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A review on Toxic Effect of Triazine on Hematological Parameters of *Channa punctatus* (Bloch)

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Abstract

In both agricultural and non-agricultural settings across the globe, triazines have been widely utilised as a class of herbicides for the last half-century to suppress the growth of broadleaf and certain grassy weeds. First developed by J.R. Geigy Limited in 1956, with simazine as the pioneering compound, triazines revolutionized weed management, especially in crops such as maize and sorghum. These herbicides have since found widespread applications in the cultivation of various crops, including fruit, legumes, and even non-crop agriculture. Despite their effectiveness, the extensive and prolonged use of triazines has raised environmental concerns, particularly regarding their persistence and toxicity in aquatic ecosystems. Additionally, triazines have been utilized in aquaculture for controlling aquatic weeds and algae, but this use also contributes to environmental contamination. Recent research has extended into understanding the immunotoxicological effects of triazines, particularly in aquatic organisms like fish. The impact of triazines on the hematological and immunological systems of fish has been a focus of study due to their potential to disrupt immune functions, including changes in blood cell profiles, immune cell activity, and overall resistance to pathogens. For example, exposure to triazines has been linked to altered levels of phagocytic activity, oxidative burst responses, and immune cell proliferation. Hematological tests, such as the measurement of blood cell counts, nonspecific antibodies, and enzymes with bacteriolytic activity (e.g., lysozyme), provide valuable insights into the extent of immunomodulation caused by triazines. Furthermore, laboratory-based studies investigating the effects of triazines on immune activation in fish have demonstrated changes in both specific and nonspecific immune responses, which could increase susceptibility to infectious diseases. This paper discusses the historical development, agricultural use, environmental impact, and immunological consequences of triazine herbicides, with a particular emphasis on their effects on hematological and immune functions in aquatic organisms. The findings underscore the need for further research into the ecotoxicological risks of triazines, especially in relation to aquatic health and disease susceptibility.

Keywords: freshwater fishes, Triazines, Hematology, *Channa punctatus*, herbicide

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Introduction

For more than half a century, people all over the world have turned to triazine-based compound—an important class of herbicides—to control broadleaf and certain grassland weeds. Their widespread use has made them and will continue to make them a serious problem in the environment, especially in aquatic habitats. The continuous application of synthetic chemicals in modern agriculture has resulted in various environmental concerns, particularly in aquatic ecosystems. Among these chemicals, herbicides, which are commonly used to manage unwanted vegetation, have become a focal point due to their widespread usage and persistence in the environment. Among these, the triazine group of herbicides, including atrazine, simazine, and terbuthylazine, are among the most commonly used globally. Triazines are highly effective in controlling weeds, yet their persistent nature and ability to contaminate water bodies have raised significant environmental concerns. Their detrimental effects on non-target aquatic organisms, particularly fish, have been well documented in recent years. This is especially concerning because of their potential to bioaccumulate in aquatic food chains, thereby affecting not only individual species but also entire aquatic ecosystems. One of the primary concerns regarding pesticide contamination is its impact on the health of aquatic organisms. Fish are often considered sentinels for environmental pollution, as they can be exposed to pollutants in their habitat and serve as a link in the food chain.

Fish exhibit a variety of responses to toxic substances, including alterations in behavior, physiology, and biochemistry. The study of these responses is crucial in understanding the broader ecological implications of pesticide contamination. Hematological parameters, such as red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration, and hematocrit, are commonly used indicators to assess the physiological impact of toxicants on aquatic organisms. Changes in these parameters can signal stress, immune suppression, or systemic damage, offering valuable insight into the sub-lethal effects of contaminants. The freshwater fish *Channa punctatus* (Bloch), commonly known as the spotted snakehead is a widely distributed species in South Asia and serves as an ideal model organism for ecotoxicological studies. Due to its adaptability to various environmental conditions and its importance in local fisheries, *Channa punctatus*has been extensively used in studies assessing the effects of pollutants. Its ecological significance and sensitivity to environmental changes make it an excellent subject for monitoring water quality and understanding the sub-lethal effects of chemicals such as herbicides. The hematological parameters of this species are particularly sensitive to environmental stressors, and as such, can serve as reliable biomarkers of exposure to toxicants like triazine herbicides.

