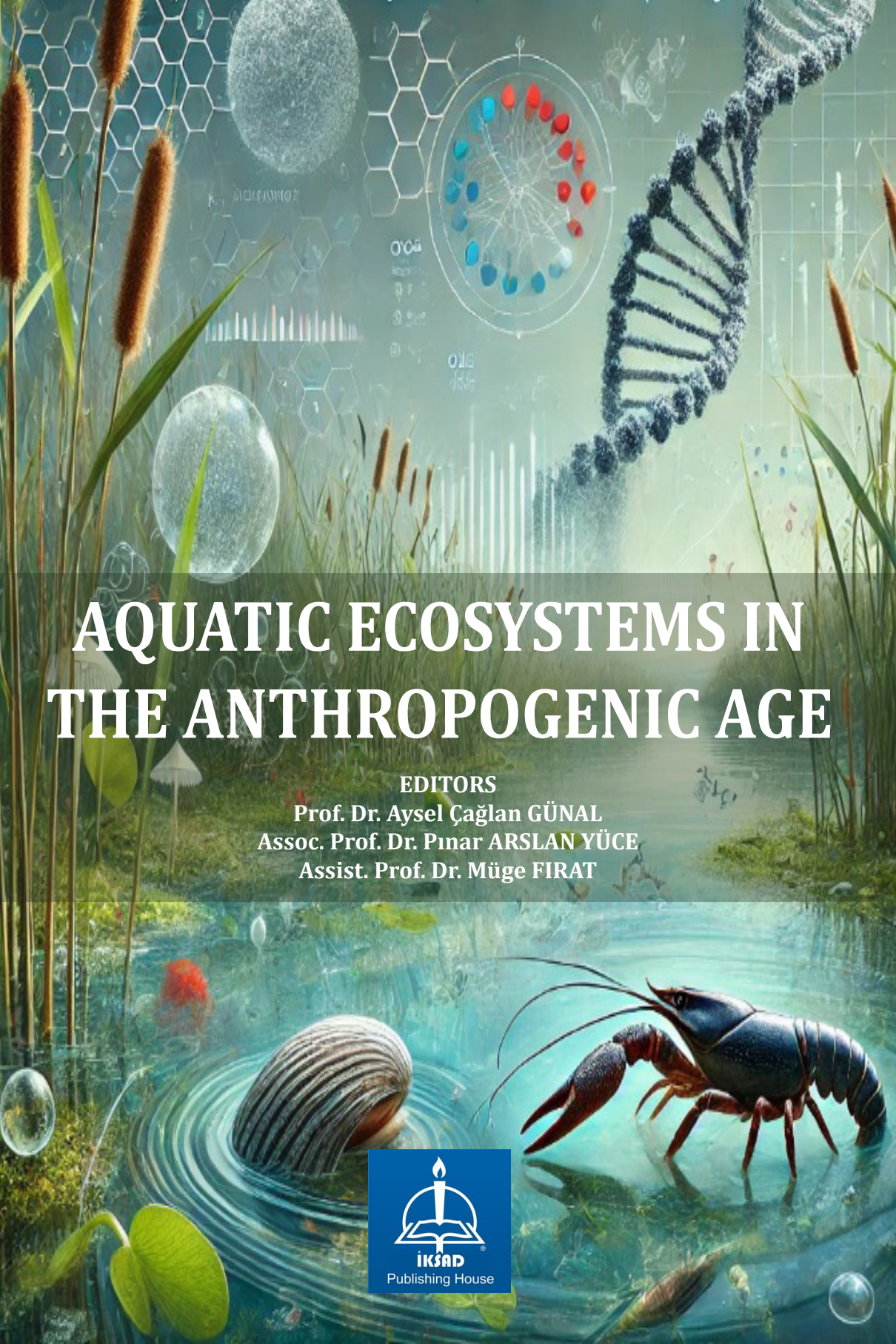

AQUATIC ECOSYSTEMS IN THE ANTHROPOGENIC AGE

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Dear Readers

Over the past few centuries, human activities have affected the nature of the world, and inevitably the aquatic ecosystems with lakes, wetlands, rivers, marine environments, and other aquatic habitats have been particularly vulnerable to these changes. Contaminants on the aquatic ecosystems caused effects on organisms in direct or indirect way.

Aquatic ecosystems are vital to planet health playing crucial role for sustainability of the biodiversity, regulating the climate and providing resources to many species especially humans. However, in the Anthropogenic Age—an era marked by the overwhelming influence of human behavior on the Earth's ecosystems—these vital systems face unprecedented challenges especially in the aquatic ecosystems.

This book presents the studies of the complex and multifaceted issues of human-induced pollution and the alternative scientific approaches for aquatic toxicology in aquatic ecosystems. We have tried to prepare a book for any readers to be interested in the human effects on the aquatic ecosystems and balance between new learners and even for the subject experts. It serves as a call to action and a reminder that while the Anthropogenic Age has brought about profound change, it is also an opportunity to reshape our relationship with the environment for the better. The health of our aquatic ecosystems depends on the choices we make today—and in these choices, there lies hope for a more sustainable and harmonious future.

We would like to express our gratitude to all the researchers who contributed to the preparation of this book. We hope that the book will benefit the researchers in this field and the health of our aquatic ecosystems.

Editors

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CHAPTER 1

EFFECTS OF NICKEL IN FRESHWATER ON AQUATIC VERTEBRATES

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INTRODUCTION

Water is a fluid that holds various elements dissolved and suspended within it in solid, liquid and gas forms. The amounts and types of these substances in water play roles in determining water quality. It is important to assess the standards of quality in this ecosystem that impact aquatic organisms and are impacted by them directly (Alley, 2007; Boyd, 2019). Freshwater sources on the Earth's surface play a role in the environment and the economy. They are essential for drinking water supply and irrigation in agriculture and supporting aquatic life forms (Gül, 2021).

