Fish immunology: Understanding the Interaction Between Pollutants and the Immune System

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SUMMARY

The aquatic environment is represented as a reservoir for numerous toxicants, posing a substantial hazard to aquatic organisms. Among various ranges of pollutants, such as endocrine disrupters, pesticides, organic pollutants, and heavy metals have been exposed to have immunotoxic effects. Furthermore, alterations in the functioning of immune system cells, antibody production and cytokine signaling have been observed that elucidate the pathways that show the compromised immune defenses of fish because of their exposure to these contaminants. Further research revealed that the presence of molecular and cellular biomarkers indicates the long-term consequences of toxicity in fish immune system by the activation of respiratory burst activity and phagocytosis. In vitro and in vivo studies have promoted advanced techniques to comprehend the certainty between toxicants and aquatic organisms. Additionally, bioremediation techniques have been implemented to mitigate pollutant exposure and improve fish immune resilience. This chapter delves into a deeper understanding of the complex interactions between environmental contaminants and fish immune system and undergoes sustainable management practices with future directions aimed at safeguarding the aquatic system.

INTRODUCTION

The immune system is a collection of humoral and cellular components that protect the body from foreign substances including bacteria, toxins, or cancerous cells. It is activated by various sorts of endogenous or exogenous stimuli. Fish immune systems are classified as innate and adaptive (memory) systems, which are further subdivided into humoral factors (soluble substances) and cell-mediated defense. However, in order to eliminate foreign invaders or activate defense mechanisms these two systems work in coordination (Magnadottir et al., 2011).

Fish lack lymph nodes and bone marrow; hence their immune system is categorized as lymphoid. In contrast to mammals which have a myeloid classification for their tissues and organs. Furthermore, during larval development, thymus, spleen, kidneys (largest lymphoid organ) and gut associated lymphoid tissues (GALT) compose the lymphoid organs (Lange et al., 2004). According to Rombout et al. (1984), morphological dissimilarities have been observed among approximately 24,000 known species of fish.

Pakistan's drinking water supplies are becoming more and more contaminated, with potentially disastrous consequences for both the environment and public health. Thus, technical regulations are based on standards. Therefore, well recognized global guidelines/standards for water quality as designed by the World Health Organization are well-known to counteract this problem. For this purpose, WHO set optimum limits for Zn, Cu, and Mn in drinking water as 3 mg/L, 2 mg/L, and 0.5 mg/L respectively, Moreover, WHO recommended safe values for iron, cadmium, nickel, lead, and arsenic should not be exceeded above 0.3 mg/L, 0.003 mg/L. 0.02 mg/L, 0.01 mg/L and 10 µg/L respectively (WHO, 2017).

FISH IMMUNOLOGY

Components of the fish immune system

The vertebrate immune system consists of two segments: the innate immune system and the adaptive immune system (Romo et al., 2016). Therefore, acquired immunity functions through antigen-specific receptors whereas, innate immunity functions by recognizing pathogen-associated molecular patterns of harmful microbes.

Innate immunity

The main defense system in fish is innate immunity which responds to infections quickly but does not offer persistent protection (Turvey & Broide, 2010). Fish innate immune system components can be broadly classified into three categories: humoral, cellular, and physical/surface variables (Uribe et al., 2011). Humoral immune cells include a wide range of non-specific defensive chemicals, such as complement components, cytokines, natural antibodies, antimicrobial peptides, transferrin, lectins, and lysozyme

How to Cite: Batool M, QU Ain & S Imran, 2024. Fish immunology: Understanding the interaction between pollutants and the immune system. In: Zoology: Advancements and Research Trends (Ijaz MU, N Ehsan, M Imran & S Yousaf, eds): FahumSci, Lahore, Pakistan, pp: 206-214. ISBN: 978-627-7745-02-8. https://doi.org/10.61748/Zool.2024/26

specified for inhibition or degradation of microorganisms. In contrast to this, physical or surface elements that play a vital role against infection resistance are the mucus layer, scales, and the epithelial cells that line the alimentary canal, gills, skin, and cellular constituents of the immune system in fish (teleosts) comprise natural killer cells, thrombocytes, macrophages, monocytes, dendrite cells, granular leucocytes, lymphocytes, eosinophilic granule cells, and mast cells. Additionally, fish have Melano-macrophage centers and rodlet cells (Garcia-Fernandez et al., 2011).

