DISCOVERY

To Cite:
Mondal N, Ghosh A. Effects of Nitrogen-based fertilizers on fish health: Evaluation of stress factors and immune response. *Discovery* 2023; 59: e109d1353
doi: https://doi.org/10.54905/disssi.v59i333.e109d1353

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Peer-Review History
Received: 13 June 2023
Reviewed & Revised: 17/June/2023 to 29/August/2023
Accepted: 04 September 2023
Published: 06 September 2023

Peer-Review Model
External peer-review was done through double-blind method.

Discovery
pISSN 2278–5469; eISSN 2278–5450

Effects of Nitrogen-based fertilizers on fish health: Evaluation of stress factors and immune response

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ABSTRACT

Increasing demand for food induces the human population to use excessive fertilizers in agricultural practices. A large quantity of Nitrogen fertilizer is used in agricultural fields compared with phosphate and potassium fertilizers, and unabsorbed excess accumulates in water bodies. Nitrogen-based fertilizers are degraded into ammonia and ammonium ions, which are toxic for fish. The gills and skin of fish are the primary sites of toxic exposure, and through gills, ammonia enters into the fish's bloodstream and reaches different organs and tissues. Ammonia exposure to fish has evidence to damage the fish's red blood cells as well as blood stem cells or hematopoietic stem cells in the liver and head-kidney, which are the major fish organs and showed tissue-specific toxicity. Unionized forms of ammonia can efficiently cross cell membranes and exert more toxicity. Ammonia exposure showed oxidative damage, i.e., lipid peroxidation, protein and DNA denaturation and stress hormone-mediated toxicity, which in turn affect the immune system. Ammonia toxicity disrupts the glutamine metabolic pathway, thereby causing cellular proliferation and death, which is associated with an inflammatory response; mediated through inflammatory cytokines like IL-1, IL-8, and TNF release in exposed fish. Present review deals with such lethal impacts of ammonia on fish health, where we are concerned as the direct consumer of fish and fish products.

Keywords: Fertilizer, Ammonia toxicity, Fish, Oxidative Stress, Immunity, Cytokines

1. INTRODUCTION

The seesaw of nature and its elements are the gospel truth. This is the anthropogenic era on earth where we are intended to meet the ever-increasing human demand for resources. According to the United Nations, the worldwide population counted 8 billion in the middle of November 2022, which was just about 2.5 billion in 1950, and it may increase up to 9.7 billion in 2050. India was declared as the most populous country in the world in April 2023. Therefore, sustainable development and food security are a challenge for the human population. Around the world, 8.9 percent of the world population faces an

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undernourishment threat, while according to the *Global Food Security Index 2022,* 16.3 percent of the Indian population is facing an undernutrition threat. Because of these exponential growths of the world population, the production of sufficient food is required. Swaminathan stated that malnutrition-related diseases are also alarming in India, while half of India's population is in undernourished condition, of which approximately 360 million people are severely malnourished. Therefore, to increase crop production, heavy use of synthetic fertilizers emerged as the primary means throughout the world.

Fertilizer used in Indian Agriculture

In India, Green Revolution is the success story of newly discovered Modern Varieties (MVs) crops in the late 1950s to mid-1960s. In the early stage of the Green Revolution, scientists produced new varieties of rice, wheat, and maize, and after the 1970s, the Green Revolution widely explored other crop plants (Evenson and Gollin, 2003). In Africa, most MV crops, when incorporated into a new region it was adopted by local farmers very quickly and successfully. Another essential element of the Green Revolution was the high-quantity synthetic fertilizer production and its use. Plants require sixteen essential macro and micronutrients to be consumed from all three spheres of the earth. The important Macronutrients are Nitrogen, Phosphorous, and Potassium (collectively called NPK), while the crucial micronutrients are chlorine, zinc, molybdenum, manganese, boron, copper, and others (Nadeem et al., 2018).

The use of fertilizers is an ancient concept, and it existed from the Neolithic age for more production of food grains induced by humans as a supplement for plant growth. Still, the era of the Green Revolution heightened this practice to a new level with synthetic fertilizers. In the era of the Green Revolution in 1966 – 1968, the consumption of fertilizers doubled in respect to the previous years in India. NPK trio are equally crucial for crop production, but the used amounts of these trio combinations differ. In the consumption table, India got the second position for N & P consumption, while the fourth position for K consumption worldwide (Prasad, 2009). Data from the Government of India, Department of Agriculture as well as Cooperation & Farmers Welfare and Consumption from the West Bengal Government, stated that, with the start of the 21st century, the production and use of fertilizers in India has widely increased.