Established quality standards and regulations for water resources. Institutions such as the United States Environmental Protection Agency (USEPA, 2018), the European Union Water Framework Directives (EU WFD, 2006; 2013), the United Nations Economic Commission for Europe (UNECE, 1993), The Organisation for Economic Co-operation and Development (OECD, 2007; 2009), The Canadian Council of Ministers of the Environment (CCME, 2015), as well as Türkiye's Surface Water Quality Regulation (YSKY, 2016) and Water Pollution Control Regulation (SKKY, 2004), have set various criteria for the protection and management of water resources (Figure 1). Standards for quality can differ among regions and societies because of varying weather conditions and societal norms, for living standards. People generated pollutants are an element influencing the cleanliness of water. Heavy metals pose a risk to water quality when they enter aquatic environments through both natural means and industrial actions (Gül, 2021).

Nickel is known as a heavy metal naturally in water bodies, soils and air. It is extensively utilized in industries and has the capability to seep into water sources through various means such as atmospheric deposition during rain and erosion processes or via discharge of wastewater. Although nickel occurs naturally in environments at minimal levels, human actions have initially led to its accumulation at higher concentrations. Significant human activities responsible for nickel movement into rivers involve mining, metal plating, burning fossil fuels, and using subpar waste disposal methods (Gül, 2021).

Figure 1. International and national water quality criteria regulations

Pure nickel is hard, silver-coloured metal that forms chemical bonds with other metals to create alloys. It is the 22nd most abundant element and may be found in all aquatic ecosystems. Under oxic conditions, nickel is either bound to dissolved organic matter or adsorbed onto insoluble iron or manganese hydroxides. In anoxic conditions, it forms insoluble sulfides (Pyle & Couture, 2012). The most commonly encountered alloys are arsenide, a binary compound with arsenic, and nickel sulfate. Mining activities can lead to the contamination of water resources with nickel. In the metal plating industry, nickel is used as a catalyst in coatings. Nickel salts are soluble in water, which facilitates their dispersion into aquatic environments. Improper disposal of nickel-containing waste, particularly into rivers, can cause significant contamination. Surface water concentrations as high as 1 mg/L have been reported, whereas typical concentrations in such waters are between 5–20 µg/L. In older houses, the presence of nickel may contaminate from the uses of nickel-alloy pipes, impacting domestic water supplies (Güler & Çobanoğlu, 1997).

Nickel poses a substantial threat to aquatic ecosystems at high concentrations (Abel, 1996). Aquatic organisms inhabiting areas near pollution sources are particularly vulnerable to both the direct and indirect toxic effects of nickel.

Nickel is trace amounts in the atmosphere. It is considered a nutrient for plants and terrestrial animals, however is not essential for aquatic organisms. Nevertheless, increasing evidence suggests that nickel might serve as a potential nutrient for fish (Pyle & Couture, 2012). Some studies have reported to rare in fish tissues, as a micronutrient involved in physiological activities (Boyd, 2019). A lack of nickel has been linked to health problems in humans, such as chronic bronchitis and shortness of breath. Certain nickel-containing compounds are regarded as potentially carcinogenic. Drinking water standards generally permit a maximum nickel concentration of 0.04 mg/L (Özdilek, 2002; Türkmen, 2003).

Aquatic vertebrates, particularly their gills, digestive systems, and skin, are directly exposed to the toxic effects of heavy metals. Such exposures can disrupt physiological, biochemical, and behavioral processes, while also endangering population dynamics, food chains, and the sustainability of ecosystems. Investigating the effects of heavy metals in freshwater ecosystems is important to water resource sustainability and ecosystem protection.

Nickel in aquatic environments can provoke harmful biochemical reactions in organisms, even at low concentrations. Aquatic vertebrates may encounter nickel either in dissolved forms within water or as accumulations in sediments. Nickel can be taken by the gills or skin or through food intake. Then binds to albumin and short peptides before traveling through the bloodstream and building up in the kidneys (Pyle & Couture, 2012). When dissolved nickel ions are directly absorbed into the body system it can disrupt the balance of ions and osmosis while also triggering stress and causing harm to cells. Studying the effects of nickel pollution from rivers on aquatic vertebrates is crucial, for safeguard in marine life and ensuring that ecosystems are sustainable.

Aquatic vertebrates can be exposed to nickel's toxic effects both directly and indirectly. Direct exposure occurs through contact surfaces of dissolved nickel in water, such as gills, skin, and olfactory epithelium. Indirect exposure occurs through interaction with contaminated sediments or consumption of prey organisms with nickel accumulation. This contamination can lead to the trophic transfer of

nickel, increasing the potential for biomagnification along the aquatic food chain (Pyle & Couture, 2012).

Nickel In Surface Water Sources

The permissible amounts of nickel in surface water resources may vary from country to country. The values of many prominent water quality regulations worldwide and the permissible levels in our country are given in Table 1.

Table 1. Amounts of nickel allowed in some international and national water quality standards.

Quality Criteria	Quality Grade	Nickel Concentration
USEPA (2018)	1st Grade	≤8.3 (µg/L)
	2nd Grade	
	3rd Grade	
	4th Grade	
	5th Grade	≤100 (µg/L)
EU WFD (2006, 2013)		34 (µg/L)
UNECE (1993)	1st Grade	<15 (µg/L)
	2nd Grade	15-87 (µg/L)
	3rd Grade	87-160 (µg/L)
	4th Grade	160-1400 (µg/L)
	5th Grade	>1400 (µg/L)
OECD (2009)	1st Grade	10 (µg/L)
	2nd Grade	25 (µg/L)
	3rd Grade	50 (µg/L)
	4th Grade	100 (µg/L)
	5th Grade	>100 (µg/L)
YSKY (2016) and SKKY (2004)	1st Grade	≤ 20 (µg/L)
	2nd Grade	50 (µg/L)
	3rd Grade	200 (µg/L)
	4th Grade	>200 (µg/L)
CCME (2015)		200 (µg/L)

The criteria of the Surface Water Quality Regulation and Water Pollution Control Regulation used in Türkiye are similar to other international criteria. Only a serious excess in the nickel rate that can be found in 4th and 5th class waters determined by UNECE (1993) stands out.

