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Behavioural and Morphological Changes in *Centropristis striata* (Sea bass) Under Acute Exposure to Gaseous Sulphur dioxide (SO_2), Nitrogen dioxide (NO_2), and Conglomeration of SO_2+ NO_2

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Abstract

This study investigates the acute toxicity of gaseous sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) on 8-week-old sea bass (*Centropristis striata*) in a synthetic marine water environment. SO_2 was generated by reacting sodium sulfite with hydrochloric acid, producing concentrations ranging from 2 ppm to 45 ppm, while NO_2 was generated using copper metal flakes and concentrated nitric acid. LC_{30}, LC_{50}, and LC_{90} were determined after 24, 48, 72, and 96 hours for each gas, indicating the concentrations at which 30%, 50%, and 90% mortality occurred, respectively. Results showed a concentration-dependent increase in mortality rates for both SO_2 and NO_2, with 100% mortality observed at higher concentrations within the experimental duration. Behavioral observations included equilibrium loss, jumpings, gulping air, restlessness, erratic swimming, opercular movements, and sluggishness, which intensified over the 96-hour period. Morphological changes such as body patches, skin discoloration, scale shedding, mucus secretion, sedimentation of chemicals, and gill clumping were more pronounced with NO_2 exposure and further exacerbated by the mixture of SO_2 and NO_2. The combined exposure demonstrated synergistic effects, leading to enhanced

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mortality rates and more severe behavioral and morphological alterations. These findings provide crucial comprehension of the toxic effects of gaseous pollutants on sea bass, emphasizing the importance of considering combined impacts in environmental risk assessments. The observed changes serve as indicators of environmental pollution, highlighting the need for effective mitigation strategies to protect aquatic ecosystems.

Keywords: Gaseous industrial emissions, Sulphur dioxide (SO_2), Nitrogen dioxide (NO_2), Behavioral changes and Morphological changes

1. Introduction

The marine environment is facing increasing challenges due to anthropogenic activities, such as industrial processes, transportation, and fossil fuel combustion, which are leading to the release of various gaseous pollutants into the atmosphere. Among these pollutants, sulphur dioxide (SO_2) and nitrogen dioxide (NO_2) are major contributors, adversely affecting marine ecosystems, including fish populations. Sulphur dioxide (SO_2) is a common air pollutant derived from burning fossil fuels containing sulfur compounds. Atmospheric deposition of SO_2 can lead to acid rain, resulting in acidification of marine environments. Acidification can negatively impact fish physiology, affecting osmoregulation, respiration, and reproduction (Novaczek 2012). Additionally, SO_2, along with other air pollutant exposure, has been linked to oxidative stress and tissue damage in fish (Jayamanne 1986). Nitrogen dioxide (NO_2) is another prevalent air pollutant resulting from combustion processes. It can contribute to the formation of nitrogen oxides (NOx) and, upon deposition in marine ecosystems, can have detrimental effects on fish health. NO_2 exposure has been associated with impaired respiratory function, changes in immune response, and altered behavior in marine fish species (Julio and Alvaro 2006). Exposure to SO_2 and NO_2 with other pollutants has been linked to reduced growth in marine fish, as observed in juvenile grass carp and Amazonian fish (Alcaraz and Espina, 1997; Bartlett et al., 1987). These pollutants also induce physiological changes, affecting gill structure, respiration, and ion regulation (Bartlett et al. 1987; Brownell 1980). Additionally, SO_2

exposure changes behavior and lowers heart rate and blood pressure in juvenile *Morone saxatilis* (striped bass) and *Brevoortia tyrannus* (Atlantic menhaden) under different temperatures (Leenwood 1984). Morphological alterations, such as swim bladder abnormalities and liver/spleen damage, have been documented in zebrafish, rainbow trout, and juvenile Atlantic salmon due to SO_2 and NO_2 exposure, respectively (Das et al., 2004; Eddy et al., 1983). This highlights the multifaceted impact of gaseous pollutants on the growth, physiology, and morphology of marine fish. In addition to SO_2 and NO_2, other gaseous pollutants, such as ozone (O_3) and carbon monoxide (CO), can impact marine fish. The combined effects of multiple pollutants may exacerbate negative outcomes, emphasizing the need for holistic research approaches. According to the aforementioned findings, the present work was suggested and was aimed at studying the toxicity of sea bass (*Centropristis striata*) exposed to 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 behavioral, morphological and physiological parameters.

2. Material & Methods

2.1. Preparation of pseudo-marine water (Stock standard)

Plastic fish tanks, specifically Azolla grow bags with a thickness of 750 gsm and a capacity of 800 liters, were procured from a local plastic manufacturer. Prior to their use, the tanks underwent an extensive cleaning protocol. Initially, they were thoroughly rinsed with running tap water to remove dust and surface residues. Following this, a 2% potassium permanganate ($KMnO_4$) solution was applied to sanitize the tanks and eliminate microbial contamination. To ensure the complete removal of any chemical residues from the sanitizing agent, the tanks were subsequently rinsed with double-distilled water. Each tank was then filled with 600 liters of brackish water, to which 1.2 kilograms of sea salt was added, achieving a salinity concentration of 15 ppt (parts/thousand). The accuracy of the salinity levels was verified using both a salinity hydrometer and a digital probe conductivity meter, ensuring precise measurement. In preparation for the cultivation of Sea bass (*Centropristis striata*), phytoplankton, beneficial microbes, and other growth enhancers were introduced into each tank. To simulate a natural marine environment while

preserving native flora, a synthetic water sample was prepared. This involved combining 50-milliliter aliquots of mangrove water and collected seawater with the tank water. This process aimed to create pseudo-marine conditions that are conducive to the health and growth of the aquatic ecosystem, closely mimicking natural habitats.

2.2. Selection of model organism

Sea bass (*Centropristis striata*) was selected as the model organism for this study due to several key advantages that make it an ideal candidate for investigating the acute toxicity of gaseous pollutants such as SO_2 and NO_2. Sea bass is a widely distributed species found in various marine and estuarine environments, making it a representative species for studying the impacts of environmental pollutants on marine ecosystems (Wenner et al., 1986; Shepherd & Packer, 2006). Their sensitivity to changes in water quality and chemical pollutants means that their physiological and behavioral responses can serve as early indicators of environmental stress (Foss et al., 2007). This sensitivity makes sea bass an effective bio-indicator species for monitoring and assessing the impact of pollutants (Arellano-Aguilar & Macías, 2009).

Additionally, sea bass are of significant economic importance due to their role in commercial and recreational fisheries. Sea bass also play a crucial ecological role as both a predator and prey species in marine food webs (Collette & Klein-MacPhee, 2002). Studying the effects of pollutants on sea bass can help in understanding the broader implications for marine biodiversity and fisheries management (Halvorsen et al., 2012). There is also a substantial body of scientific literature on the biology, physiology, and ecology of sea bass, providing a solid foundation for experimental design and data interpretation (Necaise, 2001). This established knowledge base allows for more accurate and meaningful analysis of experimental results, enhancing the reliability and relevance of the study findings.

Furthermore, sea bass can be relatively easily maintained in laboratory settings, allowing for controlled exposure experiments. Their manageable size, hardy nature, and adaptability to captive conditions facilitate experimental manipulations and repeated observations, ensuring the feasibility and consistency of toxicity studies (Brewer & Konar, 2005). In summary, the selection of sea bass as a model

organism offers several advantages, including ecological relevance, sensitivity to pollutants, economic and ecological importance, a well-established research background, and practical ease of maintenance. These attributes make sea bass a valuable species for investigating the acute toxic effects of gaseous pollutants on marine life.

2.3. Experiment-1

2.3.1. Preparation of gaseous SO_2

Figure 1: Experimental setup of production and exposure of gaseous sulphur dioxide to the aquatic environment

The clean and autoclaved gas-generating apparatus was taken and filled with 5 gm of analytical grade sodium sulfite and tightly closed with a lid. Through the inlet of the gas generating apparatus, 5 ml of concentrated HCl was added and closed the inlet immediately. The outlet of the gas generating apparatus was connected to the gas reservoir chamber to collect the released gas (Figure 1). The balanced chemical equation for the reaction is as follows.

$$2\ Na_2SO_3 + 2\ HCl \blacktriangledown \quad 2\ NaCl + H_2O + 2\ SO_2$$

2.3.2. Dissolving SO2 gas in pseudo marine water

The collected gas allowed dissolving (Frank and Raymond 1967) in synthetic marine water to get various concentrations i.e. 2 ppm (parts/million) to 45 ppm. The concentration of SO_2 was determined by standard pararosiline method described by Sirisha et al. (2013).

