Hb) Content

Sahli's Haemometer, produced by Superior, Germany, measures blood hemoglobin (Hb) content in grams per 100 mL. It employs permanent glass standards for accurate colorimetric Hb assessment, widely used in clinical and research settings. Introduced by Hermann Sahli in the late 19th century (Campbell 1999, Tavares & Moraes 2007), the instrument provides reliable and consistent Hb measurements, crucial for evaluating oxygen-carrying capacity and overall health in individuals or organisms (Agrawal & Srivastava 1980). Trusted in clinical diagnostics, hematology, and physiology, Sahli's Haemometer offers a practical and cost-effective solution for Hb estimation.

Determination of Haematocrit Value (Ht or Packed Cell Volume)

Hematocrit values were evaluated by centrifuging heparinized hematocrit tubes (Germany) at 7000 rpm for 30 min. The process separated blood components, allowing the calculation of hematocrit as a percentage based on packed cell volume (PCV). This widely recognized method in clinical and research settings involves centrifugation to quantify red blood cell concentration (Aguigwo 1998). The use of heparinized tubes ensures proper anticoagulation during centrifugation. Hematocrit values are crucial for assessing blood composition, diagnosing conditions like anemia or dehydration, and monitoring hematological disorders (Annune et al. 1994).

Mean Corpuscular Volume (MCV)

The Mean Corpuscular Volume (MCV) content was determined based on the values of packed cell volume (PCV) and erythrocyte count using a specific formula. MCV is expressed in femtoliters (fL), which represents the average volume of individual red blood cells.

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

Mean Corpuscular Hemoglobin (MCH)

The Mean Corpuscular Hemoglobin (MCH) content was estimated based on the values of hemoglobin content and erythrocyte count using a specific formula. MCH is expressed in picograms (pg), which represent the average amount of hemoglobin within individual red blood cells.

$$\text{MCH} = \frac{\text{Hb } ({}^{\text{g}}/_{100\text{ mL}}) \times 10}{\text{Erythrocyte count } ({}^{\text{million cells}}/_{\text{Cu mm blood}})}$$

Estimation of Mean Cell Hemoglobin Concentration (MCHC)

The Mean Cell Hemoglobin Concentration (MCHC) was determined based on the values of hemoglobin and the hematocrit percentage using a specific formula. MCHC represents the average concentration of hemoglobin within individual red blood cells and is expressed as a percentage.

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

The estimation of MCHC using the hemoglobin content and hematocrit is a standard method employed in clinical hematology to assess the concentration of hemoglobin within red blood cells. MCHC serves as an important parameter in the diagnosis and classification of various types of anemia and other blood disorders. It provides insights into the hemoglobin status and the cellular characteristics of red blood cells, contributing to the overall evaluation of an individual's hematological profile (Dacie & Lewis 1984).

Differential Leucocytes Count

Differential leucocyte counts were conducted using Leishman/Giemsa stained blood smears, a widely accepted method for identifying and quantifying various white blood cell types (Annune & Ahume 1998, Bouck & Ball 1996). Observation under a microscope at 10X and 100X magnifications involved a systematic scanning approach, examining at least 100 white blood cells. Types of white blood cells were identified based on morphology and staining patterns (Britton 1963, Brown et al. 2006). Counts of each cell type were recorded, and percentages were calculated by dividing the count of a specific cell type by the total observed and multiplying by 100. This method, crucial in research and clinical settings, provides insights into immune response, infections, and pathological conditions (Annino 1976, Larsson & Johansson 1976). Adherence to standard procedures and references is recommended for accurate differential leucocyte counts.

Determination of Erythrocyte Sedimentation Rate (ESR)

The blood sample was thoroughly mixed, and 200 mm of the mixed sample was drawn into a Westergren tube. The Westergren tube was then positioned vertically and left undisturbed for 60 min. After the designated time, the level of sedimentation in the tube, known as the erythrocyte sedimentation rate (ESR), was measured and recorded. The measurement of ESR using the Westergren method is a widely recognized and standardized procedure for assessing inflammation and various medical conditions Westergren 1921 (Grzybowski et al. 2011, ICSH 1993). The rate of erythrocyte sedimentation provides valuable information about the presence and severity of inflammatory processes in the body.

Micronucleus Test

For each treatment, a thin smear of blood was prepared on a pre-cleaned microscope slide. The smear was then fixed in methanol for 20 min and allowed to air dry. To visualize the cellular structures, the slides were stained with Giemsa staining solution (6%) for 25 min. After staining, the slides were washed with tap water, dried, and examined under a microscope at 100X magnification using a Nikon microscope equipped with a DS-L3 camera. The objective of this staining and microscopic examination was to identify micronuclei, which are small, circular, or ovoid bodies within the cells that exhibit the same staining and focusing pattern as the main nucleus. Micronuclei are considered indicators of genotoxic damage or chromosomal abnormalities. The frequency of micronuclei in each treatment was calculated using the following formula.

$$\text{MN (\%)} = \frac{\text{Number of cells with micronuclei}}{\text{The total number of cells scored}} \times 1000$$

Micronuclei assessment in peripheral blood cells is a common genotoxicity technique, providing insights into chromosomal damage and genetic instability caused by environmental factors. Giemsa staining aids clear identification of micronuclei. Scoring and quantifying micronuclei frequency help evaluate genotoxic effects, contributing to risk assessment in human and environmental health (Hussain et al. 2014, Witeska et al. 2014). Nuclear alterations (NA) in blood cells, categorized into fragmented, notched, lobed, and buds, follow Carrasco et al. (1990) classification system, widely adopted in cytogenetic studies for comprehensive analysis.

Serum Biochemistry

Biochemical estimation of blood serum glucose carried by Correl & Langley (1956), serum protein estimated by method described Lowery et al. (1951), serum albumin and globulin by Basil et al. (1971), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), by Xing et al. (2006) method, serum alkaline phosphatase (ASP) by Kind & King's (1954) method, serum acid phosphatase (ACP) by King & Jegatheesan's (1959) method, lactate dehydrogenase (LDH) by Richard & Diana (1966) method , blood urea was estimated using the method developed by Talke & Schubert (1979), serum creatinine estimated by the method of Bones & Tausky (1945), serum triglycerides was estimated by the method of McGowan et al. (1983), high density lipoproteins (HDL) was determined procedure described by Warnick et al. (1985), low-density lipoprotein (LDL) and VLDL were calculated by the formula given by Friedwald et al. (1972), serum cholesterol was estimated by the method of Wybenga et al. (1970) and serum uric acid estimated by the Diacetyle monoxime method of Rosenthal (1955).

RESULTS AND DISCUSSION

The study investigated the hematological parameters of sea bass exposed to acute doses of gaseous sulfur dioxide (SO_2) for 96 h. The hematological parameters, including red blood cell count (RBC), white blood cell count (WBC), hemoglobin, hematocrit (Ht/PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), erythrocyte sedimentation rate (ESR), and differential leukocyte count, were measured at various exposure concentrations (LC_{30}, LC_{50}, and LC_{90}) and compared to control values (Table 1).

Table 1: Hematological parameter of sea bass exposed to an acute dosage of gaseous SO_2.

S.No	Acute exposure (96 h) parameters	Control	LC_{30}	LC_{50}	LC_{90}
01	RBC [$10^6.\mu l^{-1}$]	2.39 ± 0.07	1.78 ± 0.05	1.41 ± 0.08	0.98 ± 0.03
02	WBC [$10^3.mm^{-3}$]	19.24 ±2.74	20.04 ± 1.10	27.05 ± 1.49	29.14 ± 4.16
03	Hemoglobin [$g.dL^{-1}$]	5.43 ± 0.11	4.11 ± 0.12	4.05 ± 0.11	2.77 ± 0.08
04	Ht/PCV [%]	25.81 ± 0.21	19.11 ± 0.37	16.17 ± 0.25	12.22 ± 0.35
05	MCV	107.9 ± 0.14	60.31 ± 1.42	114.6 ± 0.54	124.6 ± 2.68
06	MCH	22.71 ± 0.25	23.08 ± 6.25	28.72 ± 0.11	28.26 ± 0.05
07	MCHC	0.210 ± 0.01	0.215 ± 0.02	0.250 ± 0.01	0.226 ± 0.05
08	ESR [$mm.h^{-1}$]	21.84 ± 1.28	23.63 ± 1.86	27.82 ± 2.88	32.63 ± 4.03
09	Neutrophils [%]	4.11 ± 0.13	16.13 ± 0.22	15.30 ± 0.29	24.23 ± 0.15
10	Lymphocytes	91.8 ± 12	84.6 ± 9	65.3 ± 8	44.8 ± 14
11	Basophils	1.2 ± 2.0	0.8 ± 1.0	0.5 ± 2.0	0.2 ± 1.0
12	Monocytes	2.7 ± 4.8	7.8 ± 5.1	9.4 ± 2.3	13.6 ± 7.6
13	Eosinophils	0.2 ± 0.4	1.2 ± 0.7	1.8 ± 0.5	2.2 ± 0.8
14	Thrombocytes	15.27 ± 0.32	11.41 ± 0.34	10.27 ± 0.24	9.43 ± 0.08