Respiratory System Exposure

It is through the gill and olfactory epithelia of aquatic organisms that nickel ions gain access to the body. These epithelial tissues play a critical role in basic physiological processes, including the diffusion of dissolved gases and ion exchange. The gill epithelium retains dissolved nickel ions in the water column, with this process influenced by environmental factors such as water pH, hardness and dissolved organic carbon (DOC) content. The bioavailability of nickel increases with low hardness and pH, leading to greater absorption in the gills.

Nickel accumulation in the gill and olfactory epithelium may cause suppression of the Na^+/K^+-ATPase enzyme, which is responsible for ion transport. As a consequence of this inhibition, osmotic balance and ion homeostasis are affected. It is known that chronically exposure of high concentrations to dissolved nickel may occur to oxidative stress. The accumulation of reactive oxygen species (ROS) in cells can lead to lipid peroxidation and subsequent damage to cellular membranes, thus gills' function may impair. Such damage may manifest in the organism as symptoms of hypoxia and metabolic stress.

Studies have demonstrated that nickel exposure can trigger cellular apoptosis in the gill epithelium and stimulate inflammatory responses by increasing cytokine release. Furthermore, organisms' growth, reproduction, and survival rates may be adversely affected. Heavy metals like nickel are particularly disruptive to biochemical and physiological processes in freshwater organisms. At toxic levels, bioaccumulation of nickel in some fish tissues, especially in the kidneys, may increase, potentially leading to biomagnification within the food chain. Consequently, the overall performance and vitality of fish can decline, rendering them more vulnerable to environmental stressors (Abel, 1996; Vigil, 2003).

Skin Exposure

Dissolved nickel ions can also be absorbed through the skin in some fish species. However, this route is generally less effective compared to the respiratory and digestive tract. Dermal exposure is usually favoured in environments with low water flow and high nickel concentrations.

The gill structures of fish have recently hatched are not yet fully developed, which makes them more susceptible to the toxic effects of environmental nickel. The higher membrane permeability of these species allows nickel ions to enter easily, which can cause developmental disorders (Pyle & Couture, 2012).

Digestive System Exposure

Aquatic vertebrates may be exposed to nickel through the consumption of contaminated food. Nickel tends to accumulate in higher trophic levels via ingested algae and benthic organisms. Once nickel enters the digestive system, it transforms into its ionic form due to the low pH in the stomach and is subsequently absorbed through the intestines (Pyle & Couture, 2012).

The absorption of nickel through the digestive system is detoxified by hepatic metabolism. However, excessive concentrations of nickel have been demonstrated to induce lipid peroxidation and DNA damage in liver cells, which can subsequently lead to hepatotoxicity and, over time, liver damage (Zambelli & Ciurli, 2013).

The manner of nickel exposure in aquatic vertebrates is contingent on the prevailing environmental factors and the physiological characteristics of the organism in question. The processes of absorption through ion transport via gill and intestinal epithelia play a pivotal role in the expression of nickel's toxic effects (Edo et al., 2024). The physiological stress imposed on organisms by chronic exposure may result in ecological consequences that extend from the individual organism to the population level. Consequently, a comprehensive understanding of the biochemical mechanisms associated with nickel exposure is essential to the protection of aquatic ecosystems.

EFFECTS ON AQUATIC VERTEBRATES

The toxic effects of nickel usually occur in the metabolic processes of aquatic organisms. Suppression of enzyme activities, oxidative stress, disturbance of ion balance, and cellular apoptosis are the main physiological disorders caused by nickel. Nickel may cause lipid peroxidation and deoxyribonucleic acid (DNA) damage by increasing the production of reactive oxygen species (ROS), leading to long-term deterioration in general health status. In addition, nickel

accumulation in gill lamellae may affect osmotic balance by blocking ion channels.

Oxidative Stress and Antioxidant Systems

Oxidative stress is a resulting from an augmentation in the generation of reactive oxygen species (ROS) accompanied by a disruption in the equilibrium between cellular antioxidant defensive mechanisms. This leads to an oxidative deterioration of proteins, lipids, nucleic acids, and other cellular constituents.

Increased ROS production at the cellular level is known to trigger the formation of free radicals such as superoxide (O_2-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH-) by interfering with the mitochondrial electron transport chain. For example, in a study on *Oreochromis mossambicus*, it was shown that ROS production increased in fish exposed to nickel nanoparticles, resulting in lipid peroxidation (LPO) (Jayaseelan et al., 2014).

In response to oxidative stress, changes occur in the activities of various antioxidant enzymes in fish tissues. Enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) neutralising ROS. However, nickel exposure may increase toxic effects by suppressing the activity of these enzymes. In a study on *Carassius auratus*, it was reported that nickel decreased SOD activity by 39-55% and GPx activity by 16-24% (Zheng et al., 2014).

Glutathione (GSH) is protecting cells from oxidative stress by acting as an antioxidant defense mechanism within cells. Nickel disrupts the balance within cells by reducing GSH levels which leads to an increase in damage occurring in the tissues. Research shows that exposure to nickel results in a decrease in GSH levels in the gill and kidney tissues of *Oncorhynchus mykiss* (Topal et al., 2017).

