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Exploring the Impact of Naphthalene (Polycyclic Aromatic Hydrocarbons) on *Anabas testudineus* (Bloch) through Dose-Specific Bioenzymological Analysis

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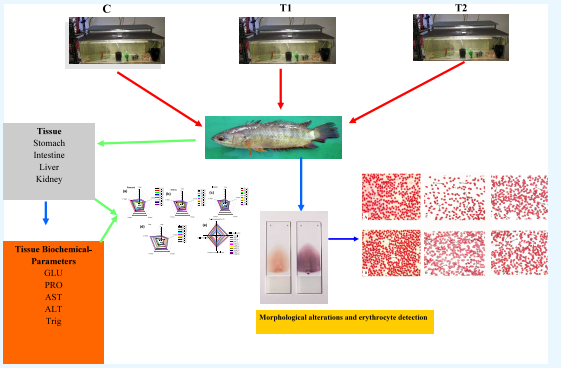
ABSTRACT: This study addresses the increasing concern about naphthalene, a polycyclic aromatic hydrocarbon (PAH), highlighting its growing threats to the environment and aquatic life. The research examines its impact on *Anabas testudineus* (Bloch) through a detailed dose-specific bioenzymological analysis. Experimental fish groups were exposed to T1 (0.71 mg/L) and T2 (1.42 mg/L) naphthalene concentrations, representing 25 and 50% of the LC_{50} value, respectively, over a 1−21 day period. Following the experiment, water samples underwent physicochemical analysis, while fish tissues were examined for diverse bioenzymological parameters. Among these parameters, aspirate aminotransferase (AST) and alanine aminotransferase (ALT) serve as crucial indicators for monitoring the physiological status of fish and addressing pollution induced by PAHs, especially naphthalene. Statistical significance was observed in morphopathological changes and erythrocyte alterations, particularly the presence of tear-drop appearance (Tr) positively interacting with swelled cells (Sc), vacuolated cells (Va), and sickle cells (Sk) ($P < 0.05$). These findings highlight tear-drop appearance (Tr) as a significant biomarker in response to naphthalene exposure. The observed changes in *A. testudineus* tissue bioenzymology, apoptosis, and erythrocytic alterations were exposure and dose-dependent. The research highlights the significance of overseeing and controlling PAH concentrations in aquatic ecosystems to ensure the well-being of *A. testudineus* (Bloch).



1. INTRODUCTION

The excessive extraction of natural resources globally poses a significant threat to both the environment and human well-being.[1,2] One of the pressing concerns worldwide is the pollution of aquatic resources, which has led to increased awareness and initiatives to preserve water bodies. Various anthropogenic activities, including uncontrolled and unplanned incomplete combustion of carbon-containing fossil fuels like coal, diesel, wood, fuel oil, and kerosene, release pollutants into aquatic environments, ultimately polluting surface waters.[3] Polycyclic aromatic hydrocarbons (PAHs), with naphthalene as a notable example, are widely distributed in aquatic ecosystems.[4] These compounds are lipophilic and are integral components of petroleum products.[5] PAHs, including naphthalene, pose a significant threat to aquatic life, leading to various difficulties such as tissue damage, necrosis, cellular damage, and ulceration.[4,6,7]

The aquatic environment is increasingly at risk due to ongoing increases in PAHs pollution. PAHs, produced as byproducts of fossil fuel combustion, are notorious for their toxic and persistent nature in the environment.[8] Aquatic organisms, including fish, shellfish, and amphibians, face particular vulnerability to PAHs as they absorb these compounds from water and sediment, where they accumulate over time.[9] Research has shown that PAHs pollution has dramatic effects on aquatic ecosystems. In fish, it has been linked to reduced growth, increased mortality, and decreased reproductive success.[10] Amphibians exposed to PAHs exhibit decreased body size and abnormal development. Additionally, PAHs can interfere with the gill structure of fish, compromising their ability to extract oxygen from the water.[11] The effects of PAHs pollution extend beyond aquatic organisms and impact the food web. PAHs are transferred up the food chain, affecting species higher in the hierarchy and leading to changes in biodiversity and ecosystem disruption.[12] To mitigate further PAHs pollution and their detrimental effects on the aquatic environment, it is imperative to reduce emissions. This can be achieved through measures such as enhancing energy efficiency, transitioning to