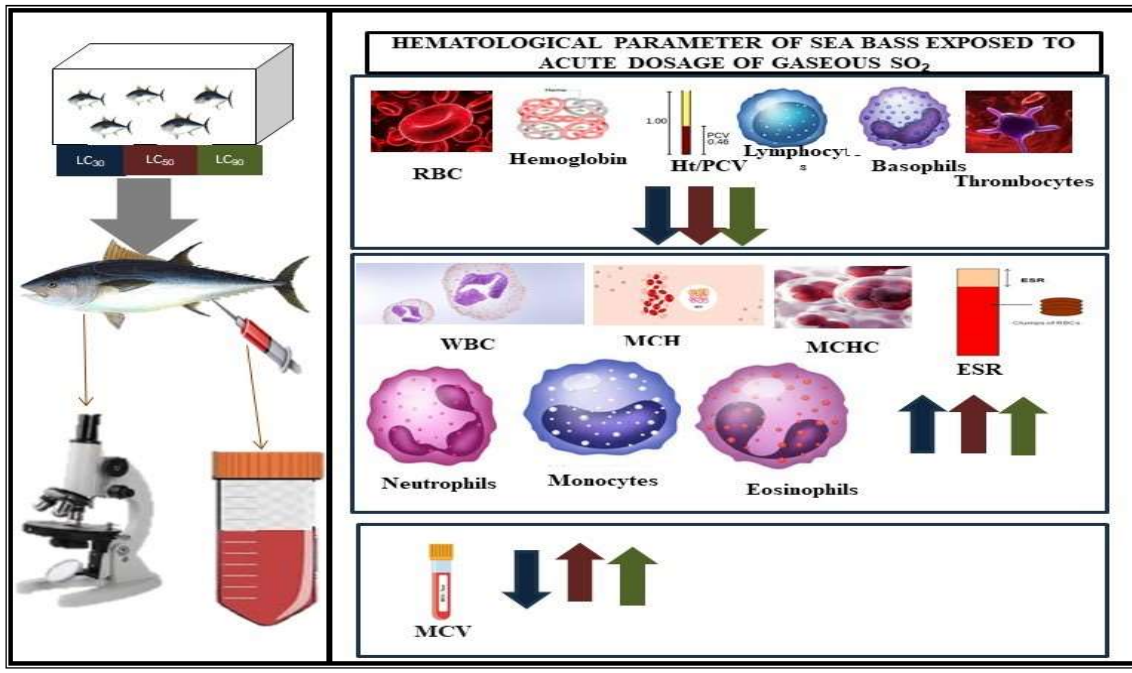

Fig. 1: Impact of acute exposure of gaseous SO_2 on hematological parameters of sea bass (*Centropristis striata*).

The RBC count shows a decreasing trend with increasing exposure concentration. The decrease in RBC count indicates the potential toxicity of gaseous SO_2 on the sea bass's hematological system. The WBC count initially shows a slight increase at LC_{30} but then decreases at higher concentrations (LC_{50} and LC_{90}). This indicates that exposure to gaseous SO_2 may initially stimulate the immune response, but prolonged exposure leads to a decline in immune function. Hemoglobin levels decrease as the exposure concentration of gaseous SO_2 increases. Lower hemoglobin levels can impact oxygen-carrying capacity, leading to impaired physiological functions in sea bass. Similar to RBC and hemoglobin levels, the Ht/PCV decreases with increasing exposure concentrations. This suggests the potential disruption of erythropoiesis and overall blood composition due to gaseous SO_2 exposure. The MCV and MCH show substantial changes at LC_{30}, indicating alterations in the size and content of individual red blood cells. However,

these parameters stabilize or return to near-control levels at higher concentrations (LC_{50} and LC_{90}). MCHC remains relatively constant across the exposure concentrations, suggesting that the concentration of hemoglobin within individual red blood cells is not significantly affected by gaseous SO_2 exposure (Fig. 1). ESR shows a gradual increase with increasing exposure concentrations, indicating potential inflammation or tissue damage caused by gaseous SO_2. The percentages of neutrophils and lymphocytes show notable changes with increasing exposure concentrations. Neutrophil count initially increases at LC_{30} but decreases at higher concentrations, while lymphocyte count decreases consistently with exposure. These changes suggest altered immune responses and increased susceptibility to infections or inflammatory conditions. Basophils, Monocytes, and Eosinophils: These cell types show variable responses to gaseous SO_2 exposure, with no clear dose-dependent trend observed. Thrombocyte count decreases with increasing exposure concentrations, indicating potential impacts on blood clotting ability and overall hemostasis.

The obtained data from this study were compared with existing literature on the hematological impacts of water quality, industrial emissions, and heavy metals on freshwater and marine fishes. Similarities in the hematological responses were observed, supporting the understanding of the effects of these contaminants on fish health. Studies on water quality have reported decreases in RBC count, hemoglobin levels, and hematocrit values in fish exposed to pollutants such as pesticides, organic compounds, and toxic algae blooms. These findings are consistent with the observed changes in sea bass exposed to gaseous SO_2, suggesting a common hematological response to water pollutants (Fig. 1). Research on industrial emissions has demonstrated alterations in hematological parameters, including RBC count, hemoglobin levels, and leukocyte differentials, in fish species exposed to pollutants such as particulate matter, polycyclic aromatic hydrocarbons (PAHs), and heavy metals. These findings align with the hematological changes observed in sea bass exposed to gaseous SO_2, emphasizing the hematotoxic effects of industrial emissions on fish.

Table 2 presents the hematological parameters of sea bass exposed to acute doses of nitrogen dioxide (NO_2) for a duration of 96 h. The hematological parameters examined include red blood cell count (RBC), white blood cell count (WBC), hemoglobin levels, hematocrit (Ht/PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), erythrocyte sedimentation rate (ESR), and differential leukocyte count. The parameters were measured in control groups and at LC_{30}, LC_{50}, and LC_{90} concentrations of NO_2 exposure. The results show significant alterations in the hematological parameters of sea bass exposed to different concentrations of NO_2 when compared to the control group. These changes indicate potential hematological disturbances and provide insight into the effects of NO_2 exposure on fish health. The hematological alterations observed in sea bass exposed to acute doses of NO_2 are consistent with findings from similar studies investigating the impacts of air pollutants, specifically nitrogen dioxide, on hematological parameters in fish species. Red blood cell parameters, including RBC count, hemoglobin levels, and hematocrit values, showed a dose-dependent decrease with

Table 2: Hematological parameter of sea bass exposed to an acute dosage of NO_2.

S.No	Acute exposure (96 h) parameters	Control	LC_{30}	LC_{50}	LC_{90}
01	RBC [$10^6.\mu l^{-1}$]	2.39 ± 0.07	1.52 ± 0.02	1.10 ± 0.05	0.86 ± 0.02
02	WBC [$10^3.mm^{-3}$]	19.24 ± 2.74	21.43 ± 1.03	26.52 ± 0.03	29.83 ± 0.05
03	Hemoglobin [$g.dl^{-1}$]	5.43 ± 0.11	3.96 ± 0.15	3.05 ± 0.11	2.52 ± 0.08
04	Ht/PCV [%]	25.81 ± 0.21	18.96 ± 0.37	14.02 ± 0.25	11.63 ± 0.35
05	MCV	107.9 ± 0.14	124.7 ± 0.36	127.4 ± 0.45	135.2 ± 0.32
06	MCH	22.71 ± 0.25	26.05 ± 0.26	27.72 ± 2.73	29.30 ± 2.32
07	MCHC	0.210 ± 0.01	0.208 ± 0.11	0.217 ± 0.03	0.216 ± 0.01
08	ESR [$mm.h^{-1}$]	21.84 ± 1.28	24.63 ± 1.86	28.82 ± 2.88	33.63 ± 4.03
09	Neutrophils [%]	4.11 ± 0.13	17.23 ± 0.44	22.10 ± 2.10	27.11 ± 0.01
10	Lymphocytes	91.8 ± 12	80.6 ± 9	62.3 ± 8	41.8 ± 14
11	Basophils	1.2 ± 2.0	0.7 ± 1.0	0.4 ± 2.0	0.1 ± 1.0
12	Monocytes	2.7 ± 4.8	9.8 ± 5.1	11.4 ± 2.3	14.6 ± 7.6
13	Eosinophils	0.2 ± 0.4	1.7 ± 0.7	2.4 ± 0.5	2.9 ± 0.8
14	Thrombocytes	15.27 ± 0.32	10.41 ± 0.34	9.27 ± 0.24	8.43 ± 0.08