When the lack of GSH it weakens ability of cells to detoxify which can potentially lead to issues, like DNA damage and death through apoptosis. Lipid peroxidation can lead to cell damage, to the structure and function of cell membranes specifically by targeting polyunsaturated fatty acids in the membrane cells and promoting the generation of malondialdehyde (MDA) a byproduct of peroxidation caused by nickel exposure. This may weaken cell membranes and interferes with essential cellular activities Research has shown an

increase in lipid peroxidation and protein carbonylation in *Galaxias maculatus* even at low nickel concentrations, as 150 μg/L (Blewett et al., 2016).

Nickel ions have been demonstrated to cause issues, in the functioning of mitochondria by increasing stress that triggers cell death known as apoptosis. Nickel interferes with the integrity of membranes leading to a decrease in ATP production. Causing an energy deficiency in cells. This situation results in the activation of caspase three enzymes which indicates cell death. Moreover, nickel has been reported to speed up the process of cell death, in *Carassius auratus* fish by activating a signaling pathway called JNK (Jun N kinase). (Zheng et al., 2014).

According to Topal et al. (2017), the impact of nickel induced oxidative stress extends to altering how genes are expressed within cells well; for instance, NF-κB transcription factors can amplify inflammatory reactions and interfere with cell division processes over time leading to cellular aging and diminished organismal function.

Histopathological Changes

The nickel exposures induce various histopathological alterations at the cellular and tissue levels in aquatic vertebrates. These alterations are commonly observed the gills, liver, kidneys, and digestive tissues, significantly affecting physiologically all of the organisms.

Nickel exposure in the gill lamellae can lead to hyperplasia, epithelial thickening, and lamellar fusion. These alterations severely restrict the gills' capacity for gas exchange and ion regulation. Structural disruption of the lamellae and cellular necrosis have been reported in the gills of *Oreochromis niloticus* exposed to nickel chloride (Marcato et al., 2014). In *Hypophthalmichthys molitrix*, nickel was found to cause mechanical damage to gill epithelia and the accumulation of blood cells (Athikesavan et al., 2006).

In the liver cells, nickel can cause lipid accumulation and cellular damage such as pyroptosis. In *O. mossambicus*, nickel nanoparticles have been reported to result in cellular necrosis, nuclear hypertrophy, and vacuolization in liver tissues (Jayaseelan et al., 2014). Anomalies in liver enzymes such as AST and ALT point to metabolic disorders and cellular degeneration (Elbahnaswy et al., 2023).

Nickel in kidney tubules may lead to degeneration and a decrease in glomerular filtration rate. Studies on *Carassius auratus* have shown reduced glomerular filtration function and tubular necrosis (Topal et al., 2017). Additionally, hyperplasia and intracellular oedema in renal epithelial cells are common effects of nickel exposure (Athikesavan et al., 2006).

In the muscle tissues of *O. mossambicus*, nickel exposure has been found to cause fibril disintegration and necrosis. Furthermore, vacuolization and oedema have been reported in muscle tissue (Jayaseelan et al., 2014).

The skin epithelium may also undergo irritation and histological damage due to the penetration of nickel ions (Athikesavan et al., 2006).

Effects on Ion Exchange and Respiration

Nickel, hampers the ion transport mechanisms, leading to structural changes and disruptions in oxygen uptake. These impair ion regulation and respiratory processes, thereby affecting the overall health of the vertebrates.

Nickel adversely affects oxygen uptake and distribution in fish. The gill damaged by nickel, leading to respiratory dysfunction. Acute nickel exposure has been reported to increase the diffusion distance in the gill epithelia of *O. mykiss*, cause hyperplasia in gill cells, and reduce oxygen uptake by 35% (Pane et al., 2003). These disruptions result in decreased arterial oxygen pressure and respiratory acidosis. It has been reported that the increased salinity, mitigate the toxic effects of nickel and limit nickel accumulation in the gills of *G. maculatus*. However, this protective effect does not entirely eliminate the negative impact on oxygen uptake (Blewett et al., 2016). However, the protective effect does not entirely eliminate the negative impacts on oxygen uptake (Blewett et al., 2016).

Nickel exposures disrupts the maintaining ion homeostasis and stabilizing ion transport mechanisms, disturbing water and electrolyte balance. Likewise, the nickel inhibits ion transport by suppressing Na^+/K^+-ATPase activity in the gills and kidneys, leading to increased osmotic stress. Low concentrations of nickel stimulated Na^+/K^+-ATPase activity in *C. carassius*, whereas higher concentrations inhibited this activity (Haverinen et al., 2023). Exposure of the nickel

can also reduce plasma Na^+ and Cl^- levels while increasing Mg^{2+} and Ca^{2+} levels. This is considered an indicator of the fish accelerating their energy metabolism to minimize ion losses, showcasing the adaptive nature of these aquatic vertebrates (Blewett et al., 2016).

The nickel exposures in the water ecosystem causes severe damages to gill tissues, such as necrosis, epithelial thickening, and lamellar fusion. In *Mugil cephalus*, these injuries have been shown to increase ion permeability and reduce osmoregulatory capacity (Jasim et al., 2022). Furthermore, the disrupted structure of cellular membranes is leading to increased permeability and accelerates ion losses due to the compromised integrity of the membrane structure (Al-Attar, 2007). It can also trigger an increase in oxidative stress and the activation of metabolic adaptation mechanisms in fish. The effects of nickel on ion transport raise energy consumption and exacerbate metabolic stress. Specifically, the imbalance between ATP production and utilization limits the fish's capacity for adaptation (Gashkina, 2024). It has been reported that nickel exposure elevates cortisol and glucose levels, reflecting the fish's effort to regulate homeostasis (Jasim et al., 2022).