In the financial year (FY) 2001-02, the total use of NPK was about 17359 thousand Tonnes (TT), while it was about 28122 TT in FY 2010-11, and in FY 2017-18, it was about 26591 TT, and notably a clear picture of dominancy of Nitrogenous fertilizer use is observed. Now we are seriously concerned about the fact that how the use of these fertilizers affects inland waterbodies and aquatic organisms, including fish species. As fish are an important livestock consumed by a large population of India, fish health is a paramount concern there.

2. NITROGEN FERTILIZER IN AQUATIC ECOSYSTEM

Fertilizers are broadly classified into two groups according to their sources i.e.: organic and synthetic fertilizers. Organic fertilizers are extensively collected or prepared from organic matter such as worm casting, compost, blood meal, etc. in contrast, synthetic fertilizers are produced in industries, primarily as by-products of petroleum industries and from inorganic substances. From the data published by FAI (2022) entitled "*Annual review of fertilizer production and consumption 2021-22*", India's recent fertilizer use trend is being observed. In the year 2021-22, Nitrogen fertilizer, Phosphate fertilizer, and potassium fertilizer were used as 19.44 million MT, 7.83 million MT, and 2.53 million MT, respectively.

The previous and most recent data on fertilizer use in India suggested that Nitrogen-containing fertilizers are excessively used rather than phosphate and potassium-containing fertilizers. Thus, the pollution regarding nitrogenous fertilizers is also higher than the other fertilizers comparatively. Intemperately used nitrogen fertilizer accumulated in nearby water bodies through the conversion to ammonium carbamate, and ammonium carbonate, where they exist as ammonium ion and ammonia. A pH-dependent conversion of ammonia to ammonium ion and vice versa is a common chemical feature Maiti, (2003), and water-logged soil is more prone to this conversion.

The total count of ionic and non-ionic ammonia is collectively called Total Ammonia Nitrogen (TAN). This pH-dependent conversion of NH3 and NH4+ was mathematically formulated by Emerson et al., (1975), and further adopted by Das et al., (2004) and rewritten as a formula, where total TAN is calculated by using NH3, temperature, and pH, which are essential to estimate the presence of nitrogen fertilizer derivatives in an aquatic ecosystem. The overburden of nitrogenous products in nature leads to health hazards and acts as environmental pollutants. In the human body, ammonia accumulates from environmental inhalation, ingestion, or food sources and may be produced through protein catabolism (Griffin and Bradshaw, 2019). In fish, ammonia enters through the gills from the ambient sources, and for the ammonotelic characteristics of fish, they excrete ammonia.

3. AMMONIA EXPOSURE AND FISH HEALTH

Fishes are mostly ammonotelic, which means that they excrete ammonia as a nitrogen waste product (on the contrary, sharks, and sting rays are ureotelic) Ip and Chew, (2010), and they may convert the ammonia into urea or glutamine (Figure 1). Thus, ammonia is the primary nitrogenous waste in fish, and it is highly toxic to fish. In the external environment of fishes, the presence of ammonia-N and its higher concentration in water is detrimental to fish health. Decomposition of organic matter, industrial waste, domestic waste, and, most importantly, agricultural runoff are significant sources of ammonia in different water bodies. In addition, in semi-intensive or intensive fish culture in a pond or closed water system, use of various synthetic chemicals and supplements are used to enhance fish growth, and act as sources of ammonia pollution in water at excess levels (Das et al., 2004; Kim et al., 2020). Ammonia toxicity is at its peak in aquaculture systems because of leftover food particles with high protein content, organic wastes, and, most importantly, their excreta.