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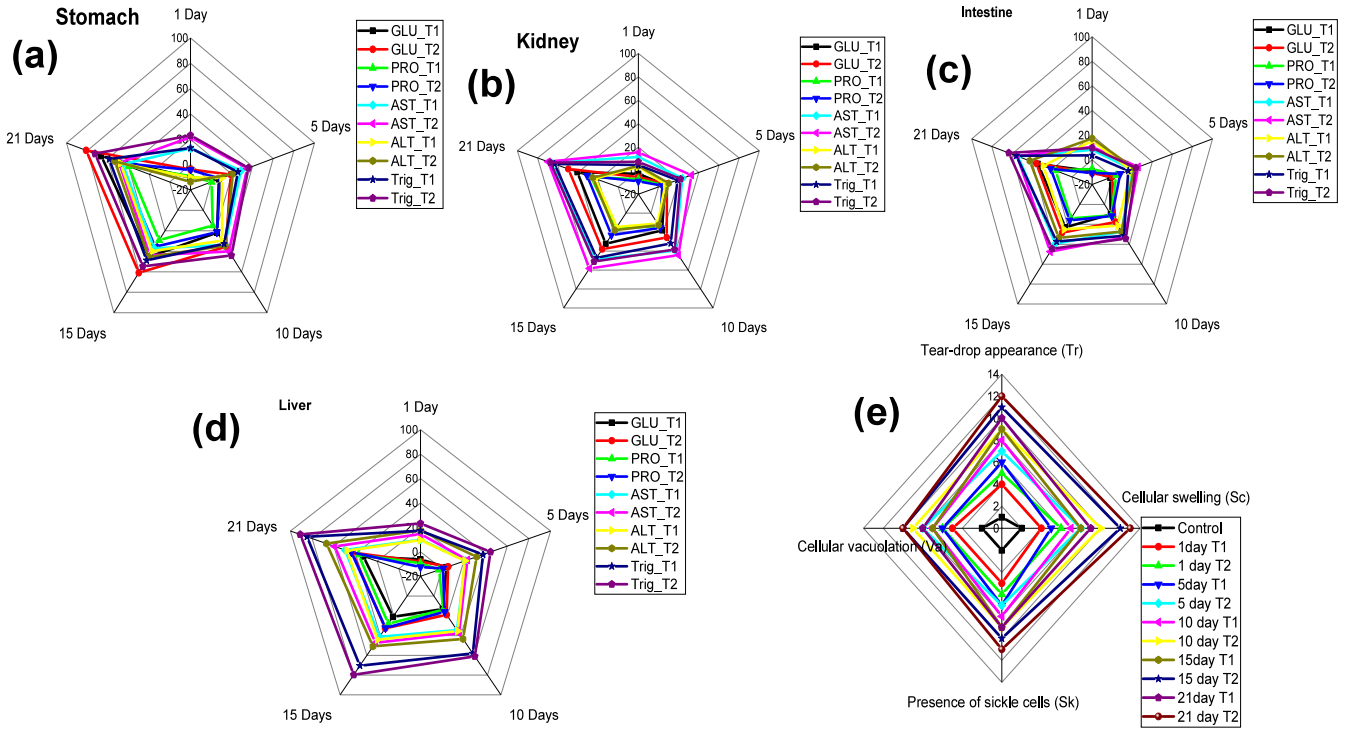

Figure 1. Result presents a comprehensive analysis of the biochemical spider plot, which was conducted in response to naphthalene exposure over a period of 0 to 21 days. This study encompasses key biochemical parameters including GLU (glucose levels), ALT (alanine transaminase), AST (aspartate transaminase), Trig (triglyceride levels), and erythrocyte alterations of different types. The data is organized according to specific organs: (a) stomach, (b) kidney, (c) intestine, (d) liver, and (e) different types of erythrocyte alterations. Note: When a value is identified as being below zero, it indicates that it can decrease in accordance with the controlled response.

renewable energy sources, and implementing more stringent policies and regulations.[13]

Naphthalene pollution significantly impacts fish health, with heightened naphthalene levels adversely affecting crucial organs such as the liver and gills. This disruption results in compromised physiological functions, hindering growth, reproduction, and overall fitness.[14,15] Prolonged exposure to naphthalene can have enduring effects on fish populations, manifesting as declines in population, alterations in community structure, and a diminished overall resilience of the aquatic ecosystem.[16,17] Research findings highlight the moderate toxicity of naphthalene to various fish species, revealing its potential to impair fish health during extended exposure. The gradual, time-dependent decline in fish health observed under chronic naphthalene exposure underscores the importance of regulating and monitoring naphthalene concentrations in aquatic ecosystems.[15] The overall health of fish populations is dependent on the aforementioned strategy. Fish living in these habitats absorb these pollutants, and there is increasing evidence of the adverse effects of PAH exposure on fish health, including reproductive issues, growth impairments, behavioral changes, as effect of fish culture, and human health.[18] Previously, extensive efforts were dedicated to understanding the consistent loss of gill structure and secondary lamellar fusion in fish exposed to diverse naphthalene concentrations.[17] Significant variations in enzyme activities, including cholinesterase (ChE), lactate dehydrogenase (LDH), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST), were observed between the control and the experimental groups. Notably, our focus extended beyond erythrocytes, apoptosis, and morphological alterations related to blood issues. Our novel approach involved exploring naphthalene accumulation directly in fish organs, shedding light on its impact through bioenzymological analyses of various fish tissues, erythrocytes, apoptosis, and morphological changes.

Exposure to naphthalene has been linked to endocrine disruption, changes in brain chemistry, organ damage, and increased mortality in fish. It interferes with neurotransmitter levels, potentially impacting hormone regulation, and disrupts hormone functions while accumulating in fish brains due to its lipophilicity.[19] These impacts on fish health also have implications for human well-being. PAHs can enter the human body through contaminated fish consumption, direct contact with polluted water, or the inhalation of particles contaminated with PAHs. The human health effects of PAHs exposure include cancer, reproductive problems, neurological disorders, and other health issues.[20]

To safeguard both fish and human health, it is crucial to reduce PAHs pollution. Strategies to achieve this goal include regulating industrial emissions, reducing fossil fuel usage, and investing in renewable energy sources. This study presents a comprehensive assessment of the bioenzymological analysis of different fish tissues, erythrocytes, apoptosis, and morphological alterations in *Anabas testudineus* (Bloch) blood under varying exposure durations to naphthalene (PAH). *A. testudineus* serves as an ideal model fish specimen, offering insights into the early warning signs of exposure to various xenobiotics in both field and laboratory settings. Its global distribution in natural aquatic environments, ease of acclimation in laboratory settings, high protein content, and commercial value make it an important subject of study in understanding the impacts of environmental stressors like PAHs.