Adaptive Immunity

Around 450–500 million years ago, the first jawed fish (cartilaginous and teleost fish) appeared with major specific components of the adaptive immune system, such as recombination activating gene (RAG), T cell receptors (TCRs), immunoglobulins (Is), and major histocompatibility complex (MHC) (Brazeau & Friedman, 2015). Similar to mammals, teleost fish possess two primary components of adaptive immunity: humoral and cellular responses. Thus, T cells are important for cellular adaptive immunity; they develop in the thymus and become effector cells like helper T (Th) or cytotoxic T lymphocytes (CTLs); on the other hand, B cells, which are necessary for humoral adaptive immunity, come from bone marrow that is produced inside an anterior kidney of teleost fish and eventually become plasma cells for production of antibodies. Moreover, antibodies can exist in two distinct forms: immunoglobulins (Is), which are soluble, and B cell receptors (BCR), which are membrane-bound (Smith et al., 2019).

KEY PLAYERS IN FISH IMMUNE RESPONSES

Leukocytes

Fish immune cells comprise Macrophages, Neutrophils, Basophils, Thrombocytes, Dendrite cells, and Antigen processing cells. Macrophages have a function in various immune responses due to their ability to activate lymphocytes and carry out phagocytosis. Macrophages have several receptors on their cell surface, such as complement, scavenger, TLRs, PRRs, and CLRs. Additionally, macrophages are a vital source of cytokines and chemokines that connect innate and adaptive immunity and facilitate a productive immune response (Secombes and Wang, 2012).

Neutrophils are the first granulocytes to be observed following macrophages; both are drawn to the damaged tissue by chemotactic signals. Using proteolytic enzymes, reactive oxygen species (ROS), and antimicrobial peptides, neutrophils phagocytose and eradicate microorganisms at the site of damage (Biller-Takashi & Urbinati, 2014).

Basophils are enormous polymorphonuclear granular leucocytes. Histamine, an inflammatory mediator, is found in their cytoplasmic granules. Moreover, they play their role in anaphylaxis and allergies (Duque & Descoteaux, 2014).

Thrombocytes are nucleated, agranular, and oval-shaped cells. They are able to phagocytose and therefore have coagulation functions. These cells assemble at the site of inflammation due to the presence of acid phosphatase activity C (Tavares-Dias et al., 1999).

Dendrite cells are a type of antigen-presenting cell that can trigger both innate and acquired immune responses, prevalent in several tissues. These cells help T lymphocytes by initiating the cell-mediated acquired response in order to transfer processed antigens through MHC class 2 receptors (Mokhtar et al., 2023). NK cell homologs in teleost fish are classified into two categories: NK-like cells and non-specific cytotoxic cells (NCCs). NCCs are small, agranular cells with morphology similar to monocytes, in comparison to NK cells, which are big and granular. Thus, NCCs contribute to antibacterial immunity by inducing cytokine synthesis and secretion whereas; a few studies on rainbow trout and catfish have shown that NK-like cells kill allogeneic and virus-infected cells (Jaso-Friedmann et al., 2001).

Antigen-presenting cells, such as B lymphocytes, mediate humoral immunity by producing antibodies against antigens (Firdaus & Zamri, 2016). On the other hand, T cells are essential for directing immune activity in cell-mediated adaptive immune system following the initial non-specific defenses and providing an immunological response upon APC recognition of the antigen (Lewis et al., 2014).

Antibodies

Antibodies cause neutralization, internalization, and elimination of pathogens. Due to the presence of effector cells that carry Fc receptors, they cause antibody-dependent cellular cytotoxicity, or ADCC (Forthal, 2014). Moreover, IgM, IgD, and IgZ/T are the three classes of Igs that have been found in teleost fish (Mashoof & Criscitiello, 2016). All jawed vertebrates with distinct characteristics contain the most ancient type of antibodies, known as IgM. Similar to mammals, teleost IgM has function in innate and adaptive immune responses, facilitating cellular cytotoxicity through ADCC, induced opsonization, complement activation, and pathogen lysis (Boshra et al., 2004). Additionally, IgM regulates agglutination, which leads to phagocytosis and the removal of pathogens. Furthermore, IgM was found in mucosal tissues, including fish skin and intestine, in addition to plasma (Ye et al., 2013).