Protein Metabolism

Nickel exposure impacts some biochemical processes in aquatic vertebrates, including protein synthesis, enzyme activities, cellular protein stability, and amino acid metabolism. The nickel lead to temporarily reduce total protein (TP), albumin (Alb), and globulin (Glb) levels in *C. carpio* species (Bozorgzadeh et al., 2023). It is suggested in the study that low-level nickel may increase protease activity, leading to a rise in free amino acid levels. Cellular damage in gill tissues, in particular, has been associated with heightened proteolytic sensitivity like transcriptional changes in *Perca flavescens*, particularly in functional categories related to protein metabolism, translation processes, and ribosome biogenesis (Bougas et al., 2013). Changes in the transcription of ribosomal proteins indicate that nickel affects cellular protein synthesis mechanisms. It has also been reported to influence liver enzyme activity in carp by the nickel, increasing the activity of enzymes such as aspartate transaminase (AST) and alkaline phosphatase (ALP) (Bozorgzadeh et al., 2023). The histopathological

effects of nickel on liver tissue correlate with these metabolic disruptions.

Even chronic, low-dose nickel exposure can weaken overall metabolic stability by disrupting protein metabolism. Studies on carp bone metabolism have revealed that nickel increases alkaline phosphatase (ALP) isoenzyme levels, thereby affecting mineral deposition (Bozorgzadeh et al., 2023).

The nickel exposure exerts multifaceted effects on the protein metabolism of aquatic vertebrates. These effects disrupt both cellular protein synthesis and organism-level metabolic functions, leading to significant biochemical imbalances in aquatic ecosystems.

Effects on Glycogen Metabolism

Nickel exposure, in carp has been associated with reduced glycogen levels in the liver and muscles. This is reported to be due to increased glycogen breakdown and the utilization of glycogen reserves to meet energy requirements at the level (Cicik & Engin in 2005). Moreover, nickel also affects enzyme activities like glucose production inhibition through glucose 6 phosphatase suppression (Lokhand & Pawale 2023).

Depleting the glycogen reserves may cause a shortage of energy in aquatic vertebrates. Some researchers have shown that fish exposed to heavy metal contamination may experience elevated blood sugar levels while seeing a decrease in glycogen levels, in their liver and muscles. This occurrence is seen as a sign of metabolic strain indicating a depletion of glycogen stores to fulfill the organism’s energy requirements (Javed & Usmani 2013).

On the energy metabolism the nickel causing the changes at the levels in glycogen metabolism and disrupting the normal functioning of enzymes like insulin and glucagon. Activities of enzymes as glycogen phosphorylase and glycogen synthase are also suppressed by nickel exposures, resulting, in the breakdown of stored glycogen. Furthermore, research shows that heavy metals can influence the expression of genes related to glycogen metabolism disturbing the metabolic equilibrium (Liu et al., 2024).

CONCLUSION

The harmful impacts of nickel, on vertebrates present a danger to the well-being of individual creatures and the balance of ecosystems at large according to this review that explored how nickel affects the physiological functions and biochemical processes in aquatic vertebrates including vital functions like breathing regulation and protein metabolism along, with glycogen storage.

Nickel exposure can harm the epithelium of vertebrates by causing structural damage and hyperplasia leading to reduced oxygen uptake, in the respiration process and disrupting osmoregulation processes through the inhibition of ion transport enzymes, like Na⁄K ATPase This disruption not results in higher metabolic energy consumption. Also negatively impacts the balance of aquatic vertebrates' internal environment.

Nickels impact, on protein metabolism involves inhibiting protein synthesis which results in free amino acid levels and initiates the breakdown of proteins, in fish biology studies show it hinders growth reproduction and overall health in fish by affecting liver enzyme functions demonstrating metabolic toxicity caused by nickel exposure.

The depletion of nickel disrupts glycogen reserves, in the body. Affects energy metabolism by reducing glycogen levels in the liver and muscles to meet energy requirements through glycogenesis process which deteriorates the fish health due, to oxidative stress and metabolic damages.

Various studies reported the examination of tissue samples, from the gills, liver, kidneys and muscles reveals that nickel may be responsible for harming cells. Signs, like cell death, inflammation, swelling and cell deterioration, highlight how harmful this metal can be.

Environmental elements could also impact the physiological impacts of nickel, on living organisms in ways There are certain factors, like water temperature, pH levels, hardness and salinity that could alter its harmful effects.

Control of nickel pollution, from discharges and various other sources, should be carefully regulated. Need to monitoring nickel levels in water bodies and ensure that our align with environmental standards.

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CHAPTER 2

EFFECTS OF HEAVY METALS FROM AQUATIC ENVIRONMENT ON HUMAN HEALTH

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INTRODUCTION

Many definitions have been made for heavy metals based on their density, atomic weight, chemical properties, or toxicity. Heavy metals are generally defined as metals or metalloids (semimetals) that have to do with possible toxicity or ecotoxicity and pollution. In actuality, metals with a density more than 5g/cm^3 are referred to be heavy metals. In medicine, the definition of heavy metals is described as all metals that possess toxic properties, regardless of their atomic weight (Aslam et al., 2011; Duffus et al., 2002).

The degree to which heavy metals impact biological processes determines whether they are considered necessary or non-essential. Essential heavy metals are those that are involved in an enzymatic reaction, are part of vitamins and hormones, and are required in a specific concentration in the structure of the organism. This group of heavy metals (Fe, Cu, Zn, Ni, and Se) become toxic once they reach a specific quantity (1–10 ppm: parts per million). In contrast, non-essential heavy metals (Hg, Cd, and Pb) can exhibit toxic effects even at very low concentrations (Andrede et al., 2017; Kim et al., 2019).