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2. RESULTS AND DISCUSSION

2.1. Bioenzymological Responses. *2.1.1. Glucose (GLU).* Naphthalene exposure had dynamic effects on glucose content in different organs of *A. testudineus*, shedding light on the fish's responses to this environmental stressor. In the stomach, exposure to naphthalene caused fluctuations in the GLU content. Under the T1 conditions, there was an initial sharp 10.37% decrease after a single day of exposure, followed by gradual recoveries over 5, 10, 15, and 21 days, with increments of 7.44, 21.71, 45.53, and 66.42%, respectively. In contrast, the T2 condition showed a milder 3.89% decrease after 1 day, followed by more robust recoveries, with the most significant increase of 81.00% observed after 21 days (see Table S1). The intestinal tract also exhibited intriguing GLU content changes in response to naphthalene. Under T1, there was a noticeable 9.14% decline after 1 day and a 2.02% decrease after 5 days, followed by progressive increases of 9.87, 23.03, and 34.09% over 10, 15, and 21 days, respectively. Meanwhile, T2 showed an even more pronounced initial drop of 10.85 and 0.02% after 1 and 5 days, respectively, followed by robust increases of 17.58, 28.38, and 35.63% over 10, 15, and 21 days, respectively, with the most significant changes after 21 days (see Table S2). In the liver, naphthalene exposure led to fluctuations in the GLU content. In T1, there was an immediate 6.25% decrease after 1 day, followed by gradual recoveries of 3.94, 13.17, 21.03, and 32.69% over 5, 10, 15, and 21 days, respectively. In T2, there was a more pronounced initial drop of 7.95% after 1 day, followed by robust recoveries of 5.43, 18.98, 32.59, and 42.83% over 5, 10, 15, and 21 days, respectively, with the most significant changes after 21 days (see Table S3).

Finally, in the kidneys, naphthalene exposure resulted in distinctive GLU content changes. Under T1, there was an initial 2.62% decrease after 1 day, followed by progressive recoveries of 6.30, 18.18, 32.30, and 40.61% over 5, 10, 15, and 21 days, respectively. In T2, there was a more pronounced initial drop of 5.40% after 1 day, followed by robust recoveries of 8.33, 25.61, 38.01, and 49.56% over 5, 10, 15, and 21 days, respectively, with the most significant changes after 21 days (see Table S4). In the study, it was observed that the GLU levels in fish organs such as the stomach, kidney, intestine, and liver were higher under condition T2 compared to under condition T1. Notably, among these organs, the stomach and liver exhibited the highest GLU levels, as illustrated in Figure 1a−d. The study's findings highlight a significant variation in GLU levels across different organs in fish.

This variation may suggest distinct metabolic activities or regulatory mechanisms within these organs. Understanding the reasons behind such variations can provide valuable insights into the physiological processes at play. These findings underscore the dynamic responses of *A. testudineus* to naphthalene exposure across various organs and conditions.[21] The initial decreases in GLU content suggest acute responses to the toxin, potentially involving detoxification processes.[22] However, as exposure continued, adaptive mechanisms appeared to kick in, resulting in increased GLU content.[23] Notably, these adaptations were more pronounced under the T2 condition, particularly after 21 days. Understanding these responses is vital for assessing the long-term impacts of environmental pollutants on fish physiology and adaptation mechanisms.[24] Further research may be needed to uncover the specific mechanisms underlying these observed changes.

2.1.2. Effect of Protein (PRO) Content. Fish PRO is essential for providing crucial nutrients, imparting biological specificity to various cell types, serving as fundamental building blocks in living organisms, and serving as the primary energy source. In the context of naphthalene exposure, the PRO content in the stomach of *A. testudineus* exhibited notable changes. Under T1 conditions, after just 1 day of exposure, there was a significant 9.09% reduction in PRO content, followed by a gradual increase over 5, 10, 15, and 21 days, with increments of 0.40, 14.83, 29.09, and 40.10%, respectively. Conversely, under T2 conditions, there was a 4.29% reduction after the first day of exposure, but subsequently, over 5, 10, 15, and 21 days, PRO levels increased by 8.76, 21.34, 35.56, and 48.94%, respectively. Notably, the most significant increase occurred after 21 days of exposure under T2 conditions, while the most substantial reduction was observed after just 1 day under T1 conditions (see Table S1). In the intestine of *A. testudineus*, a similar pattern emerged. Under T1 conditions, there was a 7.23% decrease in the PRO content after the first day of exposure, followed by gradual increases of 4.02, 10.95, 13.97, and 16.53% over 5, 10, 15, and 21 days, respectively. Under T2 conditions, a 10.72% reduction was observed after 1 day of exposure, but subsequent increases of 8.09, 11.69, 16.02, and 21.29% occurred over 5, 10, 15, and 21 days, respectively, with the most significant changes appearing after 21 days under T2 conditions (see Table S2). In the liver of *A. testudineus*, PRO content decreased by 9.37 and 2.86% under T1 conditions after 1 and 5 days of exposure, respectively. However, PRO levels gradually increased by 13.81, 27.95, and 37.25% during 10, 15, and 21 days of exposure, respectively. Under T2 conditions, there was a 12.48% decrease after 1 day of exposure, but subsequent increases of 0.76, 15.91, 32.32, and 43.75% occurred over 5, 10, 15, and 21 days, respectively, with the most significant changes appearing after 21 days under T2 conditions (see Table S3). In the kidney of *A. testudineus*, PRO content decreased by 7.18% after 1 day of exposure under T1 conditions, followed by gradual increases of 4.76, 13.67, 16.16, and 26.94% over 5, 10, 15, and 21 days, respectively. Under T2 conditions, there was a 9.11% decrease after 1 day of exposure, but subsequent increases of 3.55, 15.50, 23.21, and 30.84% occurred over 5, 10, 15, and 21 days, respectively, with the most significant changes appearing after 21 days under T2 conditions (see Table S4). This study highlights the dynamic changes in PRO content in various organs of *A. testudineus* in response to naphthalene exposure, with different patterns observed under T1 and T2 conditions. Figure 1a−d illustrates a consistent trend of higher PRO levels across various organs, with the order of abundance being stomach > liver > intestine > kidney. Notably, the stomach exhibits the highest concentration of PRO, indicating its elevated PRO content. It serves as a vital line of defense, preventing the absorption of toxic compounds into the bloodstream while simultaneously aiding in the digestion and assimilation of essential nutrients.[25] This intricate balance underscores the remarkable adaptability and resilience of the fish digestive system.[26] The findings underscore the importance of understanding how environmental factors can affect PRO levels in aquatic organisms and their potential implications for their overall health.[27] PRO becomes involved in the metabolic activation of naphthalene, leading to the formation of reactive metabolites such as epoxides. The covalent modification of PRO by these naphthalene metabolites can induce structural changes, potentially impacting their function.[28] Additionally, the binding of reactive metabolites to PRO may activate cellular stress response pathways, initiating mechanisms to counteract damage