Immunoglobulin D, a second class of Igs present in fish, shares a sequence with human IgD. However, teleost fish also exhibit unique IgD properties due to their several IgD constant domain forms (varying from 2 to 16) (Ramirez-Gomez et al., 2012). IgD that has been released and lacks V domain directly binds to the Fc receptor of basophils, acting as a pattern recognition receptor. It may therefore result in inflammatory reactions, such as the release of inflammatory cytokines, opsonizing agents, and chemicals that activate B cells and fight microorganisms. Immunoglobulin D is mostly expressed in the gills, spleen, anterior and posterior kidneys, and serum in teleost fish (Bengten and Wilson, 2015).

IgT/Z was initially revealed in zebrafish (IgZ) and in rainbow trout (IgT). The IgT/Z in the teleost fish's gut supports immune responses against bacteria and intestinal parasites. For instance, following a parasite intestinal infection, the number of IgT + B cells enhanced in gut of rainbow trout, but the number of IgM + B cells remained unchanged in the same tissue. Furthermore, IgT + B cells were discovered in lymphoid tissue associated with skin in teleost fish, and they produce IgT in the mucus of the skin (Xu et al., 2013).

Cytokines

Cytokines may act as immune response modulators related to innate as well as adaptive responses. Specifically, immune-regulatory genes often used in fish include those that produce pro-inflammatory cytokines including interleukin-1β, TNF, and IL-6. Thus, interleukin-1β is a crucial mediator of inflammation in response to infection. It has been revealed lately that it directly affects the function of the hypothalamic-pituitary interregnal axis and stimulates the release of cortisol in trout (Castro et al., 2011). Tumor necrosis factor alpha, another significant cytokine, has been cloned and studied in a number of fish species. Additionally, it has been shown that the activation of TNF-like proteins promotes neutrophil migration. Respiratory burst activity of macrophages and triggers apoptosis (Wang and Secombes, 2013). Numerous interleukins in the IL family are among the cytokines found in fish. Moreover, a wide variety of chemokines including CC and CXC sub families regulate immune cell movement to infection sites—have been identified in fish. According to Alejo & Tafalla (2011), two cytokine-induced enzymes; iNOS and COX, sequenced from teleost's involved in inflammatory responses and prostaglandins production respectively.

IMPACT OF POLLUTANTS ON FISH IMMUNE SYSTEM

Environmental pollution is one of the issues facing modern human society. As environmental pollution is rising daily because of the rapidly expanding industry, rising energy consumption, and reckless devastation of natural resources fish immunology is negatively impacted by a variety of organic and inorganic hazardous chemicals because of their continuous release into an aquatic ecosystem from both natural and man-made sources (Milla et al., 2011).

Types of environmental pollutants

Heavy metals

Heavy metals are not only hazardous but also have the ability to bioaccumulate in the food chain therefore contributing significantly to environmental pollution. According to Briffa et al. (2020), fish health is primarily affected by the toxicity of several heavy metals like mercury, arsenic, copper, chromium, zinc, lead, and cadmium, as they are the primary consumers of aquatic ecosystems. Fish exposed to chromium displayed a range of behavioral abnormalities, such as erratic swimming, mucus secretion, body color changes, appetite loss, etc. Moreover, *Cyprinus carpio* exposed to chromium at concentrations ranging from 2 to 200 μmol/L has shown cytotoxicity reduced mitogen-induced lymphocyte activation, and impaired phagocyte activities (Nisha et al., 2016). Cadmium discharges into the aquatic ecosystem through various natural and anthropogenic sources. Therefore, Cd soluble in water or sediment is absorbed by the aquatic flora and fauna via the food chain. Perera et al. (2015) reported that American eel fish (*Anguilla rostrata*) showed a substantial rise in leukocyte and lymphocyte counts after 2 months of exposure to 150 μg/L of cadmium.

Arsenic toxicity is enhanced by unbalanced pH, salinity temperature, organic matters, suspended solids, phosphate content, and other toxic substances. Freshwater fish are consistently exposed to low concentrations of arsenic which accumulate in their liver and kidney tissues. Thus, exposure to arsenic has been linked to changes in T-cell and B-cell functioning as well as a decreased capacity of Tilapia to phagocytose microorganisms (Min et al., 2014).