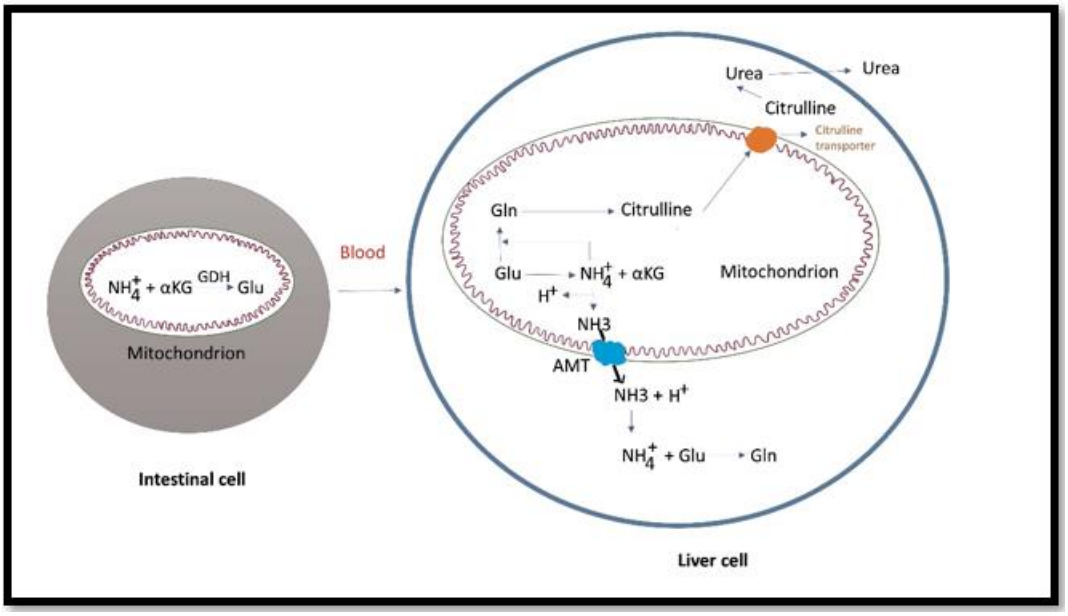

Figure 1 Ammonia detoxification in fish liver is conducted through to different catabolic pathway; one is glutamine production and another one is through urea production. In intestinal cells ammonium ion in react with α-keto glutarate and produced Glu. Through the blood circulation glutamate enters the liver cell, the detoxification centre on fish body. Liver Glu is broken down in mitochondria which produce NH4+ and α-keto glutarate. NH4+ release a H+ ion and converted into NH3. NH3 is transported through the Aquaporin 8 into the liver cytosol. Again, NH3 binds with H+ ion, which bind with Glu, to produce Gln; Gln is a non-toxic nitrogenous product. Gln also converted into urea, to release outside the fish body. (*Adapted from* Ip YK and Chew SF, (2010). Ammonia production, excretion, toxicity, and defense in fish: a review. *Frontiers in Physiology*, 1, 134).

According to Harper and Wolf, (2009), Fish health is widely dependent on their immediate surrounding water quality as well as the components present in water. Stress response in fish is commonly found in the skin, gills, liver, and urinogenital tract. Skin and gills are the primary contact site with the water and are exposed to ammonia, at the same time, the liver is the detoxification center for toxic molecules such as ammonia, and the urinogenital tract is the final sieve where the ammonia interacts and accumulation of higher ammonia in these tissues and body parts is harmful to fishes (Weiner et al., 2015). They count a set of stressors that have dominant impacts on fish morphology and physiology. Capture, recapture, handling, transport, temperature condition and level of oxygen are some major physical stressors for fish, at the same time, contaminants such as agricultural runoff, pharmaceutical effluents, and animal waste are chemical stressors where the presence of ammonia-N chemicals is inevitable.

As an impact response, the stress hormones in fishes act as a double-edged sword because it induces the production of energy with an increased metabolic rate assuming that fishes are facing any alarming condition. Due to ammonia exposure, oxygen deficiency in fish tissues is observed, which is a cause of reduced oxygen-carrying capacity of RBC (red blood corpuscle). In addition, higher exposure to ammonia in *Takifugu rubripes* showed an anemic condition (Xu et al., 2021). RBC production sites are destroyed by ammonia, so it declines the hematopoiesis process and reduces the amount of RBC in blood. On the other hand, NH4+

may directly attack RBC leading to its destruction in higher concentration. Hematological studies suggested that erythrocytes are produced in the hematopoietic tissues of the spleen and kidney region in the teleost.

Thus, any abnormality in RBC count or shape and size has a clear indication that hematopoietic tissue in the spleen or liver may be affected. Das et al., (2004), studied *Cirrhinus mrigala* (Hamilton), with different exposure to ammonia and stated that the hypoxic condition of tissue may produce a reduced number of red blood cells with distorted shape. They found that mature erythrocytes decreased while immature erythrocytes increased in number. In addition, they commented on the enzymatic changes in ammonia-exposed fishes. Regarding controlling enzymatic activities, such activities were found to be increased by about 12.15% in the brain, 39.33% liver, 18.47% kidney, and 5.8% in the gills. Due to ammonia exposure with the decrement of the RBC, leukocyte counts also decreased, indicating lower immunity (Thangam et al., 2014). Griffin and Bradshaw, (2019), stated that increased ammonia level due to the activity of glutaminase in the human liver also causes severe damage to liver cells.