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and facilitate repair, ultimately influencing the overall fate of the cell.[29] Additionally, the study aligns with previous research indicating the sensitivity of fish PRO to environmental contaminants, which can have adverse effects on fish physiology.[30]

2.1.3. Asparate Aminotransferase (AST) Activity. AST activities in fish are crucial for facilitating the conversion of amino acids and acetoacids by transferring amino groups. These processes play a vital role in gluconeogenesis and oxidation. In the stomach of *A. testudineus*, the activity of AST exhibited significant changes in response to naphthalene exposure. Under T1 conditions, the activity increased notably over 1, 5, 10, 15, and 21 days of exposure with increments of 12.77, 28.82, 33.85, 37.03, and 45.55%, respectively. In contrast, under T2 conditions, there was a subsequent increase over the same time intervals with increments of 21.62, 34.90, 40.11, 43.89, and 49.04%, respectively. The most substantial increase was observed after 21 days under T2 conditions, while the most significant reduction occurred after just 1 day under T1 conditions (refer to Table S1). In the intestine of *A. testudineus*, AST activity also responded to naphthalene exposure. In T1 conditions, there were significant increases over 1, 5, 10, 15, and 21 days, with increments of 8.16, 19.91, 27.39, 39.39, and 53.81%, respectively. Under T2 conditions, the activity increased over the same time intervals with increments of 10.54, 26.05, 32.89, 47.42, and 54.82%, respectively. Similar to the stomach, the most significant increase occurred after 21 days under T2 conditions, while the most substantial reduction was observed after 1 day under T1 conditions (see Table S2). In the liver of *A. testudineus*, AST activity was significantly affected by naphthalene exposure. In T1 conditions, the activity increased over 1, 5, 10, 15, and 21 days, with percentages of 10.00, 21.42, 34.41, 41.32, and 49.59%, respectively. Under T2 conditions, there were increases over the same time intervals with percentages of 14.59, 23.19, 37.53, 47.34, and 59.06%, respectively. As observed in other organs, the most substantial increase occurred after 21 days under T2 conditions, while the most significant reduction was seen after 1 day under T1 conditions (see Table S3). In the kidney of *A. testudineus*, AST activity responded significantly to naphthalene exposure. In T1 conditions, the activity increased over 1, 5, 10, 15, and 21 days, with percentages of 11.75, 23.69, 39.38, 49.04, and 66.38%, respectively. Under T2 conditions, there were increases over the same time intervals with percentages of 15.65, 32.69, 44.30, 58.37, and 69.58%, respectively. Once again, the most significant increase occurred after 21 days under T2 conditions, while the most substantial reduction was observed after 1 day under T1 conditions (refer to Table S4). The findings of this study highlight the significant impact of naphthalene exposure on AST activity in various organs of *A. testudineus*. The differential responses under T1 and T2 conditions demonstrate the sensitivity of these enzymes to environmental contaminants (Figure 1a−d). These enzymes play a critical role in amino acid metabolism, gluconeogenesis, and oxidation, underscoring their importance in fish physiology[31] It is reported that AST activity in the liver was decreased but increased in plasma[32,33] explained the decreased AST activity caused due to hepatocytic damage and organ inadequacy.[34] These results align with prior research that has emphasized the influence of AST activity on metabolism, particularly in the liver, and its potential regulatory effects on fish metabolic processes.[35]

2.1.4. Alanine Aminotransferase (ALT) Activity. The ALT activities in fish play a pivotal role in the conversion of amino acids and acetoacids by transferring amino groups. These enzymatic processes are essential for gluconeogenesis and oxidation. In the stomach of *A. testudineus*, exposure to naphthalene led to significant changes in the ALT activity. Under T1 conditions, there was a noteworthy 10.10% decrease in activity after just 1 day of exposure, followed by gradual increases over 5, 10, 15, and 21 days, with increments of 10.38, 29.50, 39.89, and 47.81%, respectively. Conversely, under T2 conditions, there was a 13.66% decrease after 1 day, but subsequent increases of 21.03, 34.42, 45.62, and 53.27% occurred over 5, 10, 15, and 21 days, respectively, with the most significant changes observed after 21 days under T2 conditions (see Table S1). In the intestine of *A. testudineus*, the ALT activity was significantly affected by naphthalene exposure. Under T1 conditions, there were substantial increases in activity over 1, 5, 10, 15, and 21 days, with percentages of 15.59, 18.65, 21.40, 25.07, and 28.74%, respectively. Under T2 conditions, ALT activity increased progressively over the same time intervals, with percentages of 17.43, 22.01, 27.21, 33.63, and 42.20%. Once again, the most substantial increase occurred after 21 days under T2 conditions, while the most significant reduction was observed after 1 day under T1 conditions (refer to Table S2).