Endocrine disrupters

Endocrine disrupting chemicals (EDCs) are substances, either natural or artificial, that can interfere with or mimic endogenous hormones in order to change their function. When these chemicals bind to receptors elicit a response (an agonistic action) or (an antagonistic reaction). For instance, exposure to malathion and Diazon reduced the number of lymphocytes and erythrocytes, inhibited B-cell activity, decreased antibodies production and diminished the humoral and host cell-mediated immune response in Nile tilapia, *O. niloticus* (Giron-Perez et al., 2008). Chronic exposure to various doses of TBT (1, 10, and 100 ng/L) for 8 weeks in a zebrafish model (*Danio rerio*) resulted in decreased immunoglobulin M (IgM) activity and increased expression of IL-6, NF-κB p65, TNF-α, IL-1β, and TNF-α suggesting that TBT induces oxidative stress which adversely effects on immune system (Zhang et al., 2017).

Organic pollutants

Persistent Organic Pollutants (POPs) are lipophilic, semi-volatile, and anthropogenic compounds. POPs are often known as silent killers because of their persistence and lipophilicity, hence causing chronic illness and environmental pollution. Polycyclic aromatic hydrocarbons and polychlorinated biphenyls are examples of common organic chemical pollutants (Alharbi et al., 2018). Polycyclic aromatic hydrocarbons are formed as a result of incomplete combustion of organic materials, particularly fossil fuels, oil spills, and their post-depositional modification. According to Bado-Nilles et al. (2009), European sea bass (*Dicentrarchus labrax Linne*) exposed in vitro to various PAHs showed innate and humoral responses in the form of altered complement activity and lysozyme concentration as well.

Polychlorinated biphenyls have low vapor pressure and chemical inertness, therefore, continue to seep into neighboring rivers through dry and wet sedimentation, oil tank leaks, and garbage discharge harmful for aquatic ecosystem. Aryl hydrocarbon receptor is crucial in mediating the effects of xenobiotics on the immune system. Chemicals like dioxins, PAHs, and PCBs act as agonists of AhR and when activated, suppress production of IgM and reduces B cell proliferation in fish. (Segner et al., 2021).

Pesticides

Pesticides play a crucial role in the development of agriculture and preservation of public health since the environment is highly favorable to insect breeding. Pesticide pollution in aquatic environments is a major issue, and fish are more commonly exposed to these toxicants, and have adverse effects. Insecticides have the potential to affect the host's defensive system against infections, inducing uncontrolled cell proliferation resulting impaired immunological function (Kumar et al., 2010). Extensive use of organophosphate pesticides and its widespread use make the aquatic ecosystem more hazardous. According to Diaz & Giron (2014) *O. niloticus* exposed to 0.422 and 0.211 mg/L of organophosphate pesticide (chlorpyrifos) for 96 hours experienced a considerable reduction in both the proportion of phagocytic cells and ultimately phagocytic activity. Furthermore, Fig 1 provides an overview of the considerable effect of environmental pollutants on the immunity of fish.

Route of exposure

When fish graze on pesticide-contaminated food, such as insects treated with pesticides or swim in pesticide-contaminated streams, they are exposed to pesticides directly (Sabra & Mehana, 2015). Aquatic ecosystems are contaminated by endocrine disrupting compounds from a variety of sources, such as industrial wastes, household and municipal wastes, and forest fires. Moreover, EDCs have a tendency to bio-accumulate and bio-magnify in fish tissues through the aquatic food chain (Hamid et al., 2021). The aquatic system is harmed by the introduction of heavy metal contaminants from tanneries and smelting process effluents, home sewage, and garbage leaching (Garai et al., 2021). In addition, organic pollutants from humans, animals, and waste products from canneries and wood pulp mills are thrown, or released into gutters and drains, where they may be washed into streams and rivers by runoff (Esposito et al., 2020).