They express that higher consumption of protein leads to the accumulation of ammonia in the blood, which in turn causes liver diseases such as liver cirrhosis. Peyghan and Takamy, (2002), found that ammonia exposure in *Cyprinus carpio* may have significant changes in the Alkaline phosphatase (ALP) enzyme between control and ammonia-exposed fishes. There is no significant difference between control and ammonia-exposed fishes for other serum enzymes like lactate dehydrogenase (LDH), aspartate transaminase (AST), and alanine transaminase (ALT). In addition, they found that the urea level among ammonia-exposed fishes is also higher. It was inferred that the increase of ALP level in blood is due to the damage caused in the RBC and liver due to ammonia exposure, in contrast, other enzymes are less dependent on liver function, and show less disturbance in their activities, hence showing no significant changes.

Toxicity from Nitrogenous derivatives and Oxidative Stress

Fish gills are one of the primary organs next to skin, that is exposed to the outer environment. Waterbody containing the pollutant changes in salinity, turbidity, and temperature have shown a wide impact on the gill filament (Figure 2). Thus, it may be considered that exposure to higher ammonia concentration may damage the gill tissue earliest than other organs. Cardoso et al., (1996), conducted experiments and documented the morphological changes in gill tissue. Significant changes are found between control and exposed fishes in primary filament, secondary lamella, and blood capillaries. Due to un-ionized ammonia exposure with the gill tissue damage, greater mucous secretion and mucous pavement cell hypertrophy was also found.

The study of cell plasma membrane structure found that evagination and depression in the plasma membrane of primary filament cells and secondary lamella cells occur under ammonia toxicity. Higher mucous secretion in gill tissue suggests the stress on the tissue caused by water flow and the variation in water condition as an example of an increased ammonia level (Perry and Laurent, 1989). Some experiments suggested a correlation between oxygen supersaturation and damage caused by ammonia in fish gill tissue. The increased saturation level of oxygen in water may reduce the level of gill tissue damage caused by ammonia exposure in some fish (Foss et al., 2003). A contrasting view of this phenomenon also exists, that oxygen supersaturation does not impact ammonia-exposed fish.

Japanese flounder, *Paralichthys olivaceus* is used by Dong et al., (2013), as an experimental model, and they postulated that this fish species has a significant impact of the hypoxic conditions on ammonia exposure-related damages. Also, the pH of water, CO2 level, temperature, and salinity of the water have a considerable impact on ammonia exposure. According to the previous author, hyperoxic conditions inhibit the ammonia-induced damage in fish gills due to the decreased pH in the gill arteries. A high amount of oxygen content in the water medium creates a higher partial pressure of CO2 in the gill arteries, an acid-base conversion occurs, and the pH of the blood is reduced. In turn, reduced pH in the blood may convert the un-ionized NH3 into ionized NH4+, which is less toxic than the NH3. Therefore, nitrogen, especially ammonia toxicity, is strongly associated with the oxygen environment in aquatic habitats.

Oxidative stress is a continuous process in biological systems; it is the imbalance of neutralization of Reactive Oxygen Species (ROS) by antioxidants. Oxygen is an essential requirement by living cells for the breakdown of food particles and the production of energy, which in turn, produce destructive consequences on cellular biomolecules (Chowdhury and Saikia, 2020). By accepting an electron, oxygen molecules have been converted into highly toxic and harmful oxygen radicals (O2-), despite this, due to environmental changes or chemical pollutants, other reactive oxygen molecules are produced, i.e. H2O2, .OH, NO., etc.