In the liver of *A. testudineus*, ALT activity showed significant alterations due to naphthalene exposure. Under T1 conditions, there were notable increases over 1, 5, 10, 15, and 21 days, with percentages of 9.63, 20.96, 35.42, 44.09, and 47.71%, respectively. In contrast, under T2 conditions, ALT activity increased consistently over the same time intervals, with percentages of 17.59, 31.80, 43.37, 50.84, and 66.74%, respectively. Similar to other organs, the most significant increase was observed after 21 days under T2 conditions, while the most substantial reduction occurred after 1 day under T1 conditions (see Table S3). In the kidney of *A. testudineus*, ALT activity was significantly impacted by naphthalene exposure. Under T1 conditions, there were gradual increases in activity over 1, 5, 10, 15, and 21 days, with percentages of 3.36, 6.72, 10.92, 14.56, and 22.96%, respectively. Similarly, under T2 conditions, ALT activity increased progressively over the same time intervals, with percentages of 4.20, 10.08, 12.60, 17.92, and 25.21%, respectively. As in other organs, the most significant increase occurred after 21 days under T2 conditions, while the most substantial reduction was seen after 1 day under T1 conditions (refer to Table S4). The ALT activity displays notable variations among various fish organs, as illustrated by consistently higher values observed in the upper forecast results (see Figure 1a−d). When examining a 21-day exposure in different fish organs, it becomes clear that ALT scores consistently register as higher in T2 in comparison to T1. Specifically, the ranking of organs with the highest ALT levels is as follows: stomach > liver > kidney > intestine. This observation implies that naphthalene has a more pronounced detrimental impact on the stomach and liver. In conclusion, the evidence suggests that naphthalene exerts a more harmful influence on the stomach and liver relative to other organs. In summary, this discussion provides a systematic exploration of ALT activity variations across fish organs in response to naphthalene exposure. Research conducted by[36,37] has reported a decrease in ALT activity in the liver and an increase in plasma ALT activity,[38] have also noted decreased ALT levels, potentially due to hepatocyte damage and insufficiencies in various organs. These findings emphasize the sensitivity of ALT activity to environmental contaminants and its crucial role in fish

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metabolism and health. The observed changes in ALT activity highlight the complex responses of fish to toxic substances, which can have both short- and long-term effects on their biochemical processes.

2.1.5. Triglyceride (Trig). The passage (Tables S1−S4) describes the Trig content in various fish organs of the species *A. testudineus* after exposure to naphthalene under the circumstances of T1 and T2. In this context, the Trig content is regarded as a key indicator of health state. In both T1 and T2 situations, naphthalene exposure dramatically raised the stomach's Trig content. The largest increase in the T1 state was seen on day 21 (58.77%) after a gradual increase over the course of 21 days. In T2 condition, the increase was even more pronounced, with the maximum increment occurring on day 21 (72.44%). Similar to the stomach, the Trig content in the intestine showed a significant increase during exposure. In T1 condition, there was a gradual increase over 21 days, with the maximum increase observed on day 21 (55.56%). In T2 condition, the increase was more pronounced, with the maximum increment occurring on day 21 (63.12%). The Trig content in the liver also increased significantly during exposure to naphthalene in both T1 and T2 conditions. In T1 condition, there was a gradual increase over 21 days, with the maximum increase observed on day 21 (84.71%). In T2 condition, the increase was even more pronounced, with the maximum increment occurring on day 21 (90.91%). The kidney of *A. testudineus* also showed a significant increase in the Trig content during exposure to naphthalene. In T1 condition, there was a gradual increase over 21 days, with the maximum increase observed on day 21 (63.20%). In T2 condition, the increase was more pronounced, with the maximum increment occurring on day 21 (67.34%).

It can be provided that at day 21, there is a higher level of Trig content in T2 compared to that in T1. Additionally, it suggests that this increase in Trig content may be indicative of higher fat content in the stomach and liver (Figure 1a−d). Trigs are a type of fat that is found in the bloodstream. They are also stored in fat cells and used as an energy source by the fish body. Elevated levels of Trigs in the blood can be associated with various health issues, including obesity, metabolic syndrome, and an increased risk of hematological disease. The passage also references other studies by,[39,40] highlighting the importance of Trig content as a major health status index in fish. It suggests that Trig levels in blood plasma tend to be higher in field experiments compared with laboratory experiments, likely due to natural uptake and absorption through the gut as lipids, which are then transported to the liver. Overall, the passage provides data on how naphthalene exposure affects the Trig content in various organs of *A. testudineus* and underscores the significance of Trigs as an indicator of the fish's health status. This data underscores the dynamic influence of naphthalene exposure on Trig content in the stomach of *A. testudineus*, with varying patterns reflecting the interaction between exposure duration and stress intensity.[41]

2.2. Detection of Morpho-Pathplogical and Erythrocyte Alteration. In the control condition of *A. testudineus*, blood smear analysis revealed the typical morphology of both leucocytes (L) and erythrocytes (Er). However, when subjected to environmental stressors in T1 and T2 conditions, noteworthy morphometric alterations became evident in the red blood cells (RBCs) of *A. testudineus*. These changes included the presence of vacuolated cells (Va), a tear-drop appearance (Tr), swelled cells (Sc), and sickle cells (Sk) (Figure 2).

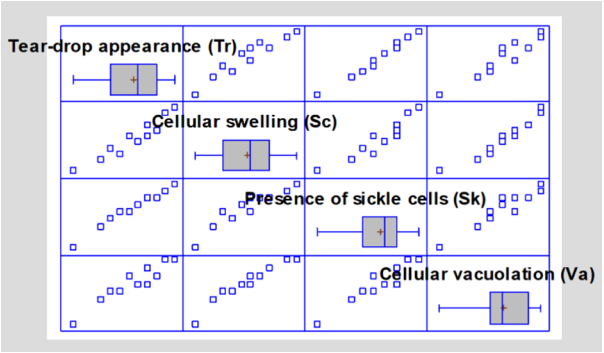

Figure 2. Illustration of Pearson product-moment correlations between variable pairs, quantifying the linear relationship strength with coefficients ranging from −1 to +1. (*P*-value below 0.05 indicates a significant nonzero correlation at a 95.0% confidence level. Notably, the following variable pairs exhibit *P*-values below 0.05: Tr and Sc, Tr and Sk, Tr and Va, Sc and Sk, Sc and Va, Sk and Va.)