IMMUNE MODULATING EFFECTS OF POLLUTANTS

Suppression of immune responses

Immunotoxic effects may be manifested either as immunosuppression led to decreased resistance against infections and neoplastic disorders or improper immune activation associated with hypersensitivity, allergies, or autoimmune diseases (Vos, 2007). Research on fish has shown that toxicants exposure suppresses the immune system's function. Xenobiotics can inhibit the immune system in two main ways: either directly by acting on immune cells and organs, or indirectly by acting as a toxin on various organs or physiological systems thus subsequently affects the immunological response (Rehberger et al., 2017).

A host resistance or pathogen challenge test can be utilized to estimate adverse effects of a chemical on fish immune system performance. In this experiment, fish are first exposed to toxicant concentrations below the lethal threshold, and then they are exposed to an infectious disease. Therefore, it is considered that the chemical is having an immunotoxic effect on fish if the chemically exposed fish exhibit a reduced resistance to pathogen infection as compared to non-exposed control fish leading to reduced growth reproduction and ultimately survival (MacGillivray and Kollmann, 2014).

Inflammation and immune activation

The process of inflammation serves as an example to indicate the impact of hazardous chemicals on fish immunity. Basically, inflammation is an adaptive mechanism that helps to eliminate infection and restore tissue damage started by receiving Signals like PAMPs from attacking microorganisms or DAMPs generated from injured tissues and the latter one is known as sterile inflammation (Netea et al., 2017). Thus, the inflammatory process then progresses to produce pro-inflammatory mediators, activate resident and migrating leukocytes, and cause pathological tissue alterations before repairing and returning to homeostasis and become chronic leading to increased morbidity and death if proper healing mechanisms will not take place. Furthermore, without directly affecting the immune system, toxic substances can also cause harm to non-immune tissues thus subsequent generation of DAMPs, which can lead to sterile inflammatory processes for instance, liver injury due to drugs in which hepatotoxicants injure hepatic cells and activate pro-inflammatory pathways, is an illustration of sterile inflammation (Vliegenthart et al., 2014).

Disruption of leukocyte function

Phagocytosis is the process in which a cell takes up various particle targets. Steps that make the phagocytic process are as follows: (1) identifying and detecting foreign particle; (2) attachment of foreign particle to phagocyte; (3) engulfing or internalizing foreign particle into a phagosome (formation of vesicle) (4) fusion of lysosome with phagosomes in order to form phagolysosome. (5) The particle's intracellular killing and digestion and death (Flannagan et al., 2012).

Phagocytic process has been studied in fish leucocytes with bacterial cells acting as target particles. In order to initiate phagocytosis process, surface molecules coated with (PAMPs) ligands interact with receptors (scavengers or Toll like receptors) that recognize and decode these cognate ligands on the bacterial cells (Esteban et al., 2015). Thus, this decoding thereby triggers engulfment into a vesicle known as

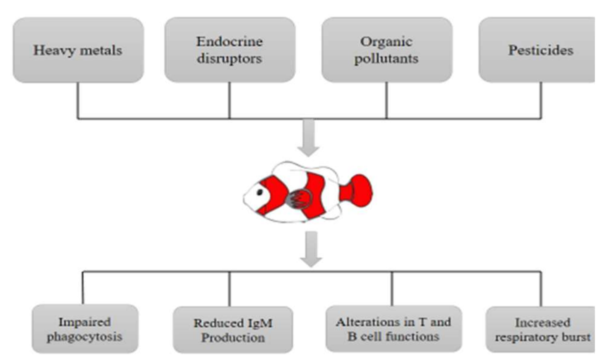

Fig 1. Effect of Environmental pollutants on fish immunity

phagosome. Following this, phagolysosomes are formed by the fusion of Lysosome with phagosome. Moreover, reactive oxygen species such as superoxide radicals, wide range of lysosomal hydrolytic enzymes and nitrogen in the form of nitric oxide intermediates kill and degrade microbes present inside these vesicles (Neumann et al., 2001).

Respiratory Burst

Oxidative burst is the process in which phagocytic cells or leukocytes consume oxygen, and it is linked to release of cytokines and an inflammatory response in fish. During this process, leukocytes increase their intracellular oxygen consumption when phagocytose, which leads to the formation of ROS (toxic but provide effective defense against microbes). Therefore, O is oxidized to O^{2-} with the help of an enzyme NADPH oxidase then along with SOD action, results in the formation of hydrogen peroxide (Biller-Takahashi et al., 2015). This H_2O_2 are able to form hypoalogens acids in biochemical reactions, which are strong oxidant compounds against microorganisms, or it can undergo the Fenton reaction (Fe^{2+} reaction with H_2O_2, Fe is released from the storage proteins by the action of O_2), which results in the formation of the hydroxyl radical (Biller-Takahashi et al., 2013).