Definition, Importance and Relevance

A review focus on synthesizing existing research concerning the impact of triazine herbicides on the blood-related physiological functions of the freshwater fish Channa punctatus, commonly known as the spotted snakehead. Triazine herbicides, such as atrazine, simazine, and terbuthylazine, are widely used in agriculture to control unwanted vegetation. However, their persistence in aquatic environments and potential to bioaccumulate in aquatic organisms has raised significant concerns about their environmental impact. This review would compile and analyze studies investigating how exposure to triazine herbicides influences key hematological parameters in Channa punctatus, including red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration, hematocrit, and other blood indices. By examining various experimental studies, the review would assess how these pollutants induce stress responses, immune suppression, and potential systemic damage in this species. The article would also highlight the significance of these hematological parameters as biomarkers for ecological monitoring and early warning systems in aquatic ecosystems affected by agricultural runoff and herbicide contamination.

Triazine Herbicides: Mechanisms of Toxicity and Environmental Impact- Triazine herbicides, especially atrazine, are among the most commonly used herbicides worldwide, primarily for controlling broadleaf weeds in crops such as corn, sugarcane, and various turfgrass applications. These chemicals are typically applied to fields via spraying, and due to their water solubility and persistence, they often run off into nearby water bodies, contaminating lakes, rivers, and streams. The impact of triazine herbicides on aquatic environments is a growing area of concern, particularly because of their ability to persist in water and bioaccumulate in aquatic organisms. While triazines are effective in weed management, their toxicological effects on aquatic species are becoming increasingly apparent. In particular, these herbicides are known to disrupt the endocrine systems of fish, affecting reproduction, growth, and development. The primary mode of action of triazine herbicides is the inhibition of photosynthesis in plants, by blocking the electron transport chain in chloroplasts. However, their effects on non-target organisms, such as fish, are mediated through different pathways, which are not fully understood but are thought to involve oxidative stress, disruption of hormone signaling, and immune suppression. Triazine herbicides can interfere with the normal functioning of various organ systems, including the liver, kidney, and immune system, in addition to affecting hematological parameters. The toxicity of these chemicals may vary depending on factors such as concentration, exposure duration, and the specific species being studied. While there has been a growing body of research on the ecological impact of triazine herbicides, particularly in aquatic systems, the specific toxic effects on hematological parameters in fish remain under-explored. Hematological changes in fish can indicate sub-lethal stress responses and may serve as early biomarkers of contamination in aquatic ecosystems. Research suggests that exposure to various pesticides, including triazines, can lead to alterations in RBC count, hemoglobin levels, hematocrit, and immune cell counts in fish, which in turn affects their overall health and survival. Hematological Parameters as Indicators of Toxicity in Fish- Hematological parameters are often used as reliable indicators of the physiological state of fish exposed to environmental stressors, including toxicants. In fish, blood is a vital medium for carrying oxygen, nutrients, and immune cells, and any disturbance in the normal functioning of the blood can lead to systemic health problems. Common hematological indices, such as RBC count, WBC count, hemoglobin concentration, and hematocrit, offer insight into the effects of environmental contaminants on the organism's health. These parameters can provide a quantitative measure of how a toxic substance, such as a herbicide, affects the blood and immune system of the fish. Red Blood Cells (RBC) play a crucial role in oxygen transport and are directly affected by chemicals that interfere with blood circulation or oxygen-carrying capacity. A decrease in RBC count can indicate anemia, which could result from toxic exposure, particularly in the gills, liver, and spleen, which are key organs in blood formation. White Blood Cells (WBC) are essential components of the immune system, helping the organism fight infections. An increase or decrease in WBC counts can indicate either an inflammatory response or immunosuppression, respectively. This is especially important in the context of triazine exposure, as herbicides have been shown to impair immune function in aquatic species. Hemoglobin (Hb) concentration is another critical parameter, as it directly correlates with the oxygen-carrying capacity of blood. Alterations in hemoglobin levels often indicate either a deficiency in red blood cells or a dysfunction in oxygen transport mechanisms, which may be caused by the toxic effects of pollutants like triazine. Hematocrit (Hct), the percentage of blood volume occupied by red blood cells, is an important parameter for assessing blood volume and oxygen transport efficiency. A decrease in hematocrit levels can be indicative of anemia or other blood-related disorders caused by exposure to contaminants. Given the significant role that these parameters play in maintaining the overall health of fish, alterations in any of them can provide valuable information about the effects of triazine herbicides on fish physiology. The study of these parameters in fish exposed to sub-lethal concentrations of triazine will help in assessing the ecological impact of herbicide pollution and the long-term consequences for fish populations in contaminated water bodies.