These observations have significant ecological implications that require careful consideration (as shown in Figure 1e). Figure 3A−L illustrates the observed changes in *A. testudineus* across various conditions. These conditions include the control condition without naphthalene exposure (A and B), conditions T1 and T2 after 1 day of exposure to naphthalene (C and D), conditions T1 and T2 after 5 days of exposure (E and F), conditions T1 and T2 after 10 days of exposure (G and H), conditions T1 and T2 after 15 days of exposure (I and J), and conditions T1 and T2 after 21 days of exposure (K and L). The presence of Er, Sc, Sk, Tr, and Va is evident, with higher Sc observed in the 21-day comparison. This indicates a noticeable increase in the level of cellular deformation in both T1 and T2 treatments over the course of 21 days.

Although the existence of Er in blood smears under both T1 and T2 conditions is expected, changes in the erythrocytes' specific features and distribution may indicate changes in the general state of the blood.[42] The statistical analysis of blood cellular deformation in *A. testudineus* subjected to naphthalene is presented in Table 1 and Table S3, which show changes in Tr, Sc, Va, and Sk. These results imply that deformation is more likely to occur given the available data.[7]

Er swelling may be a sign of changes in osmotic balance or cellular integrity, possibly as a result of elements present in both T1 and T2 situations. On the other side, the existence of Sk raises questions about the structure or function of hemoglobin, which may result in a reduction in oxygen-carrying capacity and an increased risk of clotting or circulatory issues.[43] According to[44] the emergence of Tr cells may be linked to hematopoiesis problems or bone marrow illnesses, which indicate disruptions in the regular process of cell creation and release from the bone marrow. Vacuolated cells imply the buildup of aberrant cellular contents, perhaps related to metabolic changes or cellular stressors. The modifications in the RBC morphology seen under both T1 and T2 conditions point to similar causes, but more research is needed to pinpoint the precise mechanisms and potential dose-dependent effects. This information is therefore highly valuable in the realm of ecotoxicological assessment. They highlight how sensitive *A. testudineus* is to environmental stressors and offer insightful information on the possible negative impacts of pollutants on aquatic life. RBC morphometric alterations are significant biomarkers of environmental contamination and can be used to assess the well-being of

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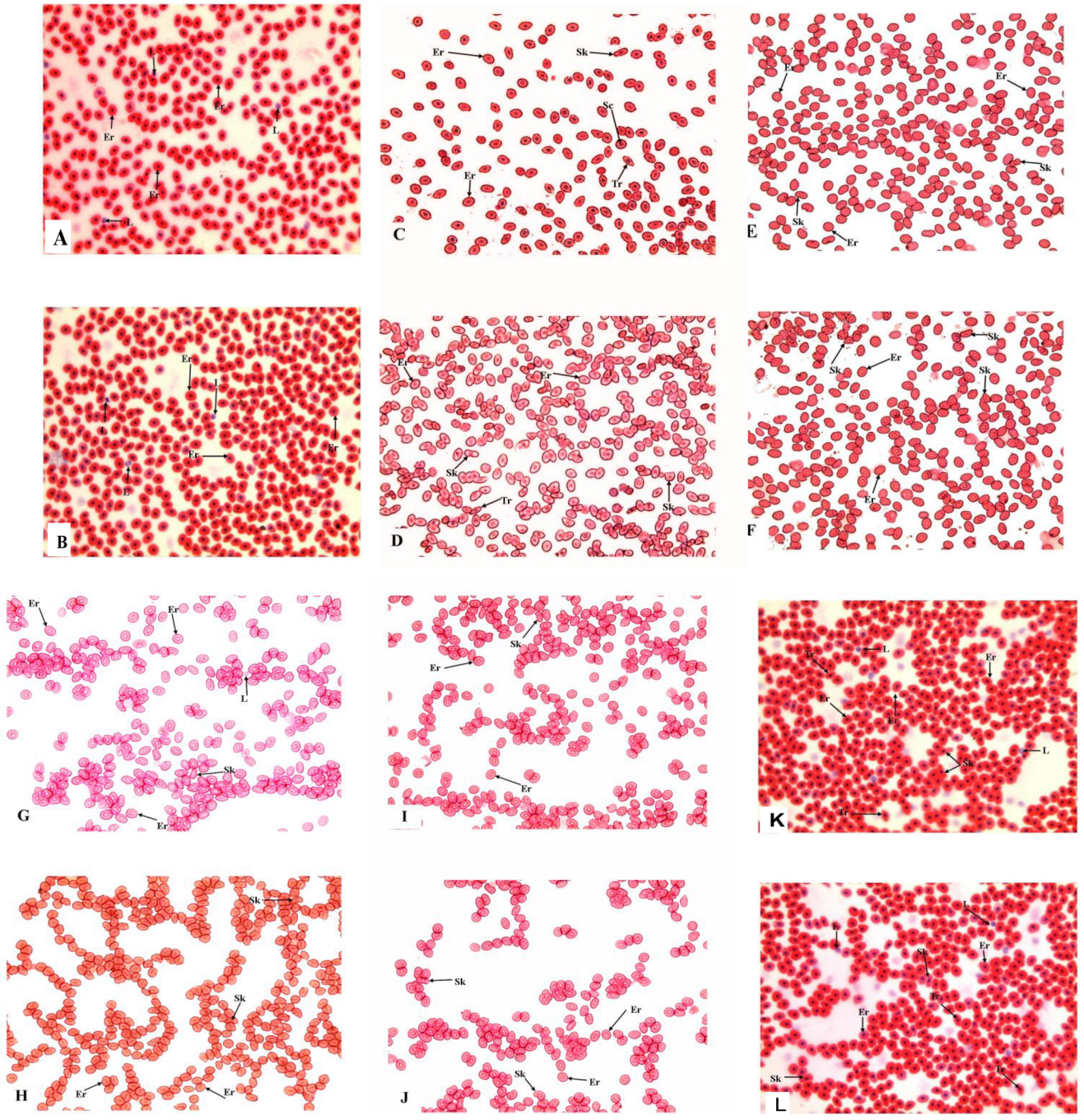