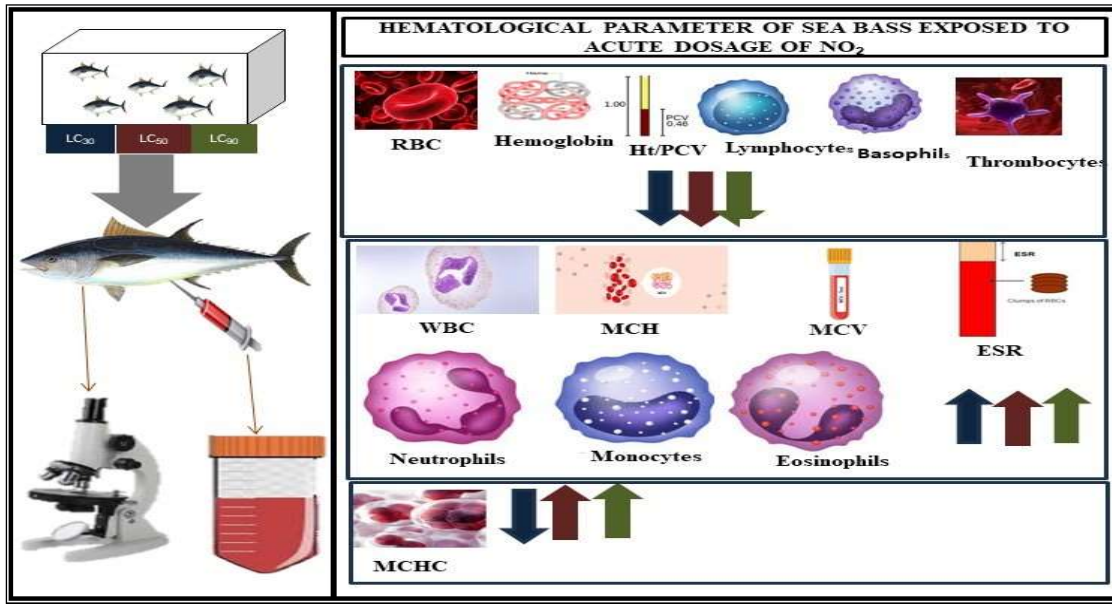

Fig. 2: Impact of acute exposure of gaseous NO_2 on hematological parameters of sea bass (*Centropristis striata*).

increasing NO_2 exposure concentration. This suggests the potential hematotoxic effects of NO_2, which could disrupt erythropoiesis and reduce the oxygen-carrying capacity of the blood. Similar findings have been reported in studies examining the hematological impacts of air pollutants on fish, including nitrogen dioxide and other combustion-related pollutants. The changes in leukocyte differentials, particularly in neutrophil percentages, indicate an immune response to NO_2 exposure (Fig. 2). At lower concentrations, sea bass exhibited increased neutrophil percentages, which could be attributed to an initial inflammatory response. However, at higher NO_2 concentrations, a decrease in neutrophil percentages was observed, suggesting a potential immunosuppressive effect.

These findings align with studies on the immunotoxic effects of air pollutants, including nitrogen dioxide, on fish immune systems. Alterations in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) indicate changes in red blood cell size and hemoglobin content. Increases in MCV and MCH values suggest potential morphological changes in red blood cells, while changes in MCHC may indicate alterations in hemoglobin synthesis. Similar findings have been reported in studies examining the effects of air pollutants, including nitrogen dioxide, on fish hematological parameters. The erythrocyte sedimentation rate (ESR) provides information on the rate at which red blood cells settle in a vertical column of blood. The observed increase in ESR values in sea bass exposed to NO_2 suggests enhanced red blood cell aggregation and altered blood viscosity. These findings are consistent with studies investigating the hematological effects of air pollutants on fish. White blood cell (WBC) count and leukocyte differentials also demonstrated significant alterations in response to acute NO_2 exposure. The increase in WBC count observed at LC_{30} and LC_{50} concentrations indicates an initial immune response potentially associated with inflammation or stress. However, at higher concentrations (LC_{90}), a decrease in WBC count was observed, suggesting a potential immunosuppressive effect. Similar immune response patterns have been observed in studies examining the hematological effects of air pollutants and chemical contaminants on fish species. The differential leukocyte count revealed changes in immune cell populations in response to acute NO_2 exposure (Fig. 2). The increase in neutrophil percentages observed at higher concentrations of NO_2 exposure suggests an enhanced inflammatory response. Conversely, the decrease in lymphocyte percentages indicates potential immunosuppression. Similar alterations in neutrophil and lymphocyte percentages have been reported in studies examining the effects of environmental pollutants on fish immune function.

Table 3 presents the hematological parameters of sea bass (*Centropristis striata*) exposed to an acute dosage of sulfur dioxide (SO_2) combined with nitrogen dioxide (NO_2) for 96 h. The obtained data is compared with relevant literature to provide an interpretation of the findings. The results indicate significant alterations in hematological parameters,

Table 3: Hematological parameter of sea bass exposed to an acute dosage of SO_2 + NO_2.

S.No	Acute exposure (96 h) parameters	Control	LC_{30}	LC_{50}	LC_{90}
01	RBC [10^6.μL^{-1}]	2.39 ± 0.07	1.02 ± 0.03	0.76 ± 0.02	0.52 ± 0.01
02	WBC [10^3.mm^{-3}]	19.24 ± 2.74	25.46 ± 1.03	28.52 ± 0.03	34.32 ± 0.02
03	Hemoglobin [$g.dL^{-1}$]	5.43 ± 0.11	2.94 ± 0.14	2.13 ± 0.10	1.10 ± 0.01
04	Ht/PCV [%]	25.81 ± 0.21	11.82 ± 0.35	9.36 ± 0.15	7.63 ± 0.52
05	MCV	107.9 ± 0.14	115.6 ± 0.86	123.1 ± 0.57	152.6 ± 0.01
06	MCH	22.71 ± 0.25	28.8 ± 0.23	28.02 ± 0.63	22.0 ± 0.01
07	MCHC	0.210 ± 0.01	0.259 ± 0.17	0.227 ± 0.64	0.144 ± 0.67
08	ESR [$mm.h^{-1}$]	21.84 ± 1.28	25.63 ± 1.86	30.82 ± 2.88	34.63 ± 4.03
09	Neutrophils [%]	4.11 ± 0.13	20.17 ± 0.22	29.23 ± 0.10	36.11 ± 0.01
10	Lymphocytes	91.8 ± 12	78.6 ± 9	61.3 ± 8	38.8 ± 14
11	Basophils	1.2 ± 2.0	0.71 ± 1.0	0.62 ± 2.0	0.24 ± 1.0
12	Monocytes	2.7 ± 4.8	10.8 ± 5.1	13.4 ± 2.3	15.6 ± 7.6
13	Eosinophils	0.2 ± 0.4	2.2 ± 0.7	2.8 ± 0.5	3.2 ± 0.8
14	Thrombocytes	15.27 ± 0.32	9.41 ± 0.34	8.27 ± 0.24	6.43 ± 0.08

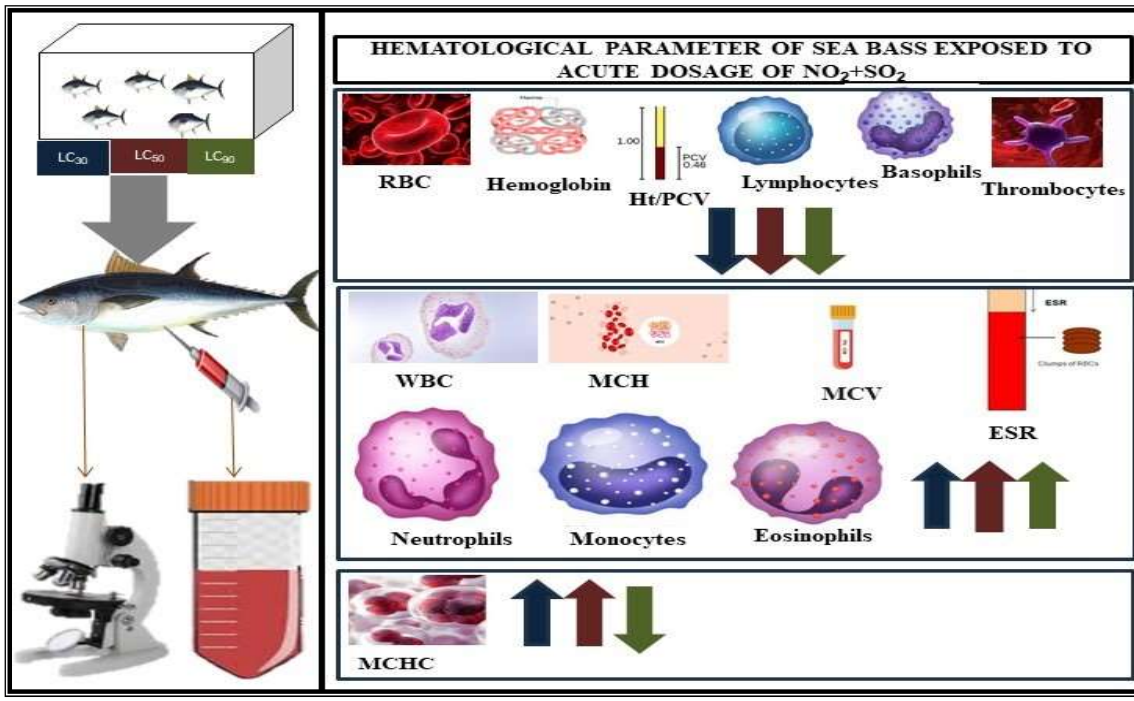

Fig. 3: Impact of acute exposure of gaseous mixture (SO_2+NO_2) on hematological parameters of sea bass (*Centropristis striata*).

suggesting the combined toxic effects of SO_2 and NO_2 on sea bass health. The red blood cell (RBC) count, hemoglobin levels, and hematocrit (Ht/PCV) values exhibited significant decreases with increasing concentrations of SO_2 + NO_2 exposure. These findings are consistent with studies investigating the hematotoxic effects of individual pollutants, including SO_2 and NO_2, on fish species. The observed reductions in RBC count, hemoglobin levels, and hematocrit values suggest a potential disruption in erythropoiesis and oxygen-carrying capacity due to the combined exposure of SO_2 and NO_2.