Hematological Effects of Triazine on *Channa punctatus* (Bloch)

The primary aim of this study is to find the toxic effects of triazine herbicides on the hematological parameters of Channa punctatus. As previously mentioned, *Channa punctatus*is a well-established model organism in ecotoxicology due to its wide distribution, ecological importance, and sensitivity to environmental pollutants. This species has been used in numerous studies to assess the effects of pesticides and other pollutants on aquatic organisms. Existing research has shown that exposure to triazine herbicides can lead to alterations in the blood composition of various fish species, but studies on *Channa punctatus* remain limited. Previous studies on other species of fish exposed to triazine herbicides have reported significant changes in RBC count, hemoglobin concentration, and WBC count. For example, Cyprinus carpio exposed to atrazine exhibited a decrease in RBC count and hemoglobin concentration, suggesting a potential toxic effect on the blood. Similarly, exposure to triazine herbicides has been linked to altered immune function, which could manifest in changes in WBC count in fish species like Tilapia. The impact of triazine herbicides on fish health is of great concern, particularly when considering the potential for bioaccumulation and long-term ecological effects. It is essential to understand how even sub-lethal concentrations of these herbicides can alter hematological parameters in Channa punctatus, as such changes can compromise the fish's health, growth, and reproductive success. This research will contribute to the growing body of knowledge on the effects of herbicide contamination on aquatic organisms and highlight the need for stricter regulation of pesticide use in and around water bodies.