Figure 3. Different types of erythrocyte alteration of the blood cell as treatments wise; (A, B) control condition of naphthalene exposure in *A. testudineus*, (C, D) T1 and T2 conditions in 1 day of naphthalene exposure in *A. testudineus*, (E, F) T1 and T2 conditions in 5 days of naphthalene exposure in *A. testudineus*. Different types of erythrocyte alteration of the blood cell as treatments wise; (G, H) T1 and T2 conditions in 10 days of naphthalene exposure in *A. testudineus*, (I, J) T1 and T2 conditions in 15 days of naphthalene exposure in *A. testudineus*, (K, L) T1 and T2 conditions in 21 days of naphthalene exposure in *A. testudineus*.

aquatic ecosystems. The changes in the RBC morphology demonstrate the necessity of ongoing study and conservation initiatives to lessen the effects of environmental contaminants on aquatic life. It is essential to comprehend these ecotoxicological reactions in order to maintain the delicate balance of aquatic ecosystems and protect the organisms that live there.

3. CONCLUSIONS

The investigation into naphthalene exposure in *A. testudineus*, an aquatic species, revealed dynamic responses in GLU levels, protein levels, AST and ALT activity levels, and Trig levels across various organs. The study observed an initial decrease in GLU levels postexposure, followed by an upward trend, with the stomach showing the most significant increase. A similar pattern was observed in PRO levels, with the liver experiencing the most prominent drop and the stomach demonstrating the highest increase. AST activity levels demonstrated a steady growth with the gut being the most susceptible organ, while ALT activity levels displayed a clear pattern, with the stomach initially experiencing a decline and all other organs subsequently

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Table 1. Statistical Overview of Blood Cellular Deformation in *A. testudineus* Exposed to Naphthalene (T1 and T2)

summary statistics	tear-drop appearance (Tr)	cellular swelling (Sc)	presence of sickle cells (Sk)	cellular vacuolation (Va)
count	1155	1155	1155	1155
average	117.45	123.63	127.54	220.09
standard deviation	3.266	3.324	2.544	2.343
coeff. of variation	43.82%	43.54%	33.72%	33.05%
minimum	12	26	42	22
maximum	566	513	611	710
range	214	233	219	368
stnd. skewness	−0.785851	0.0140659	−1.26323	−1.10591
stnd. kurtosis	−0.034364	−0.317592	0.691902	0.684656

showing an increase. Trig levels consistently increased throughout the experiment, with the liver experiencing the most substantial rise. The research also revealed a direct impact on apoptosis, Er alterations, and bioenzymological examination of *A. testudineus* tissues based on the type and amount of exposure, emphasizing the importance of considering enzymological parameters as bioindicators in ecotoxicological research, especially for pollutants like naphthalene. Exposure of *A. testudineus* to environmental stressors (T1 and T2 conditions) led to distinct morphological changes in RBCs, including Va and Tr appearance, Sc, and Sk. The study significantly advances understanding of how aquatic species metabolically respond to environmental pollutants, particularly naphthalene, and underscores the importance of monitoring and protecting these vital species and their environments.

4. MATERIALS AND METHODS

4.1. Nourishment and Collection of *A. testudineus*. A total of 160 healthy air-breathing climbing perch (*A. testudineus*) were sourced from a legally registered fish farm, Chandimata Fish Farm, Khano village of Purba Bardhaman district under the Galsi-II Block, West Bengal, India. These fish exhibited average weights and lengths for both sexes, measuring 36.32 ± 2.46 g and 12.36 ± 0.78 cm, respectively. To facilitate their adjustment to laboratory conditions, the fish underwent a 3-week acclimation period, during which they were accommodated in a 400 L aquarium. In accordance with the guidelines outlined by,[30] the fish were handled and cared for. Throughout the entire experiment, the aquarium water was refreshed every alternate day[35] to maintain optimal conditions for the fish.

4.2. Laboratory Setup for Experiment and Reagents. Healthy fish, free from disease, were carefully chosen from a 400 L acclimatized aquarium tank. Both sexes fish were then divided into three sets, each consisting of three replicates, resulting in a total of nine sets of 10 fish (05 male and 05 female) in each aquarium. Naphthalene ($C_{10}H_8$) in crystalline flakes, 99% pure (CAS number: 91-20-3), was procured from Sigma-Aldrich (Darmstadt, Germany). It exhibited solubility in methanol, and its concentration was standardized by using primary methanol before becoming water-soluble. Additionally, other chemicals, including HCl, NaOH, and H_2SO_4 for limnological parameters, were obtained from Sigma-Aldrich (analytical grade), and a specific spectrophotometer from PerkinElmer's LAMBDA 25/35/45 UV/vis system (Kasaravadavali, Thane, India) was used for analysis. Therefore, limnological parameters were provided that validate the alterations in water parameters resulting from the applied treatments (Table 2).