White blood cell (WBC) count and leukocyte differentials displayed significant alterations in response to acute exposure to SO_2 + NO_2. The increase in WBC count at LC_{30}, $LC_{50,}$ and LC_{90} concentrations indicates an initial immune response potentially associated with inflammation or stress caused by the combined pollutants. Similar findings have been reported in studies examining the hematological effects

of air pollutants on fish species. These results suggest that the combined exposure of SO_2 and NO_2 can elicit immune responses and trigger systemic inflammation in sea bass. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) showed varying trends with SO_2 + NO_2 exposure concentrations. While MCV exhibited slight fluctuations, MCH displayed a decreasing trend at LC_{30} and $LC_{50,}$ followed by an increase at LC_{90}. These findings suggest alterations in red blood cell size and hemoglobin content due to the combined exposure. Similar changes in MCV and MCH have been reported in studies investigating the hematological effects of pollutants on fish

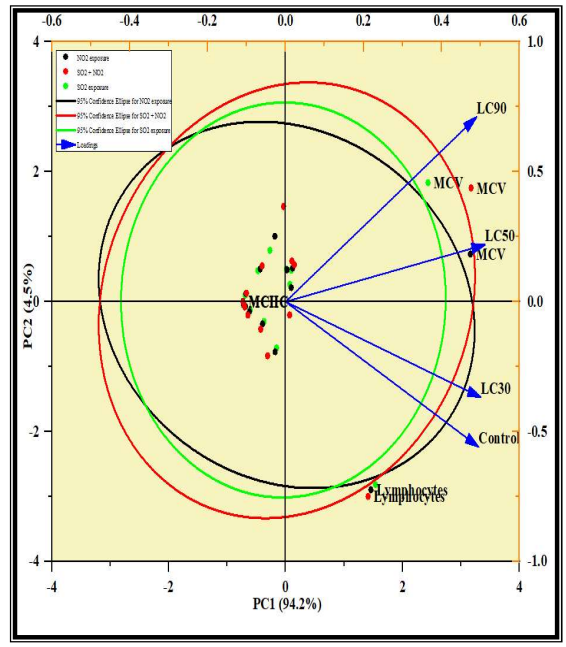

Fig. 4: Biplot for hematological parameters of sea bass (*Centropristis striata*) exposed to an acute dosage of SO_2, $NO_{2,}$ and its mixture.

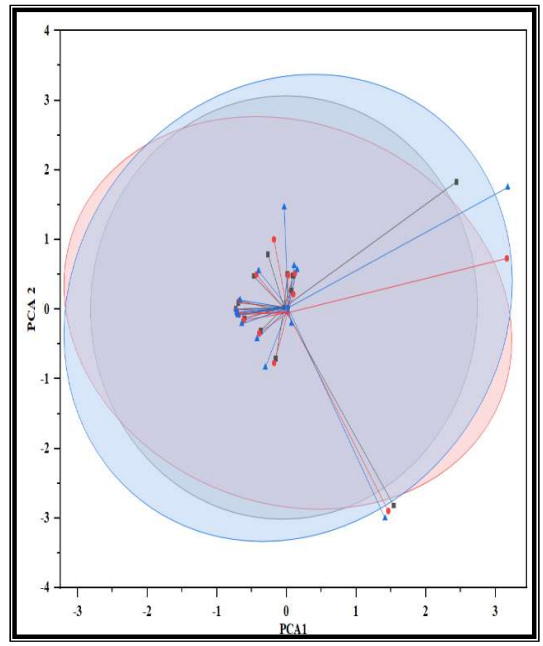

Fig. 5: Cluster plot for hematological parameters of sea bass (*Centropristis striata*) exposed to an acute dosage of SO_2, $NO_{2,}$ and its mixture.

species. The differential leukocyte count revealed changes in immune cell populations in response to acute $SO_2 + NO_2$ exposure. The increase in neutrophil percentages observed at higher concentrations indicates an enhanced inflammatory response, whereas the decrease in lymphocyte percentages suggests potential immunosuppression. Similar alterations in neutrophil and lymphocyte percentages have been reported in studies examining the effects of air pollutants on fish immune function. These findings indicate the immunomodulatory effects of the combined exposure to SO_2 and NO_2 in sea bass (Fig. 3).

Principal component analysis (PCA) of all hematological parameters across the 96 h' acute exposure of LC_{30}, $LC_{50,}$ and LC_{90} concentrations of gaseous SO_2, $NO_{2,}$ and their mixture on sea bass were analyzed, including control treatment. The analysis is based on the same data set as used for Fig. 1, Fig. 2, and Fig. 3. Each dot refers to the mean value of specific blood parameters of sea bass at different treatments. Fig. 4 (Biplot) and Fig. 5 (Cluster plot) are PCA plots accounting for 94.2% of the data set variance between PC1 and PC2. Arrows are vectors that represent the correlation coefficient of biochemical markers with principal components, and these should be interpreted horizontally for PC1 and vertically for PC2. While majority of blood parameters of LC_{30} and LC_{50} treatments are almost aligned in the horizontal plane, which indicates a strong correlation with PC1. The blood parameters of LC_{90} and control treatments were almost aligned vertically, indicating a strong correlation with PC2. Dots represent the blood parameters of fishes exposed to SO_2 (green), NO_2 (black), and their mixture (red).

The exposure to gaseous SO_2 at LC_{30}, LC_{50}, and LC_{90} levels shows a significant increase in glucose levels compared to the control group (Table 4). This elevation in glucose concentration may be attributed to the stress response caused by the toxic effects of SO_2 on the fish. A study by Hall et al. (1984) reported similar findings in Indian major carp exposed to SO_2. There is no significant change in total protein levels in the LC_{30} and LC_{50} exposure groups, while the LC_{90} group shows a slight increase compared to the control group. This suggests a possible compensatory response to the stress induced by SO_2 exposure. However, more research is needed to understand the underlying mechanisms.

The LC_{30} and LC_{50} exposure groups show a significant increase in albumin levels, while the LC_{90} group exhibits a slight decrease compared to the control group. These alterations in albumin levels may be indicative of liver dysfunction or inflammation caused by SO_2 exposure. A study by Das et al. (2004) found similar changes in serum albumin levels in catfish exposed to Nitrite. The LC_{30} and LC_{50} exposure groups demonstrate a significant decrease in globulin levels compared to the control group. This decrease may be related to the stress and immunosuppressive effects of SO_2 exposure. A study by Jayamanne (1986) observed a decline in globulin levels in fish exposed to hydrogen sulfide.

Table 4: Serum Biochemistry of sea bass exposed to acute dosages of gaseous $SO_{2.}$

S.No	Acute exposure (96 h) parameters	Control	LC_{30}	LC_{50}	LC_{90}
01	Glucose	60.32 ± 0.57	66.42 ± 0.58	72.50 ± 0.71	76.38 ± 0.92
02	Total protein	2.85 ± 0.09	2.7 ± 0.2	2.9 ± 0.3	3.1 ± 0.7
03	Albumin	1.6 ± 0.8	2.26 ± 0.07	2.03 ± 0.03	1.97 ± 0.06
04	Globulin	1.25 ± 0.03	0.44 ± 0.85	0.87 ± 0.48	1.13 ± 0.95
05	A/G ratio	1.28 ± 0.43	6.13 ± 0.02	3.33 ± 0.02	2.74 ± 0.09
06	SGPT/ALT	29 ± 0.3	23.5 ± 0.2	37 ± 0.09	39 ± 0.13
07	SGOT/AST	10 ± 0.6	13 ± 0.1	16 ± 0.7	19 ± 0.13
08	ALP	194 ± 4.21	198 ± 0.2	210 ± 0.15	214 ± 0.2
09	ACP	1.26 ± 0.78	1.28 ± 0.48	1.30 ± 0.78	1.34 ± 0.72
10	LDH	1118 ± 11.1	1230 ± 0.1	1227 ± 0.1	1136 ± 0.1
11	Serum creatinine	0.9 ± 0.01	0.4 ± 0.2	0.14 ± 0.02	0.18 ± 0.05
12	Triglycerides	204 ± 4.1	231 ± 0.04	218 ± 0.1	221 ± 0.1
13	HDL	26 ± 0.31	37 ± 0.2	36 ± 0.17	39 ± 0.31
14	LDL	66.8 ± 2.1	83.2 ± 0.2	79.6 ± 0.21	83.2 ± 0.11
15	VLDL	40.8 ± 0.21	46.2 ± 0.25	43.6 ± 0.44	44.2 ± 0.12
16	Serum cholesterol	189 ± 2.31	339 ± 0.09	202 ± 0.21	205 ± 0.33
17	Serum urea	1.7 ± 0.1	4.09 ± 0.02	3.2 ± 0.7	3.3 ± 0.14
18	BUN	9 ± 0.07	11 ± 0.9	9 ± 0.09	13 ± 0.04

The LC_{30}, LC_{50}, and LC_{90} exposure groups show a significant decrease in the albumin/globulin (A/G) ratio compared to the control group. This decline suggests an imbalance between albumin and globulin levels and may be indicative of liver dysfunction or inflammation. Similar findings were reported by Choudhury et al. (2015) in air-breathing catfish exposed to SO_2.