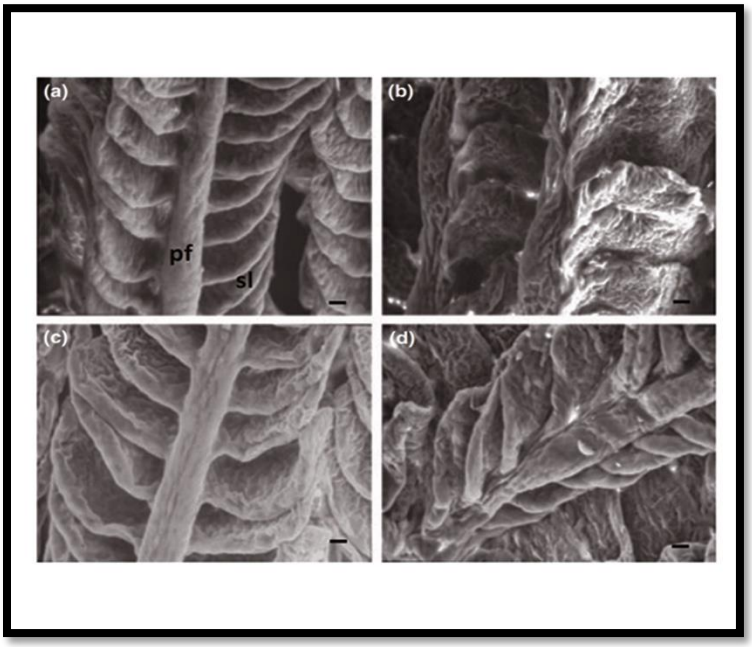

Figure 2 Primary filament (pf) and secondary lamella structure of Japanese flounder under Scanning electron microscope. (a) Absence of ammonia in normal oxygen condition, (b) Higher ammonia exposure in normal oxygen condition, (c) No ammonia exposure in supersaturated oxygen condition, (d) Higher ammonia exposure in supersaturated oxygen condition. [*Adapted from* Dong X, Zhang X, Qin J, Zong S, (2013). Acute ammonia toxicity and gill morphological changes of Japanese flounder *Paralichthys olivaceus* in normal versus supersaturated oxygen. (*Aquaculture Research*, 44(11), 1752-1759).

Sources of oxidative stress are broadly categorized into two classes; the first one is chemo-toxic sources, and the second one is environmental facts. For example, agricultural runoff, domestic sewage, industrial effluent, etc., are the chemo-toxic sources, and pH, temperature, and salinity are categorized as ecological facts. One of the main constituents of agricultural runoff is urea and used as fertilizer which may cause such oxidative stress. Oxidative stress exposure has inevitable effects on metabolic and physiological changes in fish. Oxidative stress induces the apoptotic pathway, with the induction of p53 gene expression and caspase-3 activity. However, no such evidence exists about the carcinogenic activity of ammonia; but it induces carcinogenic cell growth (Fung et al., 2013).

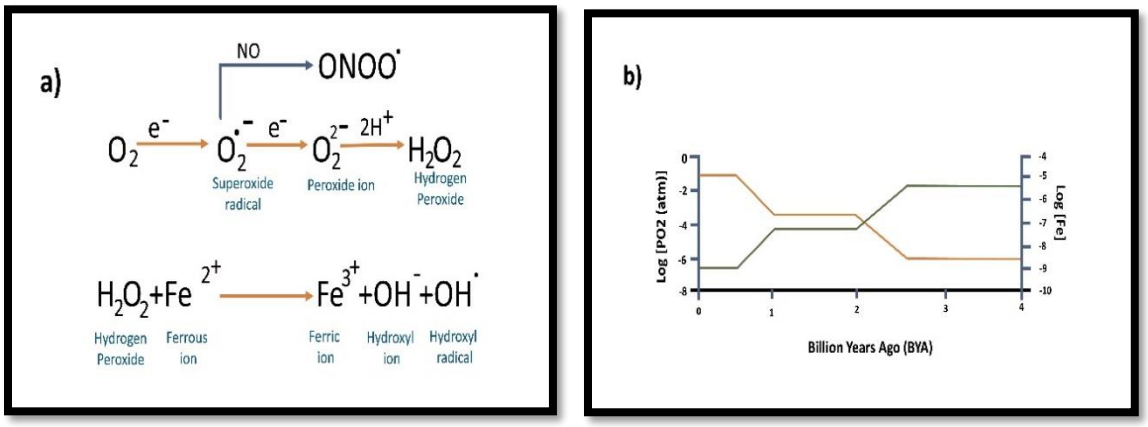

Figure 3 (A) Different forms of oxygen elements and its radical forms; the pathway of radical formation due to presence of iron. (B) Oxygen/ROS and soluble level changes with the time. (*Adapted from* Mittler R, (2017). ROS are good. *Trends in Plant Science*, *22*(1), 11-19).

Before the categorization of eubacteria from archaea, ROS production might have been started in the environment; thus, the antioxidant enzyme Super Oxide Dismutase (SOD) is found in all living metazoans. Figure 3A shows the production of ROS in the primitive oxygen-producing environment; in addition, studies suggested that in the primordial ocean level of soluble iron was

higher. Thus, the oxygen may transform into other oxygen radicals, i.e., hydroxyl radical, while Figure 3B demonstrates the changes of soluble iron and oxygen/ROS in the ocean with time. These suggested that an environmental pressure of ROS elements was present on cells from the beginning of proper cell formation.

As a result, the antioxidants are found in all eukaryotic living organisms (Mittler, 2017). The author mentioned that a balanced production and elimination of ROS are required for proper cell development because ROS have a different important function in cell signaling pathways such as ferroptosis. Higher cell proliferation requires ROS signaling, for example, stem cell and cancer cell proliferation. Component of innate immunity such as IL-1β, TNF α production requires a certain amount of ROS. Ammonia toxicity is a major cause of oxidative stress, and oxidative stress may happen in two different ways; one is through the production of ROS elements and another is the alteration of antioxidant production such as superoxide dismutase (SOD), glutathione (GSH), glutathione s-transferase (GST) etc.