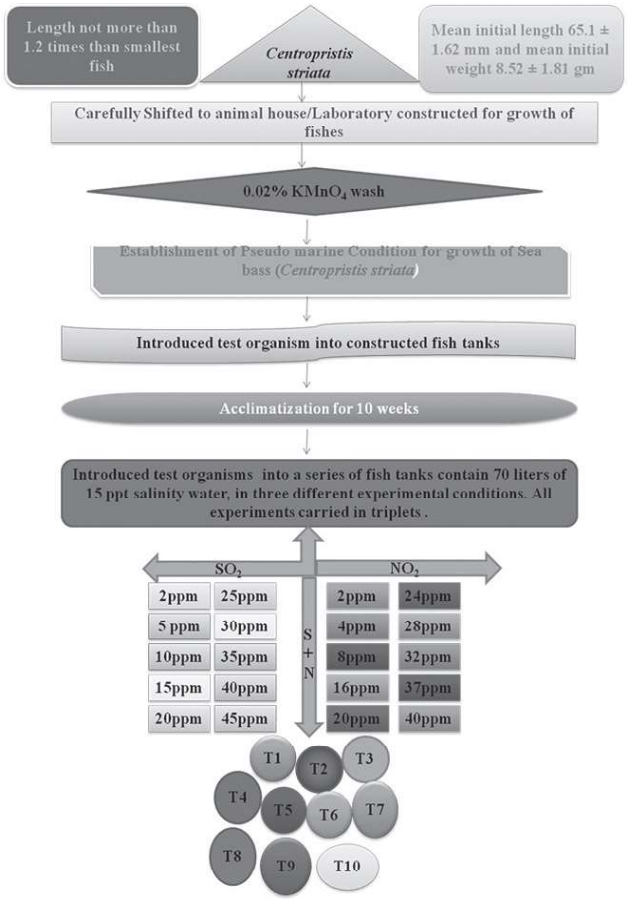

Figure 2: Schematic methodology and workflow (S+N indicates mixture of SO2 and NO2 gas, ppm indicates parts/million)

2.3.3. Determination of LC_{30}, LC_{50} and LC_{90} of gaseous SO_2

This research work was conducted in the laboratories of the Marine Biology Department, Vikrama Simhapuri University, Nellore, Andhra Pradesh. 8 weeks of sea bass grown at pseudo marine water were selected to perform the experiment. The objective of the experiment was to determine the LC_{30}, LC_{50}, and LC_{90} values for the sea bass fish after 96 hours of exposure. LC_{30} refers to the lethal concentration at which 30% of the fish population is expected to die, LC_{50} represents the lethal concentration at which 50% mortality occurs, and LC_{90} indicates the lethal concentration at which 90% mortality is observed.

Into a series of 10 plastic tanks with a capacity of 80 liters filled with 60 liters of synthetic marine water and SO_2 gas was dissolved from 2 ppm to 45 ppm concentrations to study the acute toxicity of gaseous SO_2 at different exposure periods i.e. 24, 48, 72 and 96 hours. SO_2 gas was dissolved from 2 ppm to 45 ppm, and each tank was inoculated with 30 fish irrespective of sex, length, and weight. The tanks were denoted as T_1 (2 ppm), T_2 (5 ppm), T_3 (10 ppm), T_4 (15 ppm), T_5 (20 ppm), T_6 (25 ppm), T_7 (30 ppm), T_8 (35 ppm), T_9 (40 ppm) and T_{10} (45 ppm). A set of control treatments was maintained simultaneously without any SO_2 treatment and denoted as C (Figure 2). All the experiments were carried in triplicate, and mean values were taken for results and interpretation.

2.4. Experiment-2

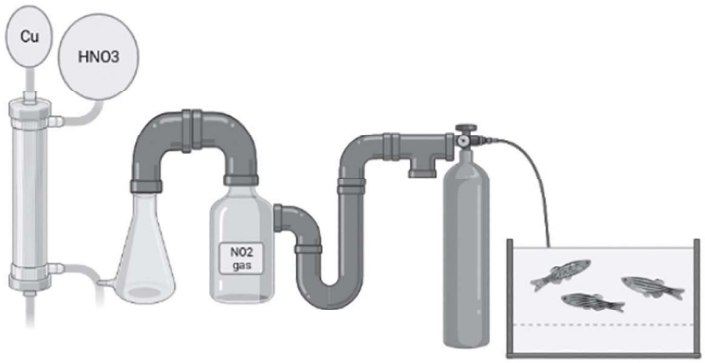

Figure 3: Experimental setup of production and exposure of gaseous nitrogen dioxide to the aquatic environment

2.4.1. Preparation of gaseous NO_2

The clean and autoclaved gas-generating apparatus was taken and filled with 5 gm of copper metal flakes and tightly closed with a lid. Through the inlet of the gas generating apparatus, 20 ml of concentrated HNO_3 was added and closed the inlet immediately. The outlet of the gas generating apparatus was connected to the gas reservoir chamber to collect the released gas.

The reaction between nitric acid and copper metal can generate gaseous nitrogen dioxide along with other products.

$$8\ HNO_3 + 3\ Cu\ (metal) \rightarrow Cu(NO_3)_2 + 4\ H_2O + 2\ NO_2$$

In this reaction, nitric acid reacts with copper metal to form copper nitrate, water, and nitrogen dioxide gas. The nitrogen dioxide gas appears to be reddish brown (Figure 3).

2.4.2. Dissolving NO_2 gas in pseudo marine water

The collected gas allowed dissolving in synthetic marine water (Doblander and Lackner 1997) to get various concentrations i.e. 2 ppm to 45 ppm. The concentration of NO_2 was determined by the standard N-(1-naphthyl)-ethylenediamine-dihydrochloride (NEDA) method described by Gandhi et al. (2014).

2.4.3. Determination of LC_{30}, LC_{50} and LC_{90} of gaseous NO_2

A series of 10 plastic tanks with a capacity of 80 liters filled with 60 liters of synthetic marine water and NO_2 gas was dissolved from 2 ppm to 45 ppm concentrations to study the acute toxicity of gaseous NO_2 at different exposure periods i.e. 24, 48, 72 and 96 hours. Then, each tank was inoculated with 30 fish irrespective of sex, length, and weight. The tanks were denoted as T_1 (2 ppm), T_2 (4 ppm), T_3 (8 ppm), T_4 (16 ppm), T_5 (20 ppm), T_6 (24 ppm), T_7 (28 ppm), T_8 (32 ppm), T_9 (37 ppm) and T_{10} (40 ppm). A set of control treatments was maintained simultaneously without any NO_2 treatment and denoted as C.

2.5. Experiment -3

2.5.1. Determination of LC_{30}, LC_{50} and LC_{90} of gaseous mixture of SO_2+NO_2

Into a series of 10 fish tanks with a capacity of 80 liters filled with 70 liters of purified water and added sea salt (1.2 kg) to reach a salinity 15 ppt/L. To this prepared synthetic marine water 30 sea bass fishes were inoculated by dissolving both gaseous SO_2+NO_2 (Figure 4) with various concentrations and denoted as follows\

Table 1: Concentrations of SO_2 and NO_2 for determination of lethal toxicity

Treatment	Concentration of SO_2	Concentration of NO_2
Control	Nil	Nil
T1	2 ppm	1 ppm
T2	4 ppm	2 ppm

T3	5 ppm	4 ppm
T4	7 ppm	7 ppm
T5	8 ppm	8 ppm
T6	10 ppm	10 ppm
T7	12 ppm	12 ppm
T8	15 ppm	15 ppm
T9	20 ppm	20 ppm
T10	25 ppm	25 ppm

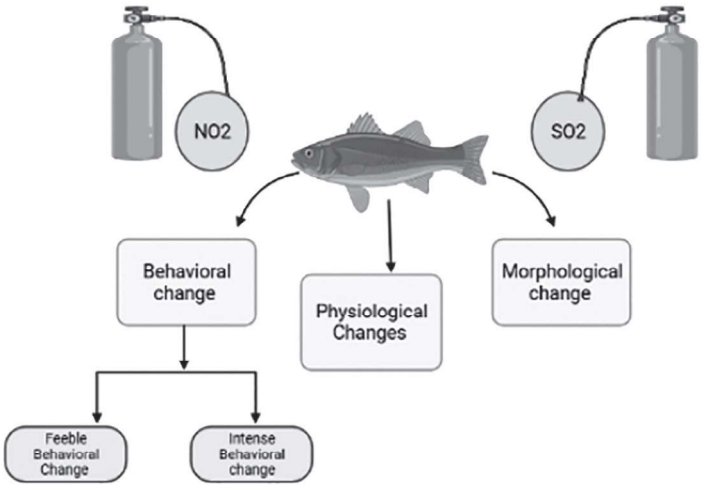

Figure 4: schematic view of experimental procedure to study the impact of gaseous emissions on sea bass

2.6. Calculation of Probit Value

The test doses have been transformed logarithmically. Empirical probit values, corresponding to the mortality percentage, have been derived from a statistical table and recorded in the respective columns of the tables. These empirical probit values have been plotted against the logarithm of the dose on regular graph paper, and a straight line passing through the points is drawn proportionally. Along this line, corresponding probit values 'Y' is recorded for the respective log dose values 'V'. The working probit value 'y' is calculated using the following formula.