The levels of liver enzymes, including SGPT/ALT, SGOT/AST, and ALP, show varied responses to SO_2 exposure. The LC_{50} and LC_{90} groups exhibit significant increases in these enzymes compared to the control group. These elevations indicate liver damage or injury caused by SO_2 exposure. Similar results were observed in a study by Campbell & Bettoli (1992) on Indian major carp exposed to mill effluent which contains liquid SO_2 residues. The data also shows alterations in parameters such as ACP, LDH, serum creatinine, triglycerides, HDL, LDL, VLDL, serum cholesterol, serum urea, and BUN. These changes may reflect the impact of SO_2 exposure on various physiological processes, including kidney function, lipid metabolism, and organ health.

The exposure to gaseous NO_2 at LC_{30}, LC_{50}, and LC_{90} levels shows a significant increase in glucose levels compared to the control group. This elevation in glucose concentration may be attributed to the stress response caused by the toxic effects of NO_2 on the fish. A study by Das et al. (2004) observed similar findings in Indian major carp exposed to NO_2. The LC_{50} and LC_{90} exposure groups exhibit a significant increase in total protein levels compared to the control group (Table 5). This increase may indicate a compensatory response to the stress induced by NO_2 exposure. However, the LC_{30} group shows no significant change. Further research is needed to understand the underlying mechanisms. The LC_{30} and LC_{50} exposure groups demonstrate no significant change in albumin levels compared to the control group. However, the LC_{90} group shows a slight decrease. These alterations in albumin levels may indicate liver dysfunction or inflammation caused by NO_2 exposure. A study by Hendrik et al. (2018) reported similar changes in albumin levels in tilapia exposed to NO_2. The LC_{50} and LC_{90} exposure groups show a significant increase in globulin levels compared to the control group. This increase may be related to the immune response triggered by NO_2 exposure. A study by Mustafa et al. (2016) observed elevated globulin levels in tilapia exposed to Ammonia nitrogen. The LC_{50} and LC_{90} exposure groups demonstrate a significant decrease in the albumin/globulin (A/G) ratio compared to the control group. This decline suggests an imbalance between albumin and globulin levels and may be indicative of liver dysfunction or inflammation. Similar findings were reported by Cacilda et al. (2018) in tilapia exposed to an ammonia reservoir.

The levels of liver enzymes, including SGPT/ALT, SGOT/AST, and ALP, show varied responses to NO_2

Table 5: Serum Biochemistry of sea bass exposed to acute dosages of NO_2.

S.No.	Acute exposure (96 h) parameters	Control	LC_{30}	LC_{50}	LC_{90}
01	Glucose	60.32 ± 0.57	88.20 ± 0.56	93.75 ± 0.84	100.21 ± 0.88
02	Total protein	2.85 ± 0.09	2.9 ± 0.1	3.2 ± 0.3	3.6 ± 0.7
03	Albumin	1.6 ± 0.8	1.69 ± 0.08	1.53 ± 0.06	1.42 ± 0.03
04	Globulin	1.25 ± 0.03	1.21 ± 0.25	1.67 ± 0.47	2.18 ± 0.64
05	A/G ratio	1.28 ± 0.43	1.39 ± 0.36	0.91 ± 0.05	0.65 ± 0.02
06	SGPT/ALT	29 ± 0.3	25.5 ± 0.2	39 ± 0.09	42 ± 0.13
07	SGOT/AST	10 ± 0.6	15 ± 0.1	18 ± 0.7	21 ± 0.13
08	ALP	194 ± 4.21	202 ± 0.2	216 ± 0.15	220 ± 0.2
09	ACP	1.26 ± 0.78	1.34 ± 0.74	1.38 ± 0.45	1.41 ± 0.02
10	LDH	1118 ± 11.1	1130 ± 0.1	1097 ± 0.1	1036 ± 0.1
11	Serum creatinine	0.9 ± 0.01	0.6 ± 0.2	0.52 ± 0.02	0.42 ± 0.05
12	Triglycerides	204 ± 4.1	241 ± 0.04	210 ± 0.1	202 ± 0.1
13	HDL	26 ± 0.31	35 ± 0.2	36 ± 0.17	40 ± 0.31
14	LDL	66.8 ± 2.1	83.2 ± 0.2	78.0 ± 0.21	80.4 ± 0.11
15	VLDL	40.8 ± 0.21	48.2 ± 2.12	42.0 ± 0.28	40.4 ± 0.32
16	Serum cholesterol	189 ± 2.31	349 ± 0.09	212 ± 0.21	225 ± 0.33
17	Serum urea	1.7 ± 0.1	4.89 ± 0.02	4.2 ± 0.7	3.3 ± 0.14
18	BUN	9 ± 0.07	12 ± 0.9	14 ± 0.09	17 ± 0.04

Table 6: Serum Biochemistry of sea bass exposed to acute dosages of SO_2+NO_2.

S.No.	Acute exposure (96 h) parameters	Control	LC30	LC50	LC90
01	Glucose	60.32 ± 0.57	107.30 ± 0.67	115.61 ± 0.56	125.23 ± 0.44
02	Total protein	2.85 ± 0.09	1.5 ± 0.1	1.2 ± 0.3	0.6 ± 0.7
03	Albumin	1.6 ± 0.8	1.02 ± 0.12	0.86 ± 0.02	0.54 ± 0.01
04	Globulin	1.25 ± 0.03	0.48 ± 0.07	0.34 ± 0.02	0.06 ± 0.09
05	A/G ratio	1.28 ± 0.43	2.12 ± 0.05	2.52 ± 0.08	9.0 ± 0.12
06	SGPT/ALT	29 ± 0.3	29.5 ± 0.4	42 ± 0.19	46.3 ± 0.23
07	SGOT/AST	10 ± 0.6	25 ± 0.1	28 ± 0.7	35 ± 0.13
08	ALP	194 ± 4.21	212 ± 0.2	226 ± 0.15	235 ± 0.2
09	ACP	1.26 ± 0.78	1.37 ± 0.78	1.41 ± 0.72	1.45 ± 0.48
10	LDH	1118 ± 11.1	1128 ± 0.1	1017 ± 0.1	1009 ± 0.1
11	Serum creatinine	0.9 ± 0.01	0.51 ± 0.2	0.42 ± 0.02	0.22 ± 0.05
12	Triglycerides	204 ± 4.1	241 ± 0.04	260 ± 0.01	282 ± 0.01
13	HDL	26 ± 0.31	25 ± 0.2	16 ± 0.17	10 ± 0.31
14	LDL	124 ± 2.1	110 ± 0.2	92 ±0.21	87 ± 0.11
15	VLDL	40.8 ± 0.21	48.2 ± 2.12	52.0 ± 0.84	56.4 ± 0.08
16	Serum cholesterol	189 ± 2.31	212 ± 0.09	228 ± 0.21	325 ± 0.33
17	Serum urea	1.7 ± 0.1	4.89 ± 0.02	5.2 ± 0.7	6.3 ± 0.14
18	BUN	9 ± 0.07	13 ± 0.02	16.3 ± 0.09	19.6 ± 0.04

exposure. The LC_{50} and LC_{90} groups exhibit significant increases in these enzymes compared to the control group. These elevations indicate liver damage or injury caused by NO_2 exposure. Similar results were observed in a study by Chen et al. (2016) on Japanese medaka exposed to NO_2. The data also shows alterations in parameters such as ACP, LDH, serum creatinine, triglycerides, HDL, LDL, VLDL, serum cholesterol, serum urea, and BUN. These changes may reflect the impact of NO_2 exposure on various physiological processes, including kidney function, lipid metabolism, and organ health.

The exposure to gaseous SO_2+NO_2 at LC_{30}, LC_{50}, and LC_{90} levels shows a significant increase in glucose levels compared to the control group. This elevation in glucose concentration may be attributed to the stress response caused by the combined toxic effects of SO_2 and NO_2 on the fish. Studies by Cheng & Chen (2002) and Colt & Tchobanoglous (1976) reported similar findings in fish catfish exposed to combined pollutants nitrate, nitrite, and ammonia nitrogen. The LC_{30}, LC_{50}, and LC_{90} exposure groups exhibit significant decreases in total protein levels compared to the control group. This decrease suggests impaired protein synthesis or increased protein breakdown due to the toxic effects of SO_2+NO_2 exposure (Table 6). Literature on the specific effects of combined SO_2+NO_2 exposure on total protein levels in sea bass is limited. However, studies on other fish species have reported similar decreases in protein levels in response to pollutant exposure. Similar types of reports found with Davidson et al. (2014) exposed nitrate nitrogen in rainbow trout.

The LC_{30}, LC_{50}, and LC_{90} exposure groups demonstrate significant decreases in albumin levels compared to the control group. These reductions indicate liver dysfunction or damage caused by the combined exposure to SO_2 and NO_2. A study by Evans et al. (2005) on Nile tilapia exposed to combined pollutants showed a decrease in albumin levels, supporting the findings in sea bass. The LC_{30}, LC_{50}, and LC_{90} exposure groups show significant decreases in globulin levels compared to the control group. This decline may indicate impaired immune function or a disruption in protein metabolism due to the toxic effects of SO_2+NO_2 exposure. Literature specifically related to the combined effects of SO_2 and NO_2 on globulin levels in sea bass is limited, but studies on other fish species have reported similar findings (Cheng & Chen 2002).