The hydroxyl radical (HO) has the ability to react with the microorganisms' proteins and amino acids, breaking down biochemical bonds. This can lead to the inactivation of enzymes, cytolysis, and cellular destruction (Halliwell, 2009). On the other hand, HO can also react with the superoxide anion radical to produce singlet oxygen ($^{-1}O_2$), the most harmful form of oxygen. Moreover, produced O_2 can engage with other molecules, such as NO, to generate peroxynitrite, chloramines, and ultimately hypochlorous acid or hypochlorite, mediated by the enzyme myeloperoxidase, which is only found in innate immune system cells (Barreiros et al., 2006).

APPROACHES TO STUDY FISH IMMUNOTOXICOLOGY

In vivo experimental methodology

Assessing the impact of contaminants on immune system of fish, freshwater air-breathing *C. punctatus* fish were obtained from nearby sources. The specimens' average weight was 14.40 ± 1.00 g, and their average length was 10.42 ± 1.02 cm. After that, the fish were kept in semi-static settings for two weeks in a laboratory setting to acclimatize them. Subsequently, atrazine was added to the aquarium by dissolving it in filtered distilled water using Pluta's method (Pluta, 1989). Ten fish specimens were randomly subjected to 0.00 mg/L as the control and to nominal concentrations of atrazine such as 25.0, 32.0, 39.0, 46.0, 53.0, and 60.0 mg/L. The fish were subsequently subjected to three sub-lethal concentrations of the herbicide (4.238 mg/L, 5.300 mg/L, and 10.600 mg/L) for 15 days, based on the 96 h LC_{50} values. Everyone, five, seven, ten, and fifteen days, five fish were sacrificed to check the toxicity (Abdul-Farah et al., 2004).

Elevated levels of lipid peroxidation have been shown in response to atrazine exposure in *C. punctatus* liver, leading to the production of increased reactive oxygen species. In addition to this, liver has been observed as a site of greater production of free radical and numerous oxidative processes. Furthermore, increased SOD, CAT and LPO activities in liver tissue showed oxidative stress which may be an adaptive response to atrazine-induced free radical toxicity (Nwani et al., 2010).

In vitro cell culture model

In vitro fish leukocyte assay is an effective method for determining whether environmental contaminants have the potential to affect fish immunity. Fish leukocytes comprise natural killer cells, dendritic cells, neutrophils macrophages and monocytes. All immune organs of fish, such as head and trunk kidneys, spleen, thymus, lymphoid tissues in organs such as gut and blood have their immune cells isolated for in vitro research (Fierro-Castro et al., 2012).

The main procedure for separating immune cells from lymphoid tissues is mechanical disaggregation of an organ. Density centrifugation is then used to separate the leukocytes having numerous adaptive and innate immune cells (Glasser and Fiederlein, 1990). Following this, culture the cells for a night; then phagocytes will adhere to culture plate, and lymphocytes will float and can be removed. Following their separation, immune cells are either cultivated in vitro for a few hours or weeks in a suspension as monolayer, or three-dimensional aggregate (Abreu et al., 2009). Prior to the trial, establish the cytotoxic concentration range for each chemical to prevent cytotoxicity from interfering with particular immunotoxin effects. In order to do this, a concentration series of each test was applied to the cells for a few hours (Segner et al., 2012).

Nitro blue tetrazolium (NBT) assay was utilized to evaluate the effect of chemical exposure on leukocytes' respiratory burst activity. Moreover, (qRT-PCR) detects the impact of chemical exposure on the levels of cytokine mRNA transcripts (IL-1β, TNFα, and IL-10). For example, the effects of Cr (VI) exposure to varying concentrations of Cr 2+ for two to six days on *Cyprinus carpio* leukocytes from head, kidney, and blood have been observed with the presence of the pokeweed mitogen at 25°C (Corsini and Roggen, 2017). Nitric oxide synthesis, respiratory burst activity, and mitogen-stimulated cell proliferation have been shown in response to concentrations of Cr (VI) (Tort at el., 2003). Furthermore, *Sparus aurata* Leukocytes from head kidney were isolated in order to assess the exposure of Ethinylestradiol up to 48hrs, in the presence or absence of bacterial DNA (VaDNA). Phagocytosis and leukocyte viability were observed. EE2 alone has not induced Respiratory burst activity but inhibition of VaDNA-induced respiratory burst activity (Cabas et al., 2012).