Due to rapid population growth and industrialization, the level of toxic heavy metals, especially in aquatic environments, has increased in recent years. Heavy metals, which constitute a part of the pollutants, along with metal compounds and various minerals, show widespread distribution in lakes, rivers, bays, and oceans, as well as in their sediments. Heavy metals cannot be easily eliminated in nature and can remain in the receiving environment for a long period without breaking down. In this way, they enter the living body and are transferred to a higher-level organism through the food chain. They are ultimately transferred to the human body (Aras et al., 2017) (Figure 1).

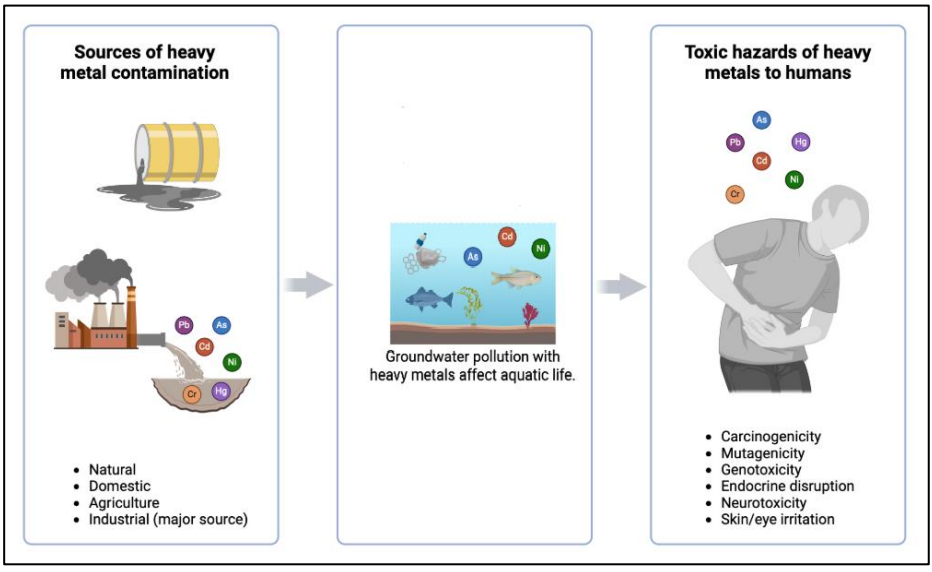


Figure 1: Pathway and impacts of heavy metal contamination on aquatic ecosystems and human health (Vasilachi et al., 2023)

The effects of heavy metals on the body are not solely dependent on the concentration of the heavy metal. At the same time, it also depends on the structure of the metal ion, its solubility value, chemical structure, redox and complex formation ability, the method of entry into the body, and the frequency of its presence in the environment. They cause disturbances in intracellular metabolic processes, exhibiting toxic effects (Gupta et al., 2016).

The generation of reactive oxygen species, oxidative damage, and subsequent development of negative health effects are the general mechanisms of heavy metal toxicity (Fu et al., 2020). Heavy metals have harmful impacts on the central nervous system (CNS). The brain, being an organ that consumes a lot of oxygen, has a high potential for the production of free radicals and reactive oxygen species. Reactive oxygen species play a significant role in neurodegeneration by targeting various biomolecules such as DNA, RNA, lipids, and proteins within nerve cells, as well as a wide range of processes like nucleic acid oxidation and lipid peroxidation, thereby altering the function of biomolecules (Singh et al., 2019). Additional conditions brought on by toxicity include autoimmune diseases (such as rheumatism, Crohn's disease, ulcerative colitis, etc.),

neurological disorders (such as depression, migraine, Alzheimer's disease, and Parkinson's disease), organic diseases (such as kidney disease, allergies, eczema, and asthma), and mitochondrial damage and apoptosis induction. The majority of the health issues brought on by heavy metals are malignancies or chronic illnesses that call for sophisticated diagnostic and therapeutic approaches (Figure 2).

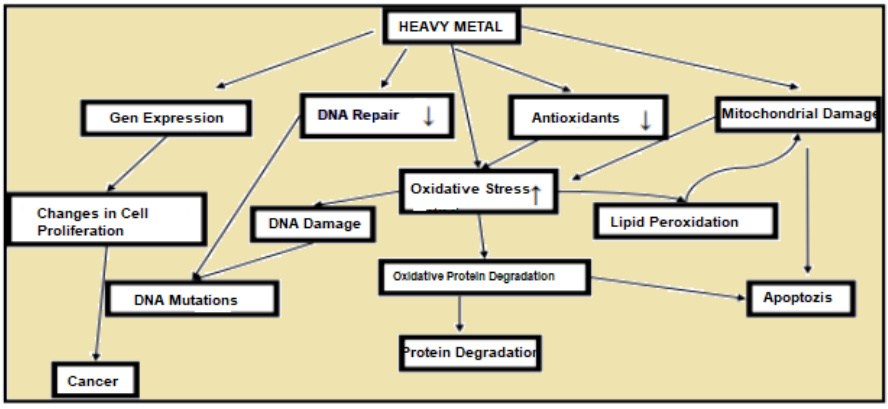
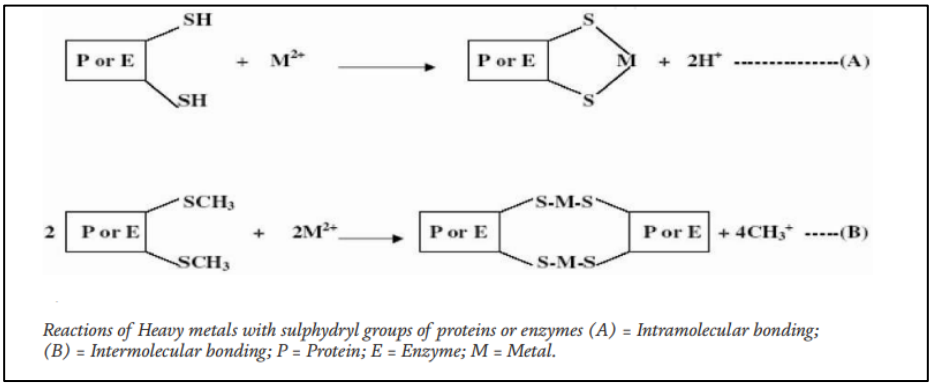