$Y = Y_0 + KP$

Behavioral indices, including opercular beat frequency (OBF), tail beat frequency (TBF), swimming velocity (SV), swimming activity Index (SAI), and Eye and Fin deformities, were computed using the formulas outlined in Table 2 to assess the behavioral changes in the fish.

Table 2: Empirical formula for calculation of behavioral responses

S. N.	Behavioural Response	Formula	Reference
01	SAI	$SAI = \frac{\text{Average number of total moves registered in a week}}{\text{Number of total moves on n day}}$	Eissa et al., 2003, 2006
02	SV	$SV = \frac{d\,(n-m)}{dt}$	Eissa et al., 2009
03	Opercular beat frequency	Beat per minute in each individual in a fish tank	Tanterpale et al., 2012
04	Tail beat frequency	Beat per minute in each individual in a fish tank	Devi and Mishra, 2013
05	Eye Deformities	Physical observation of eye color and shapes	Devi and Mishra, 2013
06	Fin deformities	Physical examination of split fins	Sameena et al., 2022.

3. Results

3.1. Determination of LC_{30}, LC_{50} and LC_{90}

This study investigates the relationship between SO_2 concentration and mortality rates. Figure 5, presents the percentage of mortality at different time intervals for varying concentrations of SO_2. The time intervals range from 12 to 96 hours, indicating the duration of exposure to the specified concentrations of SO_2.

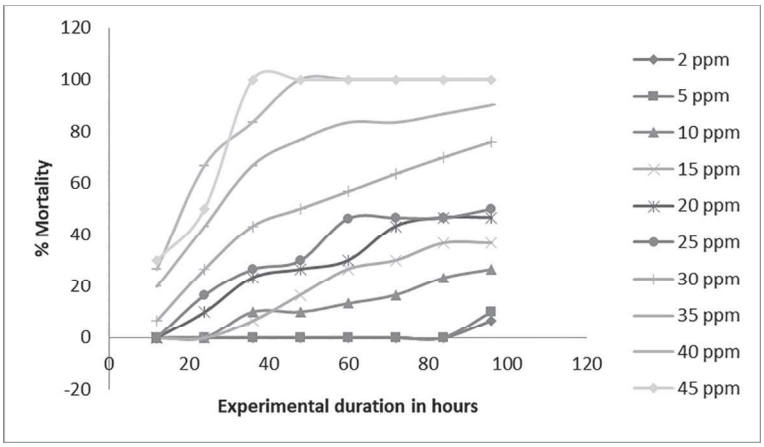

Figure 5: Mortality rate of sea bass on exposure of different concentrations of SO_2 at different time intervals

The concentrations of SO_2 tested in the study range from 2 ppm to 45 ppm. Figure 5 presents the percentage of mortality at each time interval for different concentrations of SO_2. At lower concentrations (2-10 ppm), there is no observed mortality up to 72 hours. As the concentration increases, the mortality rates gradually rise. At higher concentrations (above 30 ppm), the mortality rates reach 100%, indicating the lethal effect of SO_2 exposure. The mortality rates generally increase over time for each concentration level. Initially, at lower concentrations, there is a delay in mortality, which becomes more pronounced as the exposure time extends.

At higher concentrations, mortality rates reach a maximum earlier in the time interval. Similar studies have demonstrated that exposure to elevated levels of SO_2 can cause respiratory symptoms, exacerbate respiratory diseases, and contribute to increased mortality rates (Leenwood 1984). Long-term exposure to inorganic and organic pollutants has been associated with cardiovascular and respiratory diseases, including increased risks of premature death (Noga et al. 1994; Novaczek 2012). Research suggests that there may be a threshold concentration for SO_2, below which no immediate health effects are observed (Eeva and Meri 2002). The absence of mortality at lower concentrations in the table supports the existence of a threshold effect for SO_2 exposure. During the experiment, no fish in the control group died & acute lethal effects were caused by different concentrations of

SO_2 on sea bass, as shown in Figure 5. From the figure, it is found that the 96h LC_{30}, $LC_{50,}$ and LC_{90} values of 13 ppm, 25 ppm, and 35 ppm. Hall et al. 1982, examined the toxicity of gaseous chlorine residues on sea bass larvae and juveniles of *Atlantic menhaden*, *Brevoortia tyrannus*, providing insights into the lethal effects and potential LD_{50} values. A study by Stone and Sidell 2002, reported an LD_{50} of 60 ppm for striped bass (*Morone saxatilis*) exposed to alterations in carbon sources for 96 hours to study the hepatic energy metabolism. Leenwood et al. 1986, studied behavioural modification of estuarine fish exposed to sulfur dioxide by taking striped bass (*Morone saxatilis*) and Atlantic menhaden (*Brevoortia tyrannus*) at acclimation temperatures of 15, 20, 25, and 30°C and reported that striped bass avoided lower concentrations and Atlantic menhaden avoided higher concentrations. In their studies, they also reported that SO_2 has shown an impact on the respiratory system and enzymes involved in various mechanisms of the kidney and liver due to the dis-function of enzymes, striped bass showed higher mortality even at lower concentrations.

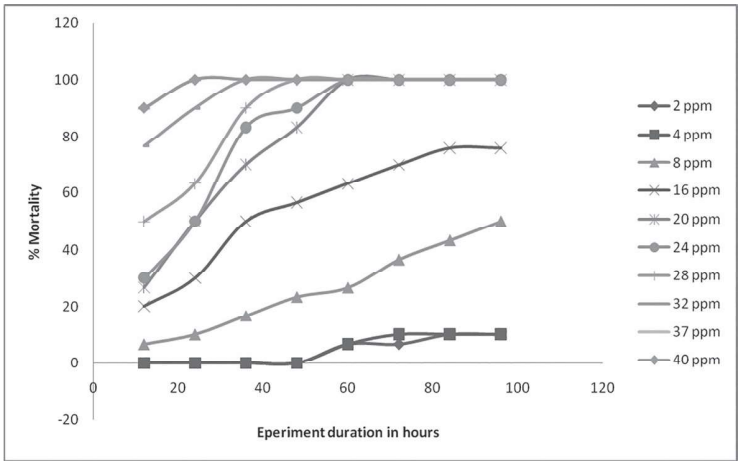

Figure 6: Mortality rate of sea bass on exposure to various concentrations of NO2 at different time intervals

Figure 6 represents the percentage of mortality of sea bass fish at various time intervals (12 to 96 hours) and concentrations of gaseous NO_2 (ranging from 2 ppm to 40 ppm). As the concentration of NO_2 increases, the percentage of mortality generally rises over time, the same phenomenon observed in the current investigation. Studies focusing on the toxic effects of NO_2 on fish species, including sea bass,

provide insights into the determination of LC_{30}, LC_{50}, and LC_{90} values. Research by Shailaja and Rodrigues (2003) investigated the acute toxicity of phenanthrene on sea bass (*Dicentrarchus labrax*), presenting relevant information for comparison. Shailaja and Rodrigues (2003), reported an LC_{50} value of 22 ppm for sea bass exposed to NO_2 for 96 hours. Based on the observed data (Fig-6), it is estimated that the LC_{30}, $LC_{50,}$ and LC_{90} values for sea bass fish fall between 6 ppm, 8 ppm, and 18 ppm, respectively. According to Sugata et al. (2013), higher concentrations of NO_2 react with water and form nitric acid. This nitric acid further produces greater concentrations of NO_2. In the presence of lethal and sub-lethal concentrations of NO_x components in aquatic ecosystems NO_x reacts with NH_2 and SH groups through its derivatives, thus being able to inhibit several enzymes & generate mutagenic or carcinogenic compounds such as nitrosamide-like compounds (De flora and Arillo 1983).