BIOMARKERS OF IMMUNE SYSTEM FUNCTION

Immune reactions develop rapidly in response to invasive organisms and are composed of a network of molecules and cells that signal the presence of contaminants or pathogens within the body of the organism. Mostly cells are leucocytes whereas; molecules such as transferrin lysozyme, acute-phase proteins, complements, (PGE2), (ROI), (COX-2), cytokines, and chemokines, etc. are involved in immune reactions (Randelli et al., 2008).

Phagocyte activities have been traced because of the inducibility of iNOS gene's that are formed in response to bacterial or parasite infection and was initially seen in rainbow trout. (Kollner et al., 2001). Additionally, *Edwardsiella tarda* was the cause of infection in ginbuna crucian carp indicated that CD8+ T cells had significantly higher levels of granzyme mRNA (Matsuura et al., 2014). While TGF-κ and IL-10, two important cytokines, are essential for Treg cell differentiation because they activate the primary transcription factor, forkhead box P3 (FoxP3). The majority of teleost fish species have been found to produce IL-10 by Tregs with significantly higher amount after two to five weeks of infection (Piazzon et al., 2017). Furthermore, Rainbow trout peripheral blood leucocytes or mitogen-activated kidney cells release soluble mediators like (MAF), which caused phagocyte respiratory burst in vitro because of the exposure to PAH (Duchiron et al., 2002).

CURRENT REGULATORY FRAMEWORKS

Remediation of toxic water is a pressing concern in numerous developing nations because of increasing pollution. Pollution control or In-situ water treatment at source site can be used to rehabilitate polluted river water. Various procedures for cleaning up contaminated river water can be distributed into five categories: chemical, biological, physical, ecological, and engineering (Gao et al., 2018).

Aeration is a physical process which enhances oxygen capacity of polluted water, therefore, plays a vital role in the cleaning of river water. Although this technique is modest, easy to handle and broadly applicable, but their adoption might be costly. In this process, microbial communities become diverse and abundant because of the presence of aeration; break down organic molecules in polluted river water. However, aeration rate and the kind of aeration technique including mobile aeration or fixed-point aeration—affect its performance. As a result, it needs to be used correctly while treating water. For instance, mobile aeration must be employed in floating bed wetland approaches, whereas fixed-point aeration must be used in sediment-rooted artificial wetland systems. Numerous earlier investigations have shown that Oeiras River in Portugal is one river whose water quality was significantly enhanced by the use of aeration techniques (Wang et al., 1999).

Table 1. Remediation methods and their limitations

Treatment Methods	Limitations
Phytoremediation	It takes a long time and a lot of land to complete
Membrane Filtration	Scaling, fouling and expensive operation
Adsorption	Adsorbent regeneration is required
Chemical precipitation	Sludge production, costs associated with managing it, and use of chemicals
Photo catalysis	Expensive equipment
Coagulation/Flocculation	Secondary pollution generates

Plantation of aquatic plants such as algae is a biological process that shows a great potential for purification of river water therefore, wastewater effluents and polluted water by adsorption, absorption, accumulation and degradation of organic and inorganic contaminants. Therefore, popular aquatic plants for treating wastewater are water hyacinth (*Eichhornia crassipes*) and reed (*Phragmites australis*) (Sato et al., 2008).

CHALLENGES IN ASSESSING IMMUNOTOXICITY FOR REGULATORY PURPOSES

Data and documentation have been reported to show the procedures needed to successfully undertake restoration in freshwater aquatic habitats but both traditional and modern technologies have distinct drawbacks as well. A thorough summary of the difficulties encountered in evaluating or eliminating contaminants from contaminated water is provided in Table 1.