Figure 2: Intracellular effects of heavy metals (Özbolat and Tuli, 2016)

Heavy metal toxicity's biochemical mechanism

Heavy metals that enter the body through food or water become acidified in the acidic environment of the stomach. In this environment, they convert into various oxidation states (e.g., Zn^{2+}, Cd^{2+}, Pb^{2+}, As^{2+}, As^{3+}, Ag^{+}, Hg^{2+}) and easily interact with structures such as proteins and enzymes, forming strong and stable bonds. Thiol groups, like the SH group of cysteine and the SCH_3 group of methionine, are the functional groups that heavy metals most commonly target (Figure 3).



Reactions of Heavy metals with sulphydryl groups of proteins or enzymes (A) = Intramolecular bonding; (B) = Intermolecular bonding; P = Protein; E = Enzyme; M = Metal.

Figure 3: Reactions of heavy metals with sulphydryl groups of proteins or enzymes (Engwa et al., 2019)

Certain enzymes can use proteins bound to heavy metals as substrates. In these situations, the protein that is bound to the heavy metal forms an enzyme-substrate complex with the enzyme and is unable to take up another substrate until it is released. Consequently, the heavy metal stays embedded in the tissue because the enzyme is inhibited, preventing the formation of the substrate's product. This condition causes the body to malfunction, become aberrant, and sustain damage. Cell damage and oxidative stress are exacerbated when thiol transferases are inhibited. Protein folding can also be inhibited by heavy metals. Chemically denatured proteins cannot refold when exposed to heavy metals as arsenic, cadmium, lead, and mercury. It has been noted that proteins misfold when exposed to heavy metals, and that misfolded proteins cannot be fixed by EDTA chelators or reduced glutathione. Mercury, cadmium, and lead are the elements that hinder protein folding the most effectively (Duruibe et al., 2007).

Heavy Metals

Mercury (Hg)

It is a heavy metal derived from the Latin word "hydragyros," meaning liquid silver, and exists in a liquid state at room temperature (Clarkson et al., 2003). The toxicity of mercury varies depending on its chemical form. Mercury exists in three forms: metallic or elemental, inorganic, and organic (methyl mercury (MeHg), ethyl mercury, and

phenyl mercury). MeHg is an element that has not formed compounds with other elements. It exists in a liquid metal state, does not dissolve in water, but can evaporate in quite toxic amounts at room temperature, and is excreted from the body very slowly (Gupta et al., 2003).

High levels of MeHg accumulate in fish and seafood. Especially large predatory fish (for example, swordfish, tuna) carry a mercury load and eventually pass it on to the human body (Crinnion, 2000). Hg is important for humans because it is a heavy metal with a high potential to cause neurotoxicity (Chin-Chan, 2015). Mercury swiftly passes through cell membranes, including the placental and blood-brain barriers, once it enters the bloodstream (Crinnion, 2000). The CNS is the main target organ of MeHg toxicity. MeHg is transferred from the mother to the fetus and reaches the fetus's brain (Farina et al., 2013). It can also cross the placenta and affect breast milk (Akcan and Dursun, 2008).

The neurological condition known as "Minamata disease" was initially identified in May 1956 in the city of Minamata, which is situated in the southwest part of Kyushu Island, Japan. It is caused by mercury poisoning, which happens to people who consume fish and shellfish contaminated with mercury from the wastewater of a chemical plant. Ataxia, hand and foot numbness, overall muscular weakness, peripheral vision loss, and speech and hearing impairment are some of the symptoms. In severe cases, paralysis, coma, and death occur a few weeks after the onset of symptoms. Impacting fetuses and producing symptoms resembling those of cerebral palsy, extensive brain damage, and microcephaly (Harada, 1995).

Mercury has been found to selectively concentrate in the granular layer of the cerebellum, the sensory neurons of the dorsal root ganglia, the amygdala, the hippocampus, and the medial basal nuclei, all of which are involved in memory processes in the human brain (Crinnion, 2000). MeHg causes an increase in extracellular glutamate levels by inhibiting glutamate uptake in astrocytes and inducing glutamate release. Excessive glutamate causes an excessive increase in intracellular Ca^{+2} concentration and consequently neurotoxicity through the overactivation of glutamate's N-methyl-D-aspartate (NMDA) receptors. Excessive intracellular Ca^{+2} activates neuronal nitric oxide synthase, which causes

mitochondria to collapse and nitric oxide levels to rise. MeHg has an impact on the electron transport chain in the mitochondria and increases the amount of ROS produced (Farina, et al., 2013).

Lead (Pb)

Lead is a neurotoxic and a heavy metal that has been utilized for thousands of years. It does not take part in biochemical reactions. At the same time, lead, which is widely used industrially, is found in both organic and inorganic forms. It is among the most dangerous heavy metals for human health. Inorganic lead is found in the atmosphere in particulate form, while organic lead is volatile and mostly mixes with food items and drinking water. For this reason, organic lead affects living organisms more than inorganic lead. Both its prevalence in industrial use and its widespread presence in environmental elements make lead an important exposure factor in terms of environmental and occupational health (Gülçin et al., 2002).