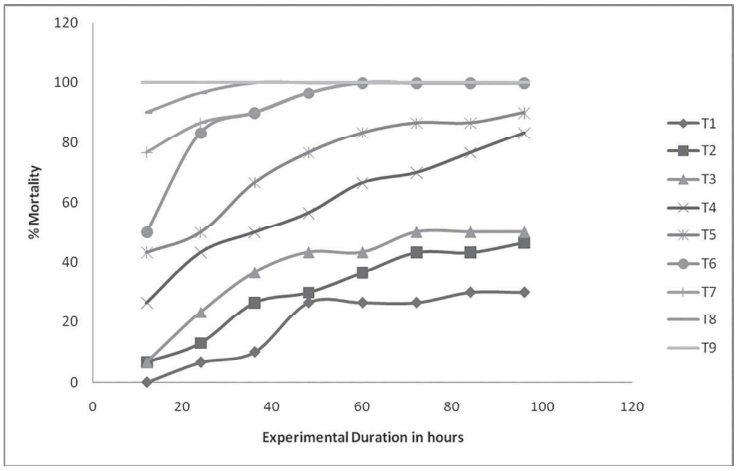

Figure 7: Mortality rate of sea bass on exposure to various concentrations of SO2 + NO2 at different time intervals

Figure 7 displays the percentage of mortality of sea bass fish at different time intervals (T_1-T_9) after exposure to a gaseous mixture of SO_2 and NO_2. Based on the data observed, the LC_{30}, LC_{50}, and LC_{90} values for the sea bass fish can be determined by identifying the corresponding concentrations at each time interval (T_1-T_9) where the percentage of mortality matches or exceeds the respective thresholds. By examining the table, the following LC values can be observed. LC_{30}: At 96 hours, the sea bass fish exhibited a mortality rate of 46.6%, indicating that

the LC_{30} value lies between T_8 and T_9. LC_{50}: At 96 hours, the mortality rate of the sea bass fish was 50%, suggesting that the LC_{50} value also lies between T_8 and T_9. LC_{90}: The sea bass fish reached a mortality rate of 100% at 72 hours (T_6), indicating that the LC_{90} value was exceeded before this time point. Therefore, the estimated LC_{30} and LC_{50} values for the sea bass fish upon exposure to the gaseous mixture of SO_2 and NO_2 after 96 hours are between T_8 and T_9, while the LC_{90} value is exceeded before the 72-hour mark.

The results of this experiment demonstrate the toxic effects of the gaseous mixture of SO_2 and NO_2 on sea bass fish. The increasing mortality rates observed over time indicate the progressive impact of the exposure. Previous studies have reported the toxicity of SO_2 and NO_2 individually on aquatic organisms. According to Vedel et al. (1998), prolonged exposure to elevated concentrations of combined ammonia and nitrite can lead to adverse effects on rainbow trout fish, including respiratory distress, tissue damage, brain glutamine/glutamate concentrations, and mortality. Similarly, studies by Parra and Yufera (1999) have highlighted the toxic effects of combined ammonia and nitrite on marine fishes (*Sparus aurata* L.; *Solea senegalensis*), including impairment of respiratory function and disruption of physiological processes. When SO_2 and NO_2 gases are combined in a mixture, as in the present study, their synergistic effects may exacerbate the toxicity and lead to higher mortality rates. The combined action of SO_2 and NO_2 can further compromise the respiratory system and other vital functions of the sea bass fish, resulting in increased mortality. The LC_{30} and LC_{50} values obtained in this study can serve as crucial indicators for assessing the potential risks posed by gaseous mixtures of SO_2 and NO_2 to sea bass fish populations in natural environments. These values can aid in establishing appropriate exposure limits and implementing effective mitigation strategies to protect aquatic ecosystems. It is important to note that the results presented here are specific to the experimental conditions and concentrations used in this study. Further investigations are warranted to explore the effects of varying concentrations, exposure durations, and potential interactions with other environmental factors to obtain a more comprehensive understanding of the toxicity of gaseous mixtures containing SO_2 and NO_2 on sea bass fish and other aquatic organisms.

3.2. Probit value

The mortality data of sea bass (*Centropristis striata*) exposed to various concentrations of gaseous SO_2 and NO_2 over a 96-hour period are presented in Tables 3 and 4, respectively. Both tables list the concentrations of SO_2 and NO_2 along with their corresponding logarithmic values (Log10 Concentration), percentage of mortality, and Probit values. For SO_2 exposure, the results indicate that as the concentration of gaseous SO_2 increased, the percentage of mortality in sea bass also increased, demonstrating a clear dose-response relationship. The control group, not exposed to SO_2, showed no mortality, serving as a baseline. At the lowest concentration tested, 2 ppm, the mortality rate was 7%, and it progressively rose with higher concentrations, reaching 100% mortality at 45 ppm. Probit values, which represent the number of standard deviations from the mean at which a certain percentage of mortality occurs, increased with the SO_2 concentrations, reinforcing the dose-response relationship. These results highlight the toxicity of gaseous SO_2 to sea bass, consistent with previous studies that have reported the adverse effects of various pollutants, including SO_2, on aquatic organisms (Saroglia et al., 1981; Bhattacharyya et al., 2013). The respiratory system of fish is particularly vulnerable to SO_2, leading to impaired respiration and eventual mortality.

Similarly, the data for NO_2 exposure showed a dose-dependent response, with higher concentrations of gaseous NO_2 leading to increased mortality rates in sea bass. The control group for NO_2 exposure also exhibited no mortality, serving as the baseline. At the lowest concentration of 2 ppm, the mortality rate was 10%, and it escalated with increasing NO_2 concentrations, culminating in 100% mortality at 40 ppm. Probit values mirrored this trend, increasing with higher NO_2 concentrations. These findings are in line with previous research emphasizing the toxic effects of NO_2 on fish, including respiratory distress and physiological disruptions leading to mortality (Machova et al., 2004; Jensen, 2003). Overall, both sets of data demonstrate a clear dose-response relationship between the concentrations of gaseous SO_2 and NO_2 and the mortality rates of sea bass, underscoring the significant impact of these pollutants. This study provides valuable information for assessing the potential risks

associated with SO_2 and NO_2 pollution in aquatic environments and contributes to the establishment of regulatory measures to mitigate these harmful effects. Further research could explore sub-lethal effects, such as physiological and biochemical changes, to gain a more comprehensive understanding of the impact of these gaseous pollutants on sea bass and other aquatic organisms (Vedel et al., 1998; Solbe et al., 1985).

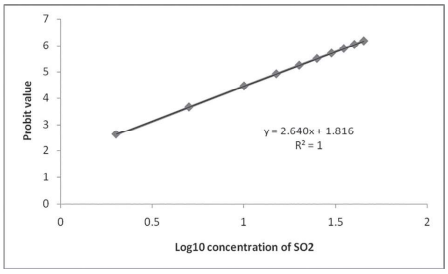

Figure 8: Regression line between the probit kill value of sea bass at different log_{10} concentrations of gaseous SO_2.

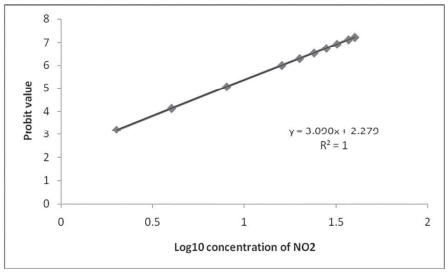

Figure 9: Regression line connecting the sea bass probit kill value at various log10 concentrations of gaseous NO_2.