It has been previously reported that total antioxidant capacity (TAC) may reduce due to ammonia toxicity. Rather than TAC, ammonia exposure can also induce glucose, triglycerides, malondialdehyde (MDA), catalase, and, most importantly for stress hormone cortisol level. Li and Qi, (2019) performed this experiment to understand ammonia exposure and its impact on GSH redox system. They stated that GSH, GSH-peroxidase, GST and GSH reductase (GR) plays an important role in redox homeostasis. The author said from their experiment that, ammonia exposure had not significantly increased H2O2 in the liver but increased dramatically in gills, in contrast, MDA level was increased in both liver and gills with increasing ammonia concentration. The level of GSH was found to increase within 24 h of ammonia exposure, while its level started to decrease after 48 h (Li and Qi, 2019).

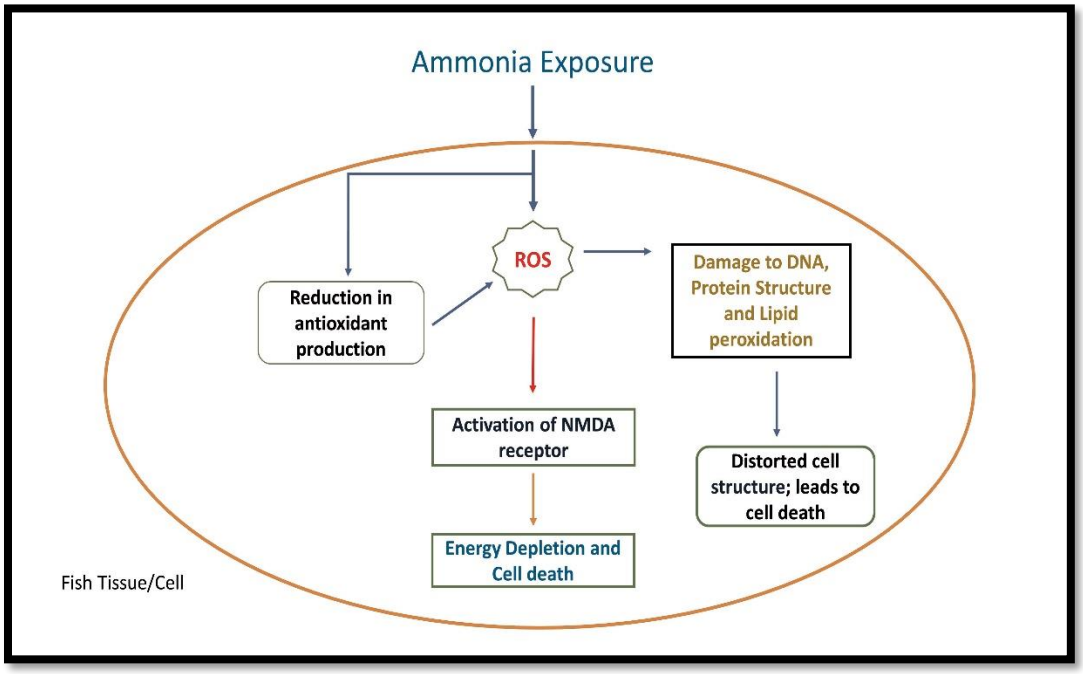

Figure 4 Induction of Oxidative stress in fish tissue due to the high ammonia exposure and cell death. Ammonia induces the production of different ROS elements and reduces the production of antioxidants which also initiate ROS accumulation in the cell. ROS elements interact with DNA, Protein and Lipid molecules, inducing their breakdown, which leads to distorted cell, and, ultimately, cell death occurs. NMDA receptor is activated by ROS elements, which leads to Na+/K+ - ATPase-dependent energy depletion and cell death initiated.

Excess ROS production indicates oxidative stress, which leads to DNA, protein and lipid structural damage (Figure 4) (Chowdhury and Saikia, 2020; Xu et al., 2021). In their view, exercise influences the pathway of ROS production, which in turn induce the antioxidant production adaptivity in human muscle cells. Exercise plays a vital role in fish ammonia toxicity levels as swimming and hyper movement increase the level of ammonia in the fish body due to the catabolism process, in contrast, the higher entrance of environmental ammonia in the fish tissue decreases the swimming performance (Randall and Tsui, 2002). Oxidative damage causes disease conditions like gill hyperplasia, in which water flow is restricted over the gill filaments.