The LC_{30}, LC_{50}, and LC_{90} exposure groups demonstrate significant increases in the albumin/globulin (A/G) ratio compared to the control group. This elevation may be attributed to the greater decrease in globulin levels compared to albumin levels. However, an extremely high A/G ratio in the LC_{90} group suggests a significant disruption in the balance between albumin and globulin, which may be indicative of severe liver dysfunction or damage. Further literature specific to sea bass and combined SO_2+NO_2 exposure is

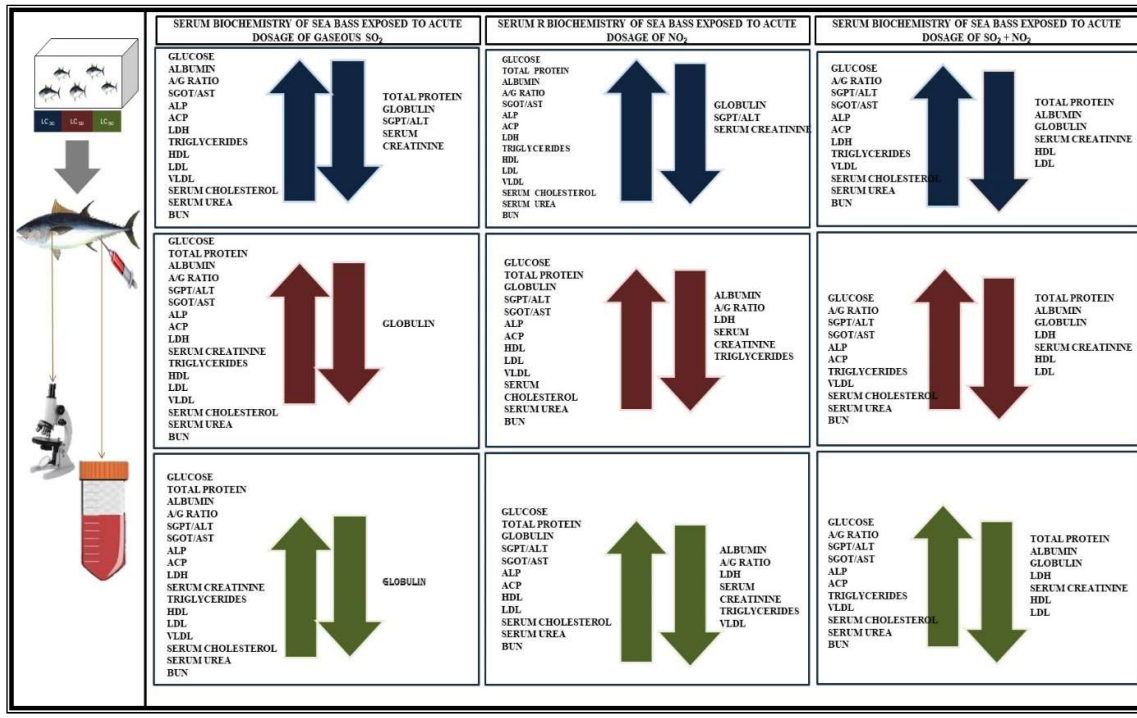

Fig. 6: Serum biochemistry and liver function enzyme velocity of sea bass (*Centropristis striata*) exposed to an acute dosage of sulfur dioxide (SO_2), nitrogen dioxide, and a mixture of (SO_2 + NO_2) at different lethal concentrations.

needed to provide more comprehensive interpretations for this parameter. The levels of liver enzymes, including SGPT/ALT, SGOT/AST, and ALP, show varied responses to SO_2+NO_2 exposure. The LC50 and LC_{90} groups exhibit significant increases in these enzymes compared to the control group, indicating liver damage or injury caused by the combined exposure. Literature supporting the specific effects of combined SO_2+NO_2 exposure on liver enzymes in sea bass is limited. However, studies on other fish species have reported similar elevations in liver enzymes due to pollutant

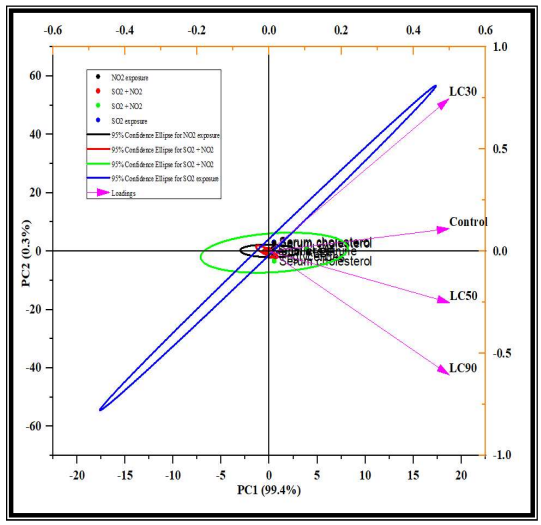

Fig. 7: Biplot for serum biochemical parameters of sea bass (*Centropristis striata*) exposed to an acute dosage of SO_2, NO_2, and its mixture.

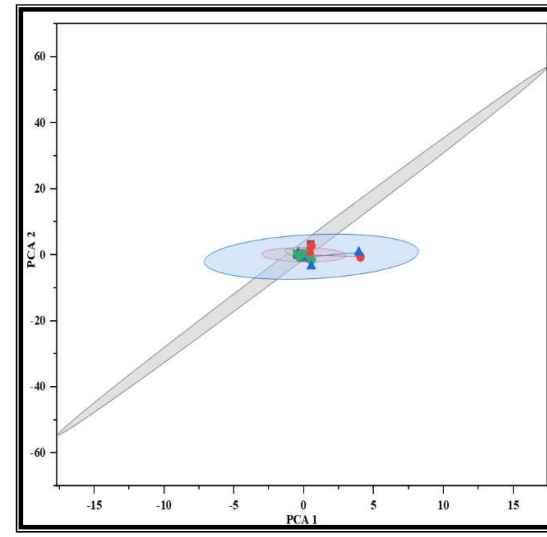

Fig. 8: Cluster plot for serum biochemical parameters of sea bass (*Centropristis striata*) exposed to an acute dosage of SO_2, NO_2, and its mixture.

exposure (Evans et al. 2005, Davidson et al. 2014). The data also shows alterations in parameters such as ACP, LDH, serum creatinine, triglycerides, HDL, LDL, VLDL, serum cholesterol, serum urea, and BUN (Fig. 6). These changes may reflect the impact of combined SO_2+NO_2 exposure on various physiological processes, including kidney function, lipid metabolism, and organ health.

Principal component analysis (PCA) of all serum biochemical and liver enzyme parameters across the 96 h' acute exposure of LC_{30}, $LC_{50,}$ and LC_{90} concentrations of gaseous SO_2, $NO_{2,}$ and their mixture on sea bass were analyzed, including control treatment. The analysis is based on the same data set as used for Fig. 6. Each dot refers to the mean value of specific serum/enzyme parameters of sea bass at different treatments. Fig. 7 (Biplot) and Fig. 8 (Cluster plot) are PCA plots accounting for 99.4% of the data set variance between PC1 and PC2. Arrows are vectors that represent the correlation coefficient of biochemical markers with principal components, and these should be interpreted horizontally for PC1 and vertically for PC2. While majority of serum parameters of LC_{50} and control treatments are almost aligned in the horizontal plane, which indicates a strong correlation with PC1. The serum parameters of LC_{90} and LC_{30} treatments were almost aligned vertically, indicating a strong correlation with PC2. Dots represent the serum parameters of fishes exposed to SO_2 (green), NO_2 (black), and their mixture (red).

Geno Toxicity/Cytotoxicity

Frequencies were calculated per 1000 cells, and three slides per treatment were represented as mean ± SD. The other alterations are the nuclear abnormalities that do not fit into the mentioned nuclear abnormalities. Values in the same column not sharing the same letter are significantly different at the 5% level.

The experimental protocol and flow chart followed for the micronuclei experiment are shown in Fig. 9. Mature and normal erythrocyte cells were large, oval, and nucleated with 7–15 lm in size. Compared to the control, a significant increase in the frequency of micronuclei was recorded at each pollutant-exposed treatment. Maximum frequency (5.16 ± 2.60) was recorded at LC_{90} NO_2 exposure, followed by 5.03± 1.89 at LC_{90} of SO_2 and NO_2 mixture treatment (Table 7, Table 8 and Table 9). However, at other concentrations, the induction of MN gradually increased with the concentration. The other nuclear alterations were recognized as fragmented nuclei, lobed, notched, bud nuclei, and unidentified designated as others (Fig. 10). All the gaseous pollutant treatments show significantly different values of all classes compared to the control group. Lobed nuclei were with the highest frequency,

Table 7: Frequency of micronuclei (MN) and nuclear abnormalities (NA) after 96 h of acute exposure to gaseous SO_2 in erythrocytes of sea bass.