Table 3: Mortality of sea bass exposed at different concentrations of gaseous SO_2

Concentration of gaseous SO_2	Log10 Concentration	Percent mortality	Probit value
Control	-	-	-
2 ppm	0.301	7	2.611
5 ppm	0.699	10	3.662
10 ppm	1.000	27	4.456
15 ppm	1.176	37	4.921
20 ppm	1.301	47	5.251
25 ppm	1.398	50	5.507
30 ppm	1.477	76	5.716
35 ppm	1.544	90	5.893
40 ppm	1.602	100	6.046
45 ppm	1.653	100	6.181

Table 4: Mortality of sea bass exposed at different concentration of gaseous NO_2

Concentration of gaseous NO_2	Log10 Concentration	Percent mortality	Probit value
Control	-	-	-
2 ppm	0.301	10	3.209
4 ppm	0.602	10	4.140
8 ppm	0.903	50	5.070
16 ppm	1.204	76	6.000
20 ppm	1.301	100	6.300
24 ppm	1.380	100	6.544
28 ppm	1.447	100	6.751
32 ppm	1.505	100	6.930
37 ppm	1.568	100	7.125
40 ppm	1.602	100	7.230

3.3. Behavioural Response

The behavioral changes in sea bass (*Centropristis striata*) following exposure to the LC50 dose of gaseous SO_2, NO_2, and mixture over different time periods (24, 48, 72, and 96 hours) are presented in Tables 5, 6, and 7. These tables provide a comprehensive overview of the behavioral responses of sea bass to these pollutants, with the control group representing baseline behavior without exposure. Exposure to SO_2 resulted in significant behavioral alterations in the fish, including equilibrium loss, surface air gulping, erratic swimming patterns, increased opercular activity, restlessness, jumping, and sluggish behavior (Table 5). After 24 hours of exposure, the fish displayed pronounced changes, such as loss of equilibrium, gulping air at the surface, erratic swimming, and notable opercular movements. These behaviors persisted and intensified over time, becoming more pronounced by 96 hours. The results indicate that gaseous SO_2 exposure profoundly impacts sea bass behavior, impairing normal swimming patterns and causing respiratory distress and physiological stress. These findings align with previous research that has documented similar adverse effects of SO_2 on fish behavior, with physiological and neurological disruptions leading to these observed changes (Eissa et al., 2009; Sameena et al., 2022; Ali et al., 2014).

Similarly, exposure to gaseous NO_2 resulted in significant behavioral alterations in sea bass (Table 6). At 24 hours, sea bass displayed noticeable behavioral changes, which intensified over the exposure period, becoming prominent by 96 hours. These changes suggest impaired motor coordination and respiratory distress caused by NO_2 toxicity, consistent with findings from other studies on the effects of NO_2 on fish behavior (Cengiz et al., 2001; Cheng & Chen, 2001). When exposed to a mixture of gaseous SO_2 and NO_2, sea bass exhibited significant behavioral changes similar to those observed with individual exposures, but with potentially enhanced severity (Table 7). The combined exposure led to equilibrium loss, surface air gulping, erratic swimming patterns, increased opercular activity, restlessness, jumping, and sluggish behavior, becoming more pronounced over time. These results indicate that the mixture of SO_2 and NO_2 has a compounded impact on fish behavior, reflecting synergistic toxic effects. Previous research supports these findings, highlighting the need to consider the combined effects of multiple pollutants on aquatic organisms (Eissa et al., 2006; Sameena et al., 2022). The results of this study provide valuable insights into the behavioral responses of sea bass to gaseous pollutants, emphasizing the need for effective monitoring and regulation of SO_2 and NO_2 emissions to safeguard fish populations and aquatic ecosystems. Implementing measures to minimize pollution and understanding the interactive effects of multiple pollutants are crucial for protecting the health and well-being of aquatic organisms.

Table 5: Behavioural response of sea bass under gaseous SO_2 exposure at LC_{50} concentration.

S. No.	Behavioural changes	Control	Experimental duration			
			24 hours	48 hours	72 hours	96 hours
1	Loss of equilibrium*	Normal	** slight	**** prominent	****prominent	****prominent
2	Gulping air at surface*	Normal	*** moderate	*** moderate	****prominent)	****prominent
3	Erratic swimming*	Normal	** slight	*** moderate	****prominent	****prominent
4	Opercular movements*	Normal	** slight	*** moderate	***moderate	****prominent
5	Restlesness*	Normal	**** prominent	*** moderate	****prominent	****prominent

6	Jumping*	Normal	**** prominent	*** moderate	****prominent	****prominent
7	Sluggishness*	Normal	**slight	**slight	***prominent	****prominent

Table 6: Impact of LC_{50} dose of gaseous NO_2 exposure on behavioural response of sea bass.

S. No.	Observed trait	Control	Experimental duration			
			24 hours	48 hours	72 hours	96 hours
1	Loss of equilibrium	Normal	*** moderate	*** moderate	**** prominent	**** prominent
2	Gulping air at surface	Normal	**** prominent	**** prominent	**** prominent	**** prominent
3	Erratic swimming	Normal	**** prominent	**** prominent	**** prominent	**** prominent
4	Opercular movements	Normal	*** moderate	**** prominent	**** prominent	**** prominent
5	Restlesness	Normal	*** moderate	**** prominent	**** prominent	**** prominent
6	Jumping	Normal	*** moderate	*** moderate	*** moderate	*** moderate
7	Sluggishness	Normal	** slight	*** moderate	*** moderate	** slight

Table 7: Effect of LC_{50} dose of a mixture of gaseous SO_2 and NO_2 exposure on behavioural response of sea bass.

S. No.	Behavioural changes	Control	Experimental duration			
			24 hours	48 hours	72 hours	96 hours
1	Loss of equilibrium	Normal	*** moderate	*** moderate	**** prominent	**** prominent
2	Gulping air at surface	Normal	**** prominent	**** prominent	**** prominent	**** prominent
3	Erratic swimming	Normal	**** prominent	**** prominent	**** prominent	**** prominent
4	Opercular movements	Normal	*** moderate	**** prominent	**** prominent	**** prominent
5	Restlesness	Normal	*** moderate	**** prominent	**** prominent	**** prominent
6	Jumping	Normal	*** moderate	*** moderate	*** moderate	*** moderate
7	Sluggishness	Normal	*** moderate	*** moderate	*** moderate	*** moderate

Table 5, Table 6, and Table 7 provide information on the behavioral responses of sea bass fish following exposure to the LC_{50} doses of gaseous SO_2, gaseous NO_2, and a mixture of gaseous SO_2 and NO_2, respectively. By comparing the behavioral changes observed in these tables, we can gain insights into the specific effects of individual gases and their combined impact on sea bass behavior. Behavioral changes were observed across all three experimental conditions (Tables 5, 6, and 7). These changes indicate the adverse effects of gaseous pollutants on the swimming ability, respiration, and overall behavior of sea bass fish. When comparing Table 5 (gaseous SO_2) and Table 6 (gaseous NO_2), it can be observed that the behavioral responses are relatively similar. Sea bass fish exposed to both gases displayed equilibrium loss, gulping for surface air, abnormal swimming, and opercular movements, with varying degrees of severity. However, in Table 6, a more prominent change in behavioral responses is observed at 48 hours and 72 hours compared to Table 5. This suggests that gaseous NO_2 may have a relatively faster onset and stronger impact on the behavior of sea bass fish compared to gaseous SO_2. In Table 7, which represents the exposure to a mixture of gaseous SO_2 and NO_2, the behavioral changes are comparable to those in Table 5 and Table 6. However, some variations in the intensity of behavioral responses can be observed. Sea bass fish exposed to the gas mixture exhibited moderate to prominent changes. Notably, the behavioral changes in Table 7 are generally more pronounced at the 96-hour exposure period, indicating a cumulative effect of the combined gases over time. The comparison of these tables suggests that the mixture of gaseous SO_2 and NO_2 may have an additive or synergistic effect on the behavioral responses of sea bass fish. This is evident from the increased severity of behavioral changes in Table 7 compared to the individual gas exposures in Table 5 and Table 6. Overall, these findings highlight the potential risks associated with exposure to gaseous pollutants, both individually and in combination, on the behavior of sea bass fish. The observed behavioral changes indicate physiological stress, impaired motor coordination, and respiratory distress. Such alterations in behavior can have significant ecological implications, affecting the survival, feeding patterns, and reproductive success of sea bass populations.

3.4. Morphological Changes

Table 8: Effect of LC_{50} dose of gaseous SO_2 on morphological changes of sea bass fish grown in pseudo marine water.

S. No.	Morphological changes	Control	Experimental duration			
			24 hours	48 hours	72 hours	96 hours
1	Patches on the body	Normal	** slight	** slight	** slight	*** moderate
2	Discoloration of skin	Normal	** slight	** slight	*** moderate	*** moderate
3	Shedding of scale	Normal	** slight	** slight	** slight	** slight
4	Mucus secretion	Normal	** slight	** slight	*** moderate	**** prominent
5	Chemical sedimentation on the body	Normal	** slight	** slight	** slight	*** moderate
6	Gills clumping	Normal	** slight	*** moderate	*** moderate	**** prominent

Table 9: Effect of LC_{50} dose of gaseous NO_2 on morphological changes of sea bass fish grown in pseudo marine water.