Within each set, there were 10 fish placed in individual 40 L capacity aquariums. One of these sets served as the control group, while the other two sets were designated for naphthalene exposure, serving as the treated groups for two different doses: T1 (0.71 mg/L for 96 h), which corresponds to 25% of the LC_{50} value, and T2 (1.42 mg/L for 96 h), representing 50% of the LC_{50} value. This entire experiment took place at the Ecotoxicological Laboratory within the Department of Environmental Science at The University of Burdwan, West Bengal, India. The experiment was conducted following the toxicology protocol established by the university.

4.3. Sampling for Bioenzymological Analysis of Fish Tissue. Every other day, considerable attention was paid to preserving and tracking physiochemical parameters during the whole trial. Different methodologies were used to examine the different biochemical characteristics of numerous tissues, specifically, the stomach, intestine, liver, and kidney. These tissues were carefully removed, completely washed in a 0.75% saline solution, and then immediately put into Teflon tubes that were cooling. These desirable tissues were collected from three replicated sets of fish that were individually exposed for the planned durations of the experiment—namely, 1, 5, 10, 15, and 21 days.

For each tissue sample, an appropriate amount of fresh fish tissue, not exceeding 0.2 g, was extracted and homogenized in 2 mL of 0.2 M phosphate buffer at a pH of 7.4. Subsequently, the homogenate was subjected to centrifugation at 8000 rpm for approximately 14−15 min at a temperature of 4 °C. The resulting supernatant was then processed for the analysis of biochemical parameters using distinct methodologies. The measurement of various bioenzymological parameters was performed as follows: Glucose (GLU) levels were assessed

Table 2. Limnological Parameter for Aquarium Tanks of Different Conditions (± S.D.)

parameters	pretreatment	control	T1	T2
temperature (°C)	31.25 ± 0.81	26.44 ± 0.56	26.36 ± 0.75	23.75 ± 0.63
pH	7.55 ± 0.03	7.035 ± 0.06	7.438 ± 0.04	7.036 ± 0.05
electrical conductivity (EC), μS/cm	421.57 ± 2.11	378.08 ± 3.01	401.34 ± 3.01	423.87 ± 0.79
total dissolved solids (TDS), mg/L	301.54 ± 0.87	279.26 ± 0.79	289.79 ± 3.79	226.76 ± 3.02
dissolved oxygen (DO), mg/L	12.57 ± 0.33	6.78 ± 0.78	6.89 ± 0.79	6.01 ± 0.78
total alkalinity (as $CaCO_3$), mg/L	19.77 ± 2.55	178.65 ± 2.79	179.67 ± 2.68	193.07 ± 3.18
total hardness (as CaCO3), mg/L	189.56 ± 2.07	175.64 ± 3.17	186.67 ± 2.94	165.87 ± 2.11
total hardness (as $CaCO_3$), mg/L	3.25 ± 0.19	2.18 ± 0.97	2.58 ± 0.86	2.79 ± 0.54
nitrate-nitrogen, mg/L	0.89 ± 0.058	0.65 ± 0.075	0.78 ± 0.064	0.69 ± 0.087

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using the Erba kit (BLT00027) (Karásek 1d, 621 00 Brno, Czech Republic). Protein (PRO) content was quantified using the method used by.[4,30] Aspartate aminotransferase activity (AST) was determined using the Erba kit (Erba cat. # FBCEM0045). Alanine aminotransferase activity (ALT) was measured using the Erba kit (Erba cat. # FBCEM0047). Triglyceride (Trig) levels were determined with the assistance of an Erba kit (BLT00058). These comprehensive procedures ensured the precise evaluation of the specified biochemical parameters within the fish tissues throughout the experimental timeline.

4.4. Erythrocytic Alteration Measurement of Blood Smeared. To assess erythrocytic changes in blood smear samples, we followed the collection and preparation methods outlined in our previously published papers.[4,7] Various alterations in erythrocytes, including normal erythrocytes (Er), leucocytes (L), the presence of swollen cells (Sc), prominent vacuolated cells (Va), sickle cells (Sk), teardrop-like cells (Tr), and hemolyzed cells (Hc), were examined using a microscope (LEICA DM 2000, Wetzlar, Germany). Apoptosis index is typically calculated by determining the ratio of apoptotic cells to the total number of cells observed in a naphthalene exposed blood cell count.[45] The formula for calculating the apoptosis index is

$$\text{Apoptosis index} = \frac{\text{Number of apoptotic cells}}{\text{Total number of cells}} \times 100 \quad (1)$$

4.5. Statistical Analysis. The Pearson product-moment correlation coefficient gauges the strength and direction of the relationship between two variables, seeking to establish a best-fit line through their data (Statgraphics Centurion XIX, The Plains, Virginia, United States). The coefficient, denoted as "*r*", reflects how closely data points align with this best-fit line. It ranges from −1 to 1, with 0 indicating no association, values above 0 signifying a positive association, and values below 0 indicating a negative association. The magnitude of the correlation is interpreted using Cohen's conventions: an *r* of 0.10 suggests a weak association, 0.30 indicates a moderate correlation, and 0.50 or higher suggests a strong correlation. Utilizing standard protocols and statistical software (Statgraphics 19), other parameters like central tendency, variability, and shape are computed.[46]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c08535.

Percentage change (±) in various biochemical parameters of the stomach, intestine, liver, and kidney in *A. testudineus* during naphthalene exposure periods, along with summary statistics for each selected data variable, which contributes to the Supporting Information of the results (PDF)

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Author Contributions

"Authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Sukhendu Dey, and authors read and approved the final manuscript." Sukhendu Dey involved in review and editing.

Notes

The author declares no competing financial interest.

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