Due to oxidative stress, oxidized protein and lipids are produced and accumulated in the cell, which results in metabolic disorders. Also, mammalian central nervous system (CNS) damage is known due to ammonia accumulation in neurons (Hegazi et

al., 2010). Evidence of neuro-glial damage mediated through ammonia toxicity is not yet well defined and needs further attention to elucidate the effect in brain tissue. In normal conditions, the neuronal cell produces ammonia which exists as an ammonium ion and is neutralized by conjugation with glutamate (Glu) to produce glutamine (Gln) by the catalyzing activity of glutamine synthetase. Gln is responsible for the hyperactivation of the N-methyl-D-aspartate (NMDA) receptor on the surface of neuronal cells, which in turn initiates NO synthesis and Na+/K+-ATPase. Due to NO accumulation toxicity level in cells is increased and Na+/K+-ATPase induces energy depletion, leading to cell death (Xu et al., 2021). This mechanism is evident in mammals, but a similar mechanism in fish is yet to be determined.

Changes in the concentration of AST, ALT, glutamate, glutamine, glutamine synthase lactate and GABA are immensely observed in ammonia-exposed fish brain tissue (Ip et al., 2005; Hegazi et al., 2010). An age-dependent fish mortality due to ammonia exposure was also tested by Gomułka et al., (2014), and the result showed that 1st to 20th-day-aged *Leuciscus idus* had an increase of LC50 value of ammonia exposure, while in 30th-day-aged fish, the LC50 value dramatically decreased. Armstrong et al., (2012), found that there was an impact of ammonia toxicity on fish reproduction success. Egg production in fathead minnow was found to be affected by ammonia exposure, and they suggested that water quality criteria must be revised depending on their findings.

Nitrogenous Toxicity, Stress, Immunity and More

Previous studies showed that ammonia exposure may induce the inflammatory cytokines; Tumor Necrosis Factor (TNF), Interleukin 1 (IL1), and Interleukin 8 (IL8) in fish. Expression of apoptotic genes like Bax, Caspase 3, Caspase 9, and tumor suppressor gene p53 is significantly higher in the ammonia-exposed group than the other. Cortisol is a major stress hormone in fish and is produced and secreted from inter-renal cells of the head kidney, involving the hypothalamo-pituitary-adrenal-interrenal axis (HPA-I). An experiment conducted by Zeitoun et al., (2016), suggested that exposure to ammonia induces the HPA-I axis and a massive increase in cortisol levels is observed in the serum.

Additionally, they mention that blood glucose levels also increased and the growth of the fish declined due to ammonia exposure. Cortisol is the steroid hormone and lipid soluble, so it can easily cross the plasma membrane to enter into a cell, and the production of cortisol is mediated by a hormonal cascade preliminary started in the hypothalamus. Rather than cortisol, Corticotropin Releasing Hormone (CRH), Adrenocorticotropin Hormone (ACTH), and Cortisone are the major stress hormone and environmental factors widely induce cortisol production (Ellis et al., 2012). Aluru and Vijayan, (2009), stated the role of cortisol in the cellular metabolic pathways. Cortisol binds with glucocorticoid receptors (GR) or mineralocorticoid receptors (MR) to activate in fish. Cortisol with GR and accessory protein enters into the nucleus and acts as a modulator to induce the gene expression of a protein involved in the metabolism, immune functions and reproduction process.

They also added that cortisol stimulation on macrophage cells shows an immune response. Cortisol is able to act on each organ of the fish body due to the presence of its receptor in most of the cell types. In fish, it was reported that cortisol induces the apoptosis of proinflammatory T cells while suppressing the antibody production from B cells. Cortisol exerts its activity widely on metabolic pathways; it helps to produce more energy through the glycolysis and gluconeogenesis process, which is required to alert the organisms in stressful condition. Secretion of cortisol is stimulated by several factors, i.e., stress, heat shock, or environmental toxicant. In acute toxicity conditions, cortisol level is a good indicator of toxicity while poorly reflecting the chronic stress response (Aluru and Vijayan, 2009, Ellis et al., 2012).

Ambient high levels of ammonia induce toxicity in fish and primarily hamper fish excretion, and fish may follow five different ways to detoxify ammonia toxicity. They may increase ammonia excretion, urea synthesis, reduced protein degradation, partial catabolism of amino acid, and glutamine synthesis. Ambient ammonia toxicity adversely impacts fish by producing proinflammatory cytokines i.e., TNF α, IL1b, etc. (Yousefi et al., 2021). It is measured that TNF α, IL 1, and IL 8 gene expression in ammonia-exposed fish is significantly higher than the control fish (Li et al., 2020). They also observed that ammonia-exposed fish efficiently express the tumor suppressor gene p53, helping to express the pro-apoptotic gene Bax and reduce the upregulation of anti-apoptotic gene Bcl 2.