S. No.	Morphological changes	Control	Experimental duration			
			24 hours	48 hours	72 hours	96 hours
1	Patches on the body	Normal	** slight	*** moderate	**** prominent	**** prominent
2	Discoloration of skin	Normal	** slight	*** moderate	**** prominent	**** prominent
3	Shedding of scale	Normal	** slight	*** moderate	*** moderate	**** prominent
4	Mucus secretion	Normal	** slight	**** prominent	**** prominent	**** prominent
5	Sedimentation of chemical on the body	Normal	** slight	*** moderate	*** moderate	**** prominent
6	Clumping of gills	Normal	** slight	**** prominent	**** prominent	**** prominent

Table 10: Effect of LC_{50} dose of a mixture of gaseous SO_2 and NO_2 on morphological changes of sea bass fish grown in pseudo marine water

S. No.	Morphological changes	Control	Experimental duration			
			24 hours	48 hours	72 hours	96 hours
1	Patches on the body	-	*** moderate	*** moderate	**** prominent	**** prominent
2	Discoloration of skin	-	*** moderate	*** moderate	**** prominent	**** prominent
3	Shedding of scale	-	**slight	*** moderate	*** moderate	**** prominent
4	Mucus secretion	-	**** prominent	**** prominent	**** prominent	**** prominent
5	Sedimentation of chemical on the body	-	*** moderate	*** moderate	*** moderate	**** prominent
6	Clumping of gills	-	*** moderate	**** prominent	**** prominent	**** prominent

Table 8, Table 9, and Table 10, present the morphological changes observed in sea bass fish following exposure to the LC_{50} doses of gaseous SO_2, gaseous NO_2, and a mixture of gaseous SO_2 and NO_2, respectively. These tables provide insights into the specific effects of individual gases and their combined impact on the morphology of sea bass fish grown in pseudo marine water. In all three tables, the control group represents the baseline morphology of sea bass fish without exposure to the respective gases. The exposure periods include 24 hours, 48 hours, 72 hours, and 96 hours to assess the progressive effects over time. Table 8 (gaseous SO_2) demonstrates that sea bass fish exposed to this gas exhibit several morphological changes. These include the formation of patches on the body, discoloration of the skin, shedding of scales, mucus secretion, sedimentation of chemical on the body, and clumping of gills. These changes are observed to varying degrees of severity, with some becoming more prominent over time. Notably, mucus secretion and clumping of gills show a moderate change at 48 hours and progress to a prominent change at 72 hours and 96 hours of exposure. Table 9 (gaseous NO_2) reveals similar morphological changes in sea bass fish. Exposure to gaseous NO_2 leads to the development of patches on the body, discoloration of the skin, shedding of scales, mucus secretion, sedimentation

of chemical on the body, and clumping of gills. These changes are generally more pronounced compared to Table 8, indicating that gaseous NO_2 may have a stronger impact on the morphological integrity of sea bass fish. The changes progress from less severe at 24 hours to prominent changes at 48 hours, 72 hours, and 96 hours of exposure. Table 10 represents the morphological changes observed in sea bass fish exposed to a mixture of gaseous SO_2 and NO_2. Similar to Table 9, the morphological changes in Table 10 are more prominent compared to Table 8, suggesting an additive or synergistic effect of the combined gases. Mucus secretion and clumping of gills show a significant increase, indicating a moderate change at 24 hours and progressing to a prominent change at 48 hours, 72 hours, and 96 hours of exposure. The comparative analysis of these results indicates that both gaseous SO_2 and gaseous NO_2 have detrimental effects on the morphology of sea bass fish when individually exposed. The mixture of these gases in Table 10 intensifies the observed morphological changes. These alterations in the fish's external appearance, such as patches, discoloration, scale shedding, mucus secretion, and sedimentation of chemicals, indicate physiological stress, impaired skin function, and potential damage to gill structures. The findings from these tables align with previous research highlighting the toxic effects of gaseous pollutants on fish morphology. For example, a study by Eissa et al. (2003), Sameena et al. 2022 and Cheng & Chen 2001, demonstrated similar morphological changes in fish exposed to various water pollutants supporting the observations in Table 8 and Table 9. Furthermore, studies investigating the combined effects of these gases, such as the research conducted by Cengiz et al. 2001, have reported exacerbated morphological alterations similar to those observed in Table 10.

The effect of gaseous SO_2, NO_2 and its mixture on tail beat frequency (TBF) of sea bass fish are presented in Table 11. The TBF decreased with increase of exposure of gaseous pollutant concentration and exposure time at all experimental conditions compare to control treatment. The TBF significantly decreased ($p<0.001$) with increase in concentration from LC_{30} to LC_{90} with an average values of 99.100/m, 55.200/m in fishes exposed to gaseous SO_2, 103.300/m to 65.483/m in fishes exposed to gaseous NO_2 and 89.883/m to 53.000/m in fishes exposed to mixture of gaseous SO_2 and NO_2. The obtained results indicating

significant reduction in tail beat frequency fishes exposed to mixture of gaseous SO_2 and NO_2 followed by fishes exposed NO_2 and fishes exposed to SO_2. From the obtained results of current experiments, it is concluded that gaseous SO_2 and NO_2 are showing less toxicity when they exposed individually buts more toxic at mixture of gases.

Table 11: Tail beat frequency obtained by the fish when exposed to gaseous SO_2, NO_2 and their mixture (Acute test)

Experiment	Time	LC_{30}	LC_{50}	LC_{90}	Control	Mean for time
Gaseous SO_2 exposure	6	106.4	94.7	80.2	55.3	93.766
	12	103.2	89.4	55.5	52.5	82.700
	24	99.4	92.1	41.4	49.3	77.633
	48	83.7	100.9	37.8	45.2	74.133
	72	108.3	97.2	80.7	37.3	95.400
	96	93.6	102.1	35.6	43.5	77.100
Mean for Concentration		99.100	96.066	55.200	47.183	
Gaseous NO_2 exposure	6	115.3	99.1	82.2	55.3	98.866
	12	110.5	85.3	72.5	52.5	89.433
	24	105.1	73.6	63.4	49.3	80.700
	48	98.3	60.5	50.2	45.2	69.660
	72	85.4	115.4	40.2	37.3	66.930
	96	105.2	110.3	84.4	43.5	71.833
Mean for Concentration		103.300	90.700	65.483	47.183	
Mixture of gaseous SO_2 and NO_2	6	95.4	88.2	78.6	55.3	87.400
	12	97.3	83.1	72.5	52.5	84.300
	24	92.6	82.4	60.2	49.3	78.400
	48	87.8	60.6	42.8	45.2	63.733
	72	94.7	74.3	34.8	37.3	67.933
	96	71.5	63.2	29.1	43.5	54.600
Mean for Concentration		89.883	75.300	53.000	47.183	

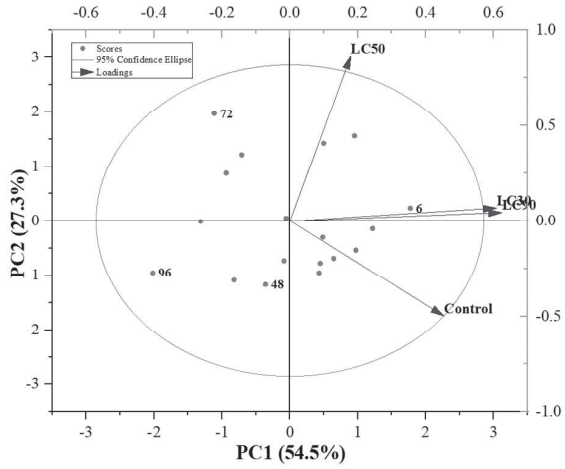

Figure 10: Biplot tail beat of sea bass (*Centropristis striata*) exposed to an acute dosage of SO_2, NO_2 and its mixture

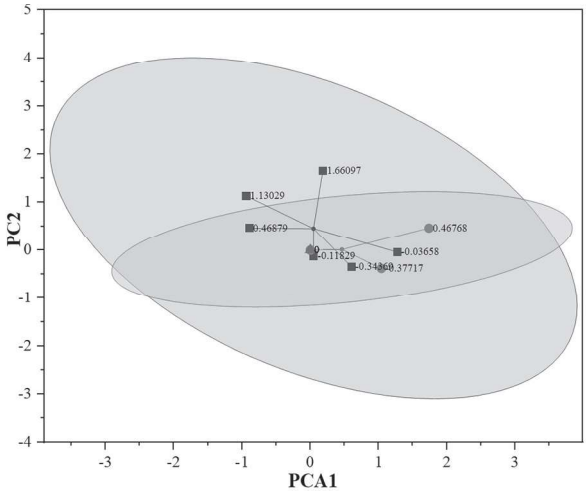

Figure 11: Cluster plot for the tail beat of sea bass (*Centropristis striata*) exposed to an acute dosage of SO_2, NO_2, and its mixture.