Review of Important Research Work

Austin (2010) monitoring on "The effects of pollution on fish health". Environmental contaminants, such as pesticides, heavy metals, and hydrocarbons, are frequently introduced into aquatic ecosystems. When significant quantities of these pollutants are discharged, they can cause immediate and noticeable impacts, such as large-scale mortality events among aquatic organisms, including fish kills due to the contamination of waterways with agricultural chemicals. In contrast, lower levels of pollutant discharge may lead to their gradual accumulation in aquatic organisms. Over time, this can result in adverse effects such as immunosuppression, reduced metabolic function, and damage to gills and epithelial tissues. Nevertheless, the direct connection between poor water quality and fish diseases remains unverified. Suspected pollution-associated diseases include conditions like epidermal papilloma, fin and tail rot, gill disorders, hyperplasia, liver damage, neoplasia, and ulceration. Lushchak (2011) observed Oxidative stress triggered by environmental factors in aquatic organisms. Reactive oxygen species (ROS) are an unavoidable byproduct of aerobic metabolism. Their concentration in cells is maintained at a balanced level through the dynamic interplay of production and elimination, ensuring a stable ROS baseline. However, this equilibrium can be disrupted, resulting in elevated ROS levels that cause damage to cellular components, a condition referred to as' oxidative stresses. Velisek *et al.,* (2011) observed on the impact of pyrethroid and triazine pesticides on fish physiology. Over the past two decades, global pesticide use has risen significantly, driven by shifts in agricultural practices and the intensification of farming. Pollution from pesticides, particularly in aquatic ecosystems, has emerged as a major environmental concern. The contamination of water bodies by pesticides, whether through direct discharge or indirect runoff, can result in fish mortality, diminished fish productivity, or the accumulation of harmful substances in edible fish tissues, posing potential risks to human health through consumption. Bhuvaneshwari *et al.,*(2012), the Parastromateus Niger fish from the Cauvery River can be used as a bio-indicator species for zinc pollution in aquatic environments since it has a greater zinc concentration as a result of river pollution. Sulodia *et al.,*(2012) worked on Histopathological Alterations in the Kidney of *Channa punctatus*(Bloch) Exposed to Folidol Stress. Pesticide levels in aquatic ecosystems are steadily rising. At elevated concentrations, these chemicals undergo bioconcentration and bioaccumulation within the tissues and organs of fish, which can have detrimental effects on human health when such contaminated fish are consumed.Velisek *et a.,*(2013) Worked on "Acute toxicity of triazine pesticides to juvenile signal crayfish (Pacifastacus leniusculus)". Acute toxicity tests were conducted following standardized chemical testing protocols (OECD Guideline No. 203) using a semi-static test system. Juvenile signal crayfish (n=672) with a weight range of 49.0–81.5 mg and a total length of 12.8–16.0 mm were utilized for the bioassay. Mortality rates were monitored daily over a 96-hour period. Each pesticide was tested at concentrations of 1, 10, 40, 70, and 100 mg.l–1. Mortality percentages were analyzed through linear regression, and median lethal concentration (LC50) values were calculated using probit analysis with EKO-TOX 5.2 software. The 96-hour LC50 values for juvenile signal crayfish were determined as 12.1 mg.l–1 for atrazine, 13.9 mg.l–1 for terbutryne, 14.4 mg.l–1 for prometryne, 19.5 mg.l–1 for hexazinone, 30.6 mg.l–1 for metribuzine, and 77.9 mg.l–1 for simazine. Among these, atrazine exhibited the highest toxicity to signal crayfish. Triazines were found to be harmful to signal crayfish, which were shown to be more sensitive to atrazine, hexazinone, and metribuzine compared to fish. Therefore, signal crayfish can serve as an effective bio-indicator for environmental contamination by these triazines. Nwani *et a.,*(2014) worked on "Induction of micronuclei and nuclear lesions in *Channa punctatus*following exposure to carbosulfan, glyphosate and atrazine". Baig *et a.,*(2014) retrospected the significance of specific nanomaterials and mineral nanoparticles, such as clays and ultra-fine mineral colloids, has been recognized for many years. Mineral nanoparticles exhibit distinct behaviors compared to their larger micro- and macroscopic counterparts of the same mineral. These differences in chemical properties are primarily attributed to variations in surface and near-surface atomic structures, crystal morphology, and surface topography, all of which are influenced by particle size at the nanoscale level. Hostovsky *et al.,* (2014) done monitoring on "The effect of the exposure of fish to triazine herbicides". The comprehensive impact of triazine herbicide exposure on fish physiology has been assessed through various parameters in numerous studies. Hematological and biochemical analyses of blood offer valuable insights into the internal environment, overall physiology, and health condition of fish exposed to triazines. Koutnik *et al.,* (2015) reviewed on "The effects of selected triazines