Their experiment also found that ammonia exposure upregulates the cytochrome c, caspase 9, and caspase 3 genes, indicating that caspase-dependent apoptosis may occur due to ammonia exposure. Yu et al., (2020), noted that complement 3 (C3) is significantly increased in the gill, spleen, and brain tissue in comparison with the control fish group, while C4 is only increased in the gill and spleen still, in the brain no significant increment is observed. A similar trend as C3 is shown by the Nitric Oxide synthase (NOS) and Immunoglobulin M (IgM) at the time of the experiment, at the same time, the level of NOS and IgM decreased after the ammonia exposure. This reduction after the increment of NOS, C4, and IgM is due to the reduced exposure in the body to

toxicants, while the level of C3 did not decrease because environmental stress induces its secretion in large amounts to maintain fish health.

They also mentioned hydrolytic enzymes such as lysozyme and AKP levels are also high after exposure to ammonia. Ammonia exposure reduces antioxidant levels in different tissue, and MDA, as the end product of lipid peroxidation, cause adverse effects on cells. Macrophage cells are well known for secreting cytokines, an essential element of inflammation due to exposure to toxicant molecules or other stressors like pressure, temperature, pH, etc. He et al., (2021), estimated pro-inflammatory cytokine, i.e., IL 1β, IL 6, and TNF α levels concerning ammonia and curcumin, and their study suggested that ammonia exposure increases this cytokine level while curcumin reduces its expression in a fish model. They also studied the COX 2 gene expression at higher ammonia level, and its elevated level suggested ammonia exposure have an impact on its secretion, and in hyperammonemia rat brain concentration of COX 2 gene expression is elevated.

In addition, Zarantoniello et al., (2021), supported and mentioned that *hsp* expression varies with the different environmental conditions, which include variations in osmotic pressure, temperature, salinity etc. Ching et al., (2009), mention that ammonia exposure results in almost a 3-fold increment in the SOD activity in fish brains. A contrasting factor is that in some fishes like, mudskipper phosphorylated p38 and p53 are unchanged in the brain, which inhibits ammonia-induced cell swelling, whereas there is no induction of NMDA receptors in fish, like mammals (Ip et al., 2005). There is very tiny evidence present about ammonia toxicity-related complications in fish brains.

The previously reviewed article by Xu et al., (2021), stated that the mammalian brain produces glutamine from glutamate in the presence of ammonia, which is not similar in fishes, it is also supported by Ip et al., (2005), that fish brain response against ammonia toxicity is different from mammalian brain, which needs to be explored. Studies are ensuring that some fishes can produce glutamate to glutamine in the presence of ammonium ions (Saha et al., 2002; Wee et al., 2007; Hegazi et al., 2010). They observed that free amino acids (FAA) such as Glutamine (Gln), Aspartate (Asp), and Alanine (Ala) level is increased in ammonia-exposed fish brain. Accumulation of FAA in the brain may be due to the high ammonium ion, which participated in amino acid synthesis in the presence of α-ketoglutamate.

4. CONCLUSION

It can be concluded that ammonia has an overall adverse impact on fish health. From the different sources of ammonia i.e., agricultural runoff, industrial effluent, and household sources, including excreta and non-feeding food residue, etc. which are finally accumulated in water bodies and show impact on fish. Ammonia shows differential detrimental effects on different fish organs. Most of the previous studies focused on the liver, gills, and kidney, where enzymatic activities were the central study area. Specifically, reporting on fish enzymes related to brain or neurotrophic factors is nearly unavailable, while cellular damage or changes are yet to be studied in detail.

Similarly, immunity is another key consideration for each vertebrate, as in fish also, while the process of involvement of the organs vital for fish immunity and their function under ammonia exposure is still unexplored. Hence, the mechanism of response of the neuroendocrine-immune axis in fish under ammonia-N derivative toxicity is still to be elucidated in detail. As consumers, humans are consuming the ammonia exposed fish, and the nitrogenous or ammonia derivatives are transported through the food chain in humans. Therefore, the fish health and human welfare study on ammonia exposure to fish and the resultant neuroendocrine-immune axis response will not only provide us with information regarding fish physiology but, in turn, support to device proper mechanisms and management strategies to maintain human health as the consumer of fish and fish products.

Acknowledgement

Authors are grateful to the institutional authorities for their support to pursue the study and the investigations in the mentioned area are continuing in the institutes.

Author Contributions

NM prepared the primary drafting with the guidance of AG and the manuscript was edited and finalized by AG.

Informed consent

Not applicable.

Ethical approval

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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