Principal component analysis (PCA) of tail beat across the 96 hours' acute exposure of LC_{30}, $LC_{50,}$ and LC_{90} concentrations of gaseous SO_2, $NO_{2,}$ and their mixture on sea bass were analysed, including control treatment. The analysis is based on the same data set as used for Table 11. Each dot refers to the mean value of specific serum/enzyme parameters of sea bass at different treatments. Figure 10 (Biplot) and Figure 11 (Cluster plot) are PCA plots accounting for 54.5% the data

set variance between PC1 and PC2. The arrows, which represent the vectors for the correlation coefficients of biochemical markers with the principal components (PCs), should be interpreted based on their orientation: horizontally for PC1 and vertically for PC2. Most individuals in the LC_{30} and LC_{90} treatment groups are nearly aligned horizontally, signifying a strong correlation with PC1. Conversely, the tail beat of individuals in the LC_{50} and control groups is almost vertically aligned, suggesting a strong correlation with PC2, which accounts for 27.3% of the variation. Dots represent the parameters of fishes exposed to SO_2 (blue), NO_2 (black), and their mixture (red).

Exposure to gaseous SO_2, NO_2 and their mixtures was observed to induce toxic effects on sea bass, resulting in altered OBF in fish across all experimental conditions. A significant reduction in OBF ($p < 0.001$) was noted with increasing concentrations of gaseous SO_2, particularly as the concentration rose from LC_{30} to LC_{90} during acute tests, as shown in Figure 12. Fish exposed to the combination of SO_2 and NO_2 exhibited a more pronounced decrease in OBF compared to those exposed to either gas individually, highlighting the mixture's heightened toxicity relative to the control treatment. The steep decline in tail beat frequency, indicative of increased sluggish movements, corresponded with higher concentrations of gaseous pollutants and their mixtures, leading to further reductions in OBF. Consequently, OBF was dependent on both the concentration of gaseous pollutants and the duration of exposure.

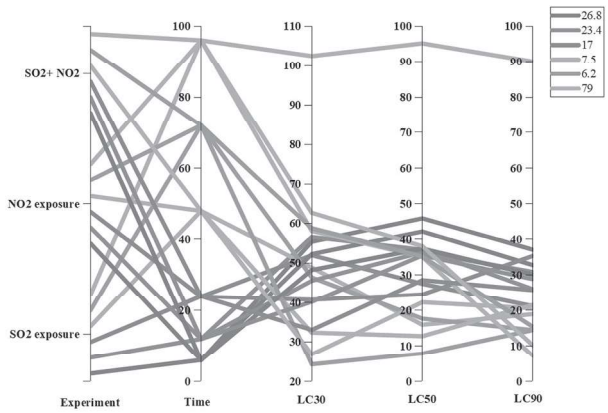

Figure 12: Opercular beat frequency obtained by the fish exposed to gaseous SO_2, NO_2 and their mixture

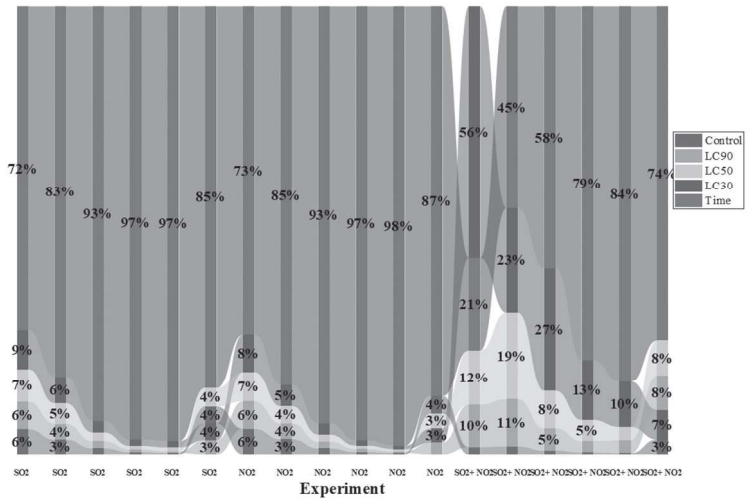

Figure 13: Swimming velocity, obtained by the fish under various concentrations of gaseous SO_2, NO_2 and their mixture

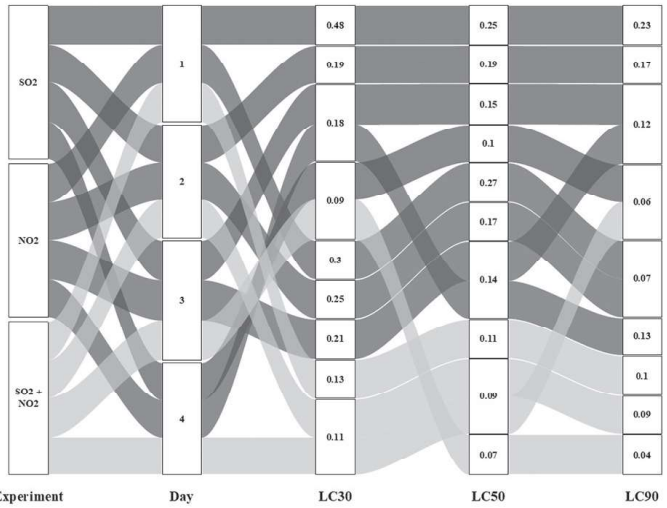

Figure 14: Swimming activity index (SAI/day) obtained by the fish when exposed to gaseous SO_2, NO_2 and their mixture (Acute test)

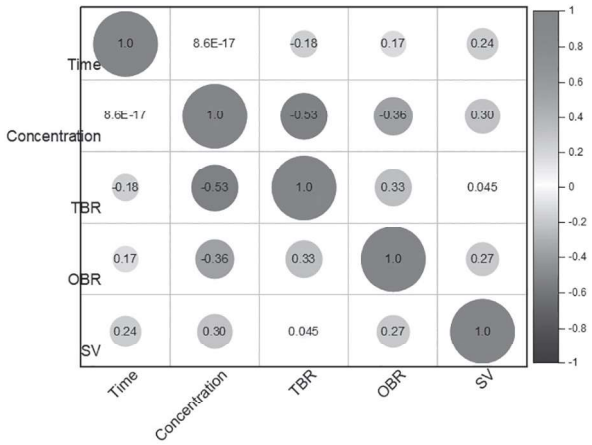

Figure 15: Correlation between exposure of time, concentration of gaseous pollutants, TBR, OBR and SV of sea bass fish at acute exposure for 96 hours.

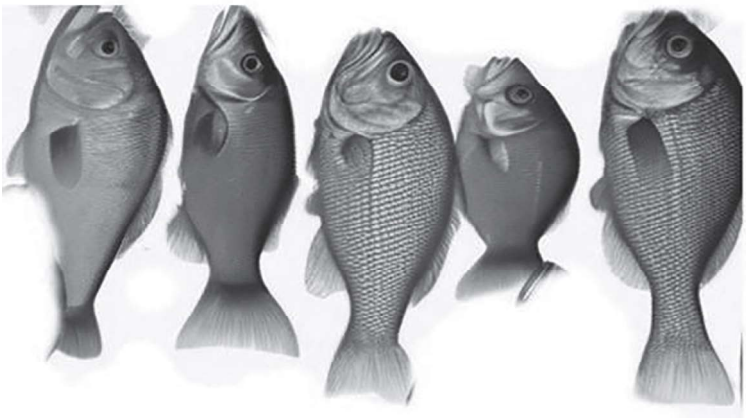

Figure 16: Morphological changes observed in sea bass at LC90, acute exposure of SO_2+NO_2 after 96-hour duration

Throughout the entire bioassay, the swimming velocity's daily mean values were computed and found to fluctuate, generally decreasing with higher concentrations of gaseous SO_2, NO_2, and their mixtures. Over the 96-hour bioassay, average swimming velocities dropped from 1.352 cm/sec to 0.940 cm/sec in fish exposed to SO_2, from 1.103 cm/sec to 0.817 cm/sec in those exposed to NO_2, and from 9.865 cm/sec to 3.599 cm/sec in fish exposed to the SO_2 and NO_2 mixture (Figure 13). The mean swimming activity index (SAI) decreased under all experimental conditions compared to the control. Activity levels declined over time and with increasing pollutant concentrations. For

fish exposed to gaseous SO_2, the SAI values dropped from 0.235 to 0.145 per day.