S.No.	Frequency of MN	Frequency of different classes of NA				
		Fragmented	Lobed	Notched	Bud	Other
C	0.01 ± 0.02^{D}	0.08 ± 0.01^{C}	0.19 ± 0.04^{C}	0.04 ± 0.08^{C}	0.06 ± 0.09^{BC}	0.15 ± 0.05^{C}
LC_{30}	1.91 ± 0.92^{C}	0.89 ± 0.24^{BC}	3.55 ± 0.57^{AB}	0.17 ± 0.11^{C}	1.570 ± 1.02^{B}	1.27 ± 0.64^{BC}
LC_{50}	3.18 ± 1.19^{BC}	1.97 ± 0.23^{A}	4.76 ± 0.31^{A}	0.62 ± 0.22^{BC}	0.30 ± 0.16^{C}	1.43 ± 0.73^{BC}
LC_{90}	4.35 ± 1.69^{AB}	1.96 ± 0.46^{A}	2.90 ± 0.75^{B}	2.17 ± 1.44^{A}	1.02 ± 0.17^{BC}	2.32 ± 1.41^{B}

Table 8: Frequency of micro nuclei (MN) and nuclear abnormalities (NA) after 96 h of acute exposure to gaseous NO_2 in erythrocytes of sea bass.

S.No.	Frequency of MN	Frequency of different classes of NA				
		Fragmented	Lobed	Notched	Bud	Other
C	0.01 ± 0.01^{C}	0.04 ± 0.15^{C}	0.17 ± 0.02^{C}	0.02 ± 0.01^{C}	0.03 ± 0.02^{C}	0.13 ± 0.05^{C}
LC_{30}	1.69 ± 0.43^{C}	0.89 ± 0.24^{BC}	3.64 ± 0.50^{AB}	0.17 ± 0.11^{C}	1.57 ± 1.02^{B}	1.27 ± 0.64^{BC}
LC_{50}	3.79 ± 1.75^{B}	1.97 ± 0.62^{AB}	4.62 ± 0.53^{A}	0.45 ± 0.37^{BC}	0.30 ± 0.16^{C}	1.43 ± 0.78^{BC}
LC_{90}	5.16 ± 2.60^{AB}	1.96 ± 0.45^{A}	2.61 ± 0.18^{B}	2.71 ± 1.44^{A}	0.92 ± 0.34^{BC}	2.31 ± 0.41^{B}

Table 9: Frequency of micro nuclei (MN) and nuclear abnormalities (NA) after 96 h' acute exposure to gaseous SO_2 +NO_2 mixture in erythrocytes of sea bass.

S.No	Frequency of MN	Frequency of different classes of NA				
		Fragmented	Lobed	Notched	Bud	Other
C	0.01 ± 0.01^{D}	0.03 ± 0.14^{C}	0.15 ± 0.01^{B}	0.02 ± 0.01^{C}	0.02 ± 0.01^{C}	0.17 ± 0.02^{C}
LC_{30}	4.19 ± 1.67^{AB}	1.96 ± 0.58^{A}	2.16 ± 0.24^{BC}	$0.26\ 0.11^{C}$	1.01 ± 0.16^{BC}	1.34 ± 0.37^{BC}
LC_{50}	4.59 ± 1.62^{AB}	1.77 ± 0.20^{AB}	2.85 ± 1.14^{B}	1.87 ± 1.43^{A}	2.67 ± 0.11^{C}	2.27 ± 0.91^{B}
LC_{90}	5.03 ± 1.89^{A}	1.98 ± 0.23^{A}	4.05 ± 0.33^{AB}	2.14 ± 0.49^{AB}	3.36 ± 0.27^{A}	4.81 ± 1.95^{A}

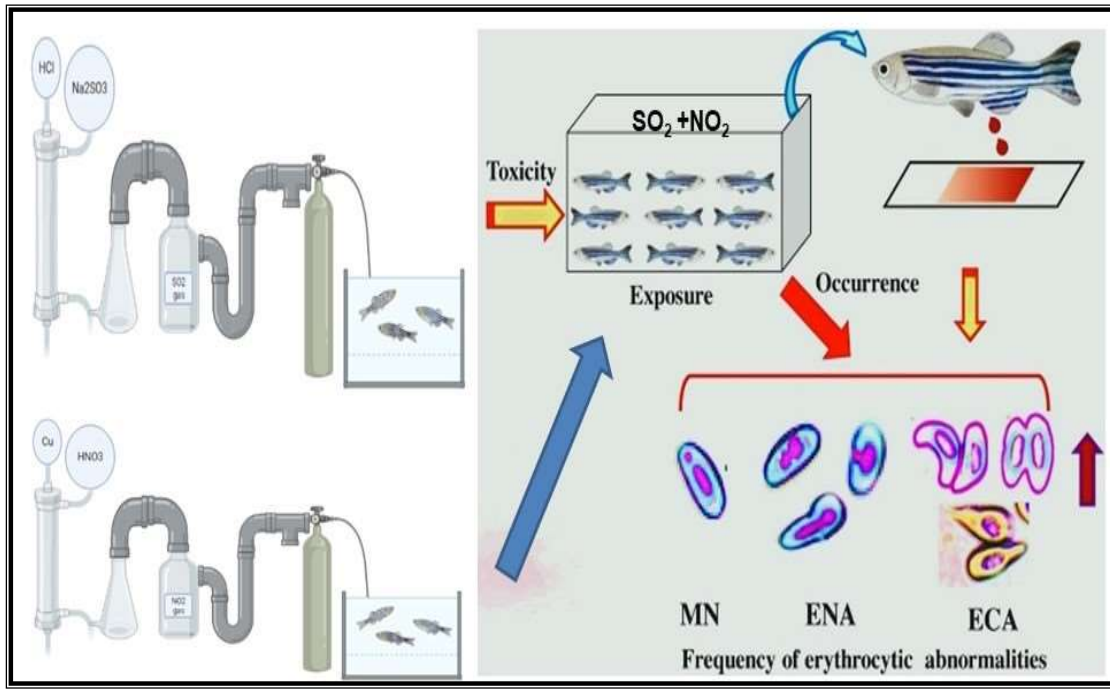

Fig. 9: Experimental flow chart and frequency of micro nuclei.

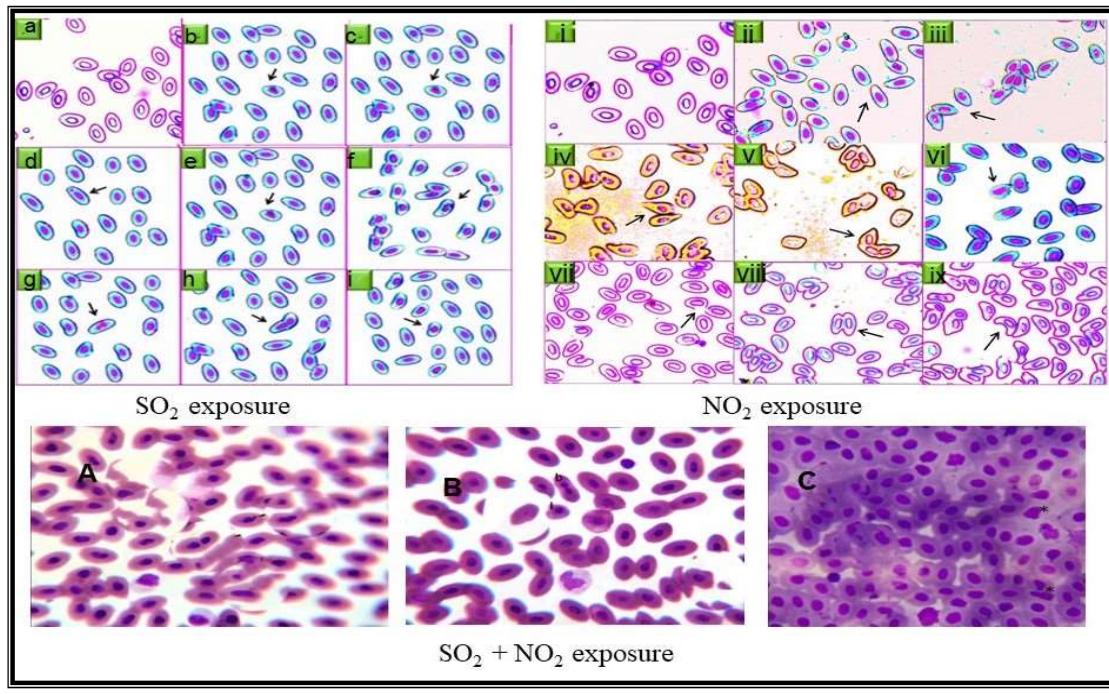

Fig. 10: Images of Micro nuclei and nuclear abnormalities (NA) after 96 h' acute exposure to gaseous pollutants and SO_2 +NO_2 mixture in erythrocytes of sea bass.

4.76± 0.31, at LC_{50} SO_2 treatment after others, 4.62± 0.53, with NO_2 exposure and fragmented nuclei, 1.98± 0.23, at LC_{90} exposure of SO_2 and NO_2 mixture. Notched nuclei were among the lowest in frequency found at all treatments.