on fish". Human-induced pollution represents a global issue of increasing concern. The rise in environmental contamination can be linked to various factors related to industrial and agricultural advancements. Triazine herbicides are one of the most widely utilized pesticides globally and belong to a predominant category of herbicides. Kumari (2016) observed Hematological parameters and Histopathological responses and recovery ability of common carp after acute vulnerability to photosynthesis and biochemical examination. Hematological parameters, such as red and white blood cell counts and hemoglobin levels, serve as essential clinical markers for assessing health and diagnosing diseases. These parameters are tightly regulated in healthy organisms and are influenced by genetic factors. Kumar *et a.*, (2017) studied variations in total protein levels in the liver and kidney of freshwater fish, *Channa punctatus*(Bloch), following exposure to Carbaryl. The concentration of pesticides in aquatic ecosystems is steadily rising, reaching levels that lead to bioconcentration and bioaccumulation in fish tissues and organs, posing significant health risks to humans upon consumption. The impact of Folidol at sublethal concentrations of 20 ppm was examined on the kidney histopathology of adult specimens of *Channa punctatus*(Bloch). Toxicological Impact of Tricholorfon on Haematological and Biochemical Parameters in Thermally Stressed Cyprinus carpio L. was the subject of research by Woo *et al.,* (2018). Commonly used in aquaculture procedures is triclosan, an organophosphate insecticide with a moderate toxicity level. Acute doses of trichlorfon (0, 0.5, 1.0, 2.0, and 4.0 mg L^{-1}) were administered to fish at temperatures of 25 °C for 1 week and 15 °C for 2 weeks, respectively. To assess its effects, numerous parameters were examined. After being exposed, the quantities of red blood cells (RBCs), haemoglobin (Hb), haematocrit (Ht), and plasma proteins were found to be significantly lower. In contrast, groups treated with trichlorfon showed significantly higher levels of glucose, calcium, mean cell haemoglobin (MCH), and corpuscular volume (MCV). Furthermore, there was a notable increase in the plasma levels of GOT, GPT, and ALP (glutamine-oxaloacetate transaminase). The results also showed that toxic stress and the progressive increase of HSP70 and CYP1A expression were related. At 15 °C, the values of Ht, MCV, and MCH, among other biochemical parameters, were lower than at 25 °C, suggesting less physical activity. Various quantities of atrazine (0.3 µg/l - environmentally appropriate concentration; 300, 1000, and 3000 µg/l) were subjected to common carp (Cyprinus carpio L.) for twelve weeks in a thorough evaluation of their health condition by Blahova *et al.,* (2020). Behavioural changes, biometric traits, oxidative stress indicators, haematological and biochemical indices, and histopathological modifications were among the biomarkers used to assess the effects of atrazine exposure. The modelling of synergistic interactions in toxicant mixtures was reviewed by Liess *et al.*(2020), who looked back at the topic and showed how certain toxicant combinations create effects that are much stronger than what the effect models anticipate. The harmful effects of atrazine on genotoxicity, haemodynamic parameters, and metabolic profiles were studied by Akhtar *et al.,* (2021) in snow trout (Schizothorax plagiostomus). The results demonstrated that the treated groups exhibited markedly reduced levels of haematocrit, haemoglobin, white blood cell, red blood cell, monocyte, and lymphocyte counts ($P < 0.05$), in contrast to the dramatically higher levels of haemoglobin C and platelet counts. The histopathological alterations in freshwater fish were investigated by Ismayil and Joseph (2021). The gills, liver, and kidneys of cats treated to imidacloprid at levels below the lethal threshold showed structural changes. Acute exposure to bisphenol A (BPA) was studied by Sharma and Chadha (2021) to determine its harmful effects on the blood cells of the freshwater fish Channa punctatus. BPA poses serious health dangers to humans and animals alike, and it's employed extensively in industrial items. Researchers used cytological, biochemical, and haematological measures to determine its toxicity. The metabolic disturbances in Daphnia magna produced by per- and polyfluoroalkyl substances (PFAS) were compared by Labine *et al.*(2022) to those that were not fatal. The persistence, prevalence, and toxicity of perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) and other PFAS, as well as other legacy pollutants, are causes for worry in aquatic environments. The sub-lethal effects of diazepam and irbesartan in glass eels (Anguilla Anguilla) were investigated by Mora *et al.* (2023) using metabolomics. Glass eels are a threatened species, and our study highlighted how their migration through urbanised estuaries exposes them to pollution all the time. After exposing freshwater food fish *Channa punctatus*to fungicides, Kumar *et al,.* (2024) examined histological changes, oxidative stress, and genotoxicity. A significant factor that contributes to the poisoning of aquatic ecosystems is the overuse of pesticides in agricultural areas.

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