The SAI of fishes exposed to NO_2 is decreased in between 0.285 to 0.097/day indicating NO_2 is more toxic than SO_2. In fishes exposed to a mixture of SO_2 and NO_2, the average SAI was also found to get decrease compared to SO_2 and NO_2 (Figure 14). As the concentration of gaseous pollutants and exposure time increases, there is significant behavioral and morphological changes were observed (Figures 15 and 16).

3.5. Fin deformities

In the current study, none of the fish in the experimental groups exhibited fin necrosis when exposed to gaseous SO_2, NO_2 or their mixture. However, several split fins were observed at the conclusion of the bioassays for both gaseous pollutants. In fish exposed to gaseous SO_2, up to 20% developed split fins after 96 hours, indicating that this concentration can be lethal and cause morphological deformities. Fish exposed to gaseous NO_2 and the SO_2+NO_2 mixture began showing fin deformities as early as 48 hours into the experiment at higher concentrations (LC_{50} and LC_{90}). This suggests that even small amounts of NO_2 and its mixtures are highly toxic and potentially lethal to sea bass.

3.6. Eye deformities

In this study, no instances of unilateral anophthalmia or exophthalmia and microphthalmia were observed. However, eye clouding was noted across all experimental conditions. Initially, this clouding caused the pupils to appear as white balls, eventually covering the entire eye. The density of the white cloudiness increased with prolonged exposure to the gaseous pollutants.

4. Discussion

According to Daoud et al. (2009), fish exposed to aquatic pollutants metabolize and store them within their tissues due to the lipophilic nature of these pollutants. These pollutants include toxicants such as metals, minerals, and dissolved gases. As a result, the biology of fish can be impaired. The objective of this study was to assess

the behavioral and morphological impairments in fish exposed to gaseous pollutants, specifically SO_2, NO_2, and a mixture of SO_2+NO_2. By observing changes in fish behavior, behavioral toxicology serves as a primary tool to study the effects of pollutants. Toxic insults to the brain directly impact fish behavior, particularly in relation to aquatic pollution (Rao 1999). The study revealed that gaseous NO_2 and SO_2 negatively affect fish physiology, leading to behavioral and morphological toxicity. Comparatively, NO_2 was found to be more hazardous than SO_2, causing severe toxicity.

Quantifying the TBF and OBF provides a quantitative measure of beats per minute in fish. These parameters serve as indicators of stress, pathology, or toxicity, as any changes in TBF and OBF can result in lethargy, sluggishness, uncoordinated movements, and erratic behavior in fish (Smith et al., 2010). Several studies have demonstrated that exposure to gaseous pollutants such as SO_2, NO_2, and a combination of SO_2 + NO_2 can lead to induced toxicity in fish, resulting in alterations in TBF and OBF. For example, a study conducted on sea bass (*Dicentrarchus labrax*) revealed a significant ($P \leq 0.001$) reduction in both TBF and OBF with increasing concentrations of gaseous pollutants and prolonged exposure time, when compared to control samples (Jensen 2003). The findings of this study align with previous research that has shown the negative impact of gaseous pollutants on fish physiology and behavior. Such alterations in TBF and OBF can be indicative of the adverse effects of aquatic pollution, highlighting the sensitivity of fish to toxic substances in their environment (Lisa et al. 2020).

The sudden increase in TBF observed can be attributed to the initial shock experienced by the fish when exposed to toxic gaseous pollutants. This shock results in quiescent movements. Similarly, the exposure to gaseous SO_2 and NO_2 causes an abrupt shock that leads to increased respiratory rates as the fish try to avoid the toxic environment, thereby elevating OBF. Our findings are in line with previous studies by Omoregie (1995), Chindah et al. (2004), and Grillitsch et al. (1999), which showed that chemical stress induces behavioral modifications and reduces TBF and OBF values under both acute and sub-lethal toxicity conditions. The experiments carried out by Devi and Mishra (2013) also reported an initial increase in

TBF in *Channa punctatus* exposed to chlorpyrifos for up to 24 hours, followed by a decline towards the end of the experiment, similar to our observations. Previous studies have shown that the toxicity of petroleum hydrocarbon compounds can damage the epithelial cells in the gill chamber (Omoregie, 1995), resulting in a decrease in opercular beat frequency (OBF) in common carp. Our findings align with the results of Harit and Srivastava (2018), Banjara and Singh (2019), Devi and Mishra (2013), Misha and Verma (2016), Chindah et al. (2004), Pandey et al. (2008), and Woke and Wokoma (2009), all of whom reported reduced opercular beats in various fish species exposed to organophosphate pesticides.

The observed decrease in TBF and OBF in all fish exposed to dimethoate and chlorpyrifos is primarily due to the inhibition of acetylcholinesterase (AChE) in the muscles, which blocks neural transmission (Devi and Mishra, 2013). This inhibition results in paralysis and impending death, leading to a slowdown in physiological processes (Fryday et al., 1996; Omoregie, 1995). It is important to note that during the initial hours of pesticide exposure, there was an increase in opercular movements to meet the oxygen demand in the fish's body. However, as the experiment progressed and paralysis set in, opercular movements decreased, causing the fish to attempt to escape the toxic environment by gulping for surface air.

An increase in SV is indicative of hyperactivity in fish, while a reduction in SV signifies lethargy, paralysis, and even death. Furthermore, Swimming Activity provides insight into the overall movement patterns of fish, serving as a descriptor of normal behavior in the absence of disease, stress, and toxicity. These indices play a crucial role in identifying abnormal behavior and deviations from typical swimming patterns in fish. Numerous studies have demonstrated the significance of SV and SAI in assessing fish behavior and its correlation with various factors. For example, Eissa et al. (2003; 2006) have utilized these indices to evaluate the effects of different stressors and toxic substances on fish behavior, highlighting the importance of monitoring SV and SAI as indicators of abnormal behavior. The present study investigated the impact of gaseous pollutants, namely SO_2, NO_2, and a mixture of SO_2+NO_2, on SV and SAI in sea bass. The results revealed a consistent decrease in both S V and Swimming

Activity as a result of exposure to these pollutants. In all experimental setups, the Swimming Velocity values exhibited a gradual decline across replicates with increasing pollutant concentration and exposure time. These reductions in SV were found to be statistically significant ($p \leq 0.001$) when compared to the control group. Similarly, the SAI also showed a significant decrease ($p \leq 0.001$) compared to the control group. These findings are consistent with previous research highlighting the adverse effects of gaseous pollutants on fish behavior. Studies by Gandar et al. (2015) demonstrated that exposure to SO2 and NO_2 resulted in a decrease in Swimming Velocity in several fish species, including sea bass. Another study conducted by Sloman and McNeil (2012) reported a significant reduction in SAI in fish treated with a mixture of gaseous pollutants. The decrease in SV and SAI observed in sea bass treated with gaseous pollutants indicates the negative impact of these pollutants on fish behavior and suggests potential stress, toxicity, or lethargy in the exposed individuals.

The observed reduction in swimming indices, such as Swimming Velocity and Swimming Activity, can be attributed to the neurotoxic stress induced by the presence of toxicants. This stress leads to the accumulation of acetylcholine at synaptic junctions, resulting from the inhibition of the acetylcholinesterase enzyme. Consequently, fishes exhibit sluggish movements, which ultimately lead to lower SAI. The change in fish locomotor behavior is a direct consequence of the accumulation of ACh, resulting in a disturbance of the nervous system and muscle coordination. Several studies support this association between depressed locomotor activity and AChE enzyme inhibition in fishes. For instance, Begum et al. (2006), Rao et al. (2005), and Kavitha and Rao (2008) have reported similar reductions in locomotor activity, which were linked to the inhibition of AChE enzyme in fishes. The findings of the current study emphasize the neurotoxic effects of the pollutants, leading to alterations in fish locomotor behavior and impaired swimming indices.

5. Conclusions

This study elucidates the acute toxicity of gaseous SO_2 and NO_2 on 8-week-old sea bass (*Centropristis striata*) and unveils the synergistic effects of their combined exposure in a pseudo-marine water

environment. Concentration-dependent mortality rates were observed for both SO_2 and NO_2 individually, with pronounced impacts at higher concentrations. The combined exposure exhibited synergistic effects, amplifying mortality rates. Behavioral and morphological changes, including loss of equilibrium, erratic swimming, and skin discoloration, were evident, accentuated by the presence of gaseous NO_2. These findings underscore the critical importance of considering combined pollutant impacts in environmental risk assessments. The observed alterations in sea bass behavior and morphology serve as sensitive indicators of environmental pollution, emphasizing the imperative for effective mitigation strategies to preserve the health of aquatic ecosystems.

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