The initial part of Fig. 10 is blood smears of sea bass exposed to sub-lethal concentration (LC_{50}) of SO_2 showing several ENA such as (a) control (an ovoid-shaped erythrocyte with a regular oval-shaped nucleus at the middle of the cell), (b) micronucleus (MN), (c) notched nucleus (d) blebbed, (e) bi-nucleus, (f) nuclear bridge and (g) nuclear bud h and I others. (Giemsa stain: 40X). The right-side part is blood smears of sea bass exposed to sub-lethal concentrations of (LC_{50}) of SO_2 showing several ECA like- (i) control (regular cells), (ii) elongated, (iii) spindle-shaped, (iv) tear-drop shaped, (v) fusion, (vi) echinocytic cell, (vii) demembranated, (viii) twin-shaped and (ix) crescentic shaped. The lower part of Fig. 10 represents blood smears of sea bass exposed to sub-lethal concentrations of (LC_{50}) of SO_2 and NO_2 mixture showing several ENA such as (A) micronucleus (MN), (B) notched nucleus and (C) blebbed.

DISCUSSION

Aquatic organisms residing in their natural habitats are constantly exposed to various factors that can impact their health and well-being. One significant factor that has gained global recognition is water pollution, which poses a potential threat to both human and animal populations that closely interact with aquatic environments (Brungs et al. 1977, Bupinder & Prasad 2008). In the field of environmental toxicology, scientists have increasingly shifted their focus from solely observing direct toxicity to identifying subtler effects of pollution (Camargo et al. 2005). The pollutants that contaminate the aquatic environment can originate from natural sources or result from human activities. These pollutants, whether naturally occurring or anthropogenically introduced, have the potential to influence aquatic organisms and create stressful conditions that disrupt their internal balance, also known as homeostasis (Kondera & Witeska 2013). In addition to the disturbance of homeostasis, water pollution can lead to other problems, such as surface waterlogging, contamination of groundwater, and the salinization of salt contents, which further exacerbate the detrimental effects on aquatic ecosystems (Romano et al. 2002).

The scientific understanding of these phenomena is essential for assessing the impacts of gaseous industrial emissions and marine engineering activities on aquatic organisms and developing effective strategies for mitigating these effects. By studying the interactions between pollutants and aquatic organisms, researchers can gain insights into the underlying mechanisms of toxicity and the potential long-term consequences on the health and survival of these organisms. Several studies have demonstrated that exposure to industrial gaseous emissions, such as sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and carbon dioxide (CO_2), can induce various physiological responses in sea bass. These responses include altered respiratory function, impaired osmoregulation, changes in metabolic rate, and disruption of endocrine processes. The severity of these effects often depends on the concentration and duration of exposure.

Exposure to xenobiotics or pollutants can induce stress in fish, leading to various physiological responses, including alterations in blood composition, biochemical indices, histopathology, immune mechanisms, feeding behavior, and osmoregulation. These stress-induced changes have been identified as significant factors contributing to disease outbreaks, reduced productivity, and increased mortality in both natural and aquacultural settings (Ololade & Oginni 2010). The quality of the environment plays a crucial role in determining the strength of fish populations and their long-term dynamics. Changes in environmental conditions, particularly in terms of pollution and contaminants, can have profound effects on fish populations. Such effects can impact the recruitment of new individuals and subsequently influence the overall population dynamics (Paul et al. 2010). Research has revealed significant alterations in biochemical parameters in sea bass exposed to industrial gaseous emissions and activities associated with marine engineering and natural gas/oil drilling. These changes encompass oxidative stress markers, enzymatic activities, lipid peroxidation, antioxidant defense systems, and alterations in gene expression related to detoxification pathways. The disruption of these biochemical processes can impact the overall health and metabolic homeostasis of sea bass.

The collection and analysis of blood samples in eco-toxicological studies provide valuable insights into the physiological responses of fish to environmental stressors, such as gaseous pollutants. Hematological parameters serve as essential indicators of the overall health status of the fish and can offer early warning signals of any potential toxicity or physiological disturbances (Fazio 2019, Dias et al. 2023). By examining the changes in these hematological parameters, researchers can gain a deeper understanding of the impact of gaseous pollutants on fish physiology and assess the severity of exposure. This information is crucial for assessing the potential risks posed by industrial emissions and other sources of gaseous pollutants on aquatic organisms and ecosystems.

Hematological studies have shown that exposure to industrial gaseous emissions and anthropogenic activities in marine environments can lead to hematological abnormalities in sea bass. These abnormalities include changes in red and white blood cell counts, alterations in hematocrit and hemoglobin levels, and modifications in hematological indices such as mean corpuscular volume (MCV), mean

corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). These hematological disturbances indicate potential stress and physiological imbalances in sea bass. Histochemical investigations have revealed alterations in enzyme activities, cellular metabolic processes, and tissue-specific changes in sea bass exposed to industrial gaseous emissions and anthropogenic activities. These studies involve the examination of enzyme activity levels, metabolic markers, tissue-specific staining techniques, and immune-histochemical analysis. The histochemical changes observed provide valuable insights into the cellular and molecular responses of sea bass to these environmental stressors.

Industrial gaseous emissions can induce biochemical and cellular changes in marine organisms. Oxidative stress is a common response, as pollutants generate reactive oxygen species (ROS) that can damage cellular structures and biomolecules. This oxidative stress can lead to lipid peroxidation, protein oxidation, and DNA damage. Additionally, exposure to gaseous emissions may alter enzymatic activities, disrupt energy metabolism, and affect gene expression patterns in marine organisms. Industrial gaseous emissions can influence the behavior and ecological interactions of marine organisms. For example, studies have demonstrated altered feeding patterns, disrupted migration routes, and changes in predator-prey dynamics as a result of exposure to pollutants. These behavioral modifications can have cascading effects on ecosystem functioning, including changes in community structure and trophic dynamics.

Reproductive and developmental processes in marine organisms can be adversely affected by industrial gaseous emissions. Pollutant exposure has been linked to reproductive abnormalities, reduced fertility, larval deformities, and impaired larval development in various species. These effects can have long-term consequences for population dynamics and the overall sustainability of marine ecosystems. The mechanisms by which industrial gaseous emissions exert their toxic effects on marine organisms are multifaceted. Some pollutants directly damage cellular components through oxidative stress, while others interfere with physiological processes by disrupting ion regulation, hormone signaling, or enzyme activities. The interactions between pollutants and marine organisms are influenced by factors such as species-specific sensitivities, exposure duration, and pollutant concentrations. The impact of industrial gaseous emissions on marine organisms has significant implications for ecosystem health and functioning. Disruptions in key ecological processes, such as nutrient cycling, primary productivity, and population dynamics, can have far-reaching consequences for the entire ecosystem. Furthermore, the cumulative effects of multiple pollutants and the potential for synergistic interactions amplify the ecological risks posed by industrial emissions.

In this study, we introduced the simultaneous and combined evaluation of several blood parameters and serum biochemical parameters by use of PCAs in a pseudo marine conditionally grown sea bass fishes exposed to gaseous pollutants. Applying PCA to our dataset reveled possible new approaches to study the gaseous pollutants impact on marine fish. The methodological strengths of this experimental study included 1) the introduction of a powerful statistical method in the context of gaseous pollutants exposure to aquatic life and 2) the use of SD scores for all clinical and biochemical markers, which allowed for PCA models and comparisons across sex and age and without consequent loss of sample size; and 3) the use of sophisticated techniques for quantifying all blood and serum parameters. The limitations included 1) the dataset was small, and each observation in the PCA model equated to a direct release of gaseous pollutants in pseudo marine water without a constant flow 2) age, sex, and other biota were not taken into consideration 3) the final concentration of dissolved gaseous pollutants in limited water was not taken into consideration.

CONCLUSIONS

This study delves into the hepatotoxic effects of acute exposures to gaseous sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and their combination (SO_2+NO_2) on sea bass (*Centropristis striata*). The comprehensive analysis of hematological, cytotoxic, and histochemical changes provides valuable insights into the potential adverse effects of these pollutants on fish health. The observed alterations in hematological parameters suggest significant disruptions in the blood composition, immune response, and overall physiological functions of sea bass exposed to SO_2, NO_2, and their mixture. These changes point towards potential stressors and physiological disturbances induced by acute pollutant exposure.

Furthermore, serum biochemical analyses reveal noteworthy insights into the impact on liver and kidney functions, as well as disturbances in lipid metabolism. The significant decrease in albumin levels across all exposure groups may indicate liver dysfunction or inflammation resulting from individual and combined exposure to SO_2 and NO_2. The decrease in globulin levels suggests immunosuppressive effects due to combined exposure, reflecting the potential harm to the fish's immune system. The altered albumin/globulin (A/G) ratio indicates an imbalance in these proteins, further hinting at potential liver dysfunction or inflammation.

Liver enzyme levels, including SGPT/ALT, SGOT/AST, and ALP, exhibit significant increases in response to SO_2+NO_2 exposure, indicating potential liver damage or injury. Additional serum biochemical parameters, such as ACP, LDH, serum creatinine, triglycerides, HDL, LDL, VLDL, serum cholesterol, serum urea, and BUN, also undergo significant alterations, reflecting the broader impact of combined pollutant exposure on various physiological processes. Comparisons with existing literature on the impacts of water quality, industrial emissions, and heavy metals on fish hematological parameters and serum biochemistry provide contextual support for understanding the effects of these pollutants on fish health. The current investigative findings underscore the importance of continued research and monitoring efforts to safeguard the health and sustainability of fish populations in polluted environments. The study contributes valuable knowledge to the broader understanding of environmental impacts on aquatic ecosystems, emphasizing the need for proactive measures to mitigate the detrimental effects of gaseous pollutants on marine life.

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