STRATEGIES FOR MITIGATION AND ENVIRONMENTAL PROTECTION

- CBWM organization is "a process where government agencies, local institutions, community groups, industry and citizens collaborate to observe and respond to issues related to common community". However, these organizations frequently operate on watersheds to assess chemical, biological and physical traits then use the data they gather to direct projects related to source water protection, restoration and mitigation (Garda et al., 2017).
- Water Pollution Prevention and Control Boards are conventions to implement Act and enforce pollution regulatory limits on waste. Therefore, these characteristics can also be used to monitor how well systemic restoration initiatives are working.
- Global concern and understanding/awareness raised through education are essential to restoring environmental balance and most likely, to successfully altering people's "throw-away" behaviors. Thus, it would be beneficial to conduct conferences and seminars to educate people about the importance of protecting the environment (Jakovcevic et al., 2014).

EMERGING POLLUTANTS AND UNEXPLORED INTERACTIONS

Recent investigations have documented emerging contaminants include growth promoters, hormones, antibiotics, antidepressants, carbamazepine, triclosan, and diclofenac in aquatic ecosystems. Therefore, water can be polluted through "leaching and runoff' of these contaminants from livestock. Thus, development of innovative treatments, such as advanced municipal wastewater treatment, and the elimination of sources like using antibiotics in animals and, in some cases, microbeads in cosmetics are two ways to mitigate the effects of new pollutants. "Over 700 newly discovered contaminants, along with their metabolites and transformation products, are identified as existing in aquatic environments throughout Europe" and require consideration (Haddaoui & Mateo-Sagasta, 2021).

INTEGRATING FISH IMMUNOTOXICOLOGY INTO ECOLOGICAL RISK ASSESSMENTS

Fish may act as bio-indicator due to environmental pollution thus plays a substantial role in evaluating the risk caused by the contaminants in aquatic environment because fish are directly exposed to toxicants exhibit uncoordinated behavior such as faster opercula activity which causes their fins to become hard and stretched (Lakra and Nagpure, 2009). After this, their body released huge amounts of mucus continuously, and soon the gills and buccal cavity were found to be covered in a thick layer of mucus. Therefore, fish eventually lost their balanced energy and became lethargic. Furthermore, environmental contaminants modulate defensive systems in aquatic organisms because of the production of ROS such as hydroxyl radical (OH), hydrogen peroxide (H_2O_2), and superoxide anion O_2^- as well (Pena-Llopis et al., 2003).

Advancements in monitoring techniques and technologies

There is a growing need for innovative approaches and technology to monitor wastewater systems due to detrimental effects that wastewater disposal practices have on our aquatic ecosystem (Xu et al., 2014). In an attempt to facilitate an understanding of mechanisms to remove phthalate acid esters from polluted environments, the use of qRT-PCR measured expression levels in genes that are probably involved in hazardous di(2-ethylhexyl) phthalate (DEHP)-catabolism pathway (Zhao et al., 2018). Furthermore, Transcriptomics or meta transcriptomics is an approach implements on the application of microbial meta transcriptomics to expose anabolic and catabolic pathways in response to various concentrations of polychlorinated biphenyls, an organochloride residues and PAH in DetroitRiver, Canada (Falk et al., 2019).

A spectrophotometric approach has been applied to the detection of river pollution. Therefore, in order to identify and monitor insecticides, fungicides, herbicides, pesticides and some of their discarded products in ground and surface waters thus, may be impacted by agricultural activities throughout Spain's wine-growing region, for instance, procedure of (LC-ESI-MS) was established (Cordier et al., 2019).

CONCLUSION

Water pollution is a growing problem in industrialized countries because of increasing industries and urbanization. Anthropogenic contaminants like heavy metals, polycyclic aromatic hydrocarbons, PCB's and pesticides have gained attention due to their disastrous effects on aquatic environment. Therefore, availability of molecular and cellular markers in fish leucocytes as well as phagocytosis and oxidative stress in response to toxicants provides strong evidence of a correlation between contaminants and fish immunity. Certain monitoring programs for evaluation and the removal of toxicants from water have been achieved effectively through various treatment technologies, including physical adsorption, aeration, aquatic weeds therefore, received significant attention. However, it is crucial to understand the effect of emerging pollutants, further investigations and advanced technologies required to determine their presence and hazardous effect on quality and safety of aquatic ecosystem.

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