Lead enters the food chain through algae and small aquatic organisms. The major absorption pathways are the gastrointestinal tract and the respiratory system. Absorption from the gastrointestinal tract varies with age; while 10% of orally ingested lead is absorbed in adults, this rate is 40% in children. In addition, lead absorption can also occur through the skin. 85-90% of the lead that enters the human body binds to the membrane of erythrocytes in the blood, 1% remains free, and the rest is transported bound to albumin. 90% of the lead particles taken in through inhalation are absorbed. Lead, which is eliminated from the body very slowly, is expelled from the blood in 30 days and from the bones in 27 years. If there is long-term exposure to lead, it is stored in the body. It primarily distributes in soft tissues and parenchymal organs. The primary sites of deposition are bones and teeth. Approximately 94% of the accumulated lead in adults is found in teeth and bones (Erickson and Thompson, 2005).

Since lead is a divalent cation, it has a high capacity to bind to sulfhydryl groups, and the products it forms inhibit enzymes and proteins. Lead also disrupts the activity of pyrimidine 5'-nucleotidase and increases the levels of pyrimidine nucleotides within erythrocytes.

This situation prevents the maturation of erythrocyte elements, reduces the number of erythrocytes, and results in anemia. This outcome is one of the best-known toxic effects of lead. Abnormal concentrations of hem precursors appear in the blood and urine. Lead inhibits the two key enzymes of heme biosynthesis: the δ-aminolevulinic acid dehydratase enzyme that catalyzes the "ALA → porphobilinogen" step and the ferrochelatase enzyme that catalyzes the "protoporphyrin IX → heme" step (Figure 4). Inhibition of the δ-aminolevulinic acid dehydratase enzyme (ALA dehydratase) causes an increase in circulating ALA levels and reduces the release of Gamma Amino Butyric Acid (GABA) from the central nervous system. When the Pb level in whole blood exceeds 20 µg/dl, ALA dehydratase activity is inhibited by 50%. Lead shortens the lifespan of erythrocytes by disrupting the Na+/K+-ATPase pump and the membrane structure of erythrocytes (Alfven et al., 2002; Ercal et al., 2001).

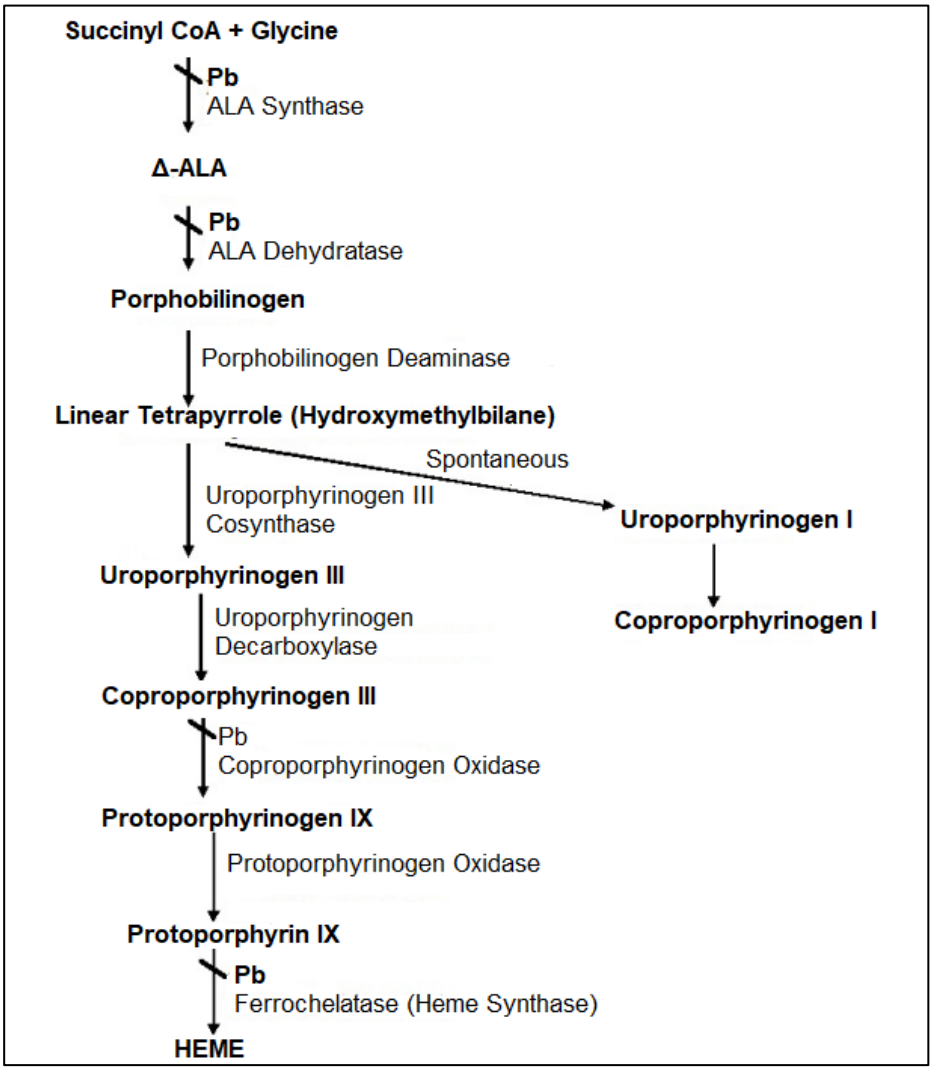


Figure 4: Steps of heme synthesis inhibition (Özbolat and Tuli, 2016)

When evaluating lead toxicity, the susceptibility of the kidneys, cardiovascular system, and hematological system to lead is particularly crucial. Additionally, lead also affects the male and female reproductive systems. Lead easily passes from the mother's blood to the placenta and fetus, accumulates in the bones, and therefore, maternal exposure can cause the newborn to be affected by lead even years later. This exposure causes delays in cognitive development as well as embryonic organ development (Flora et al., 2012) (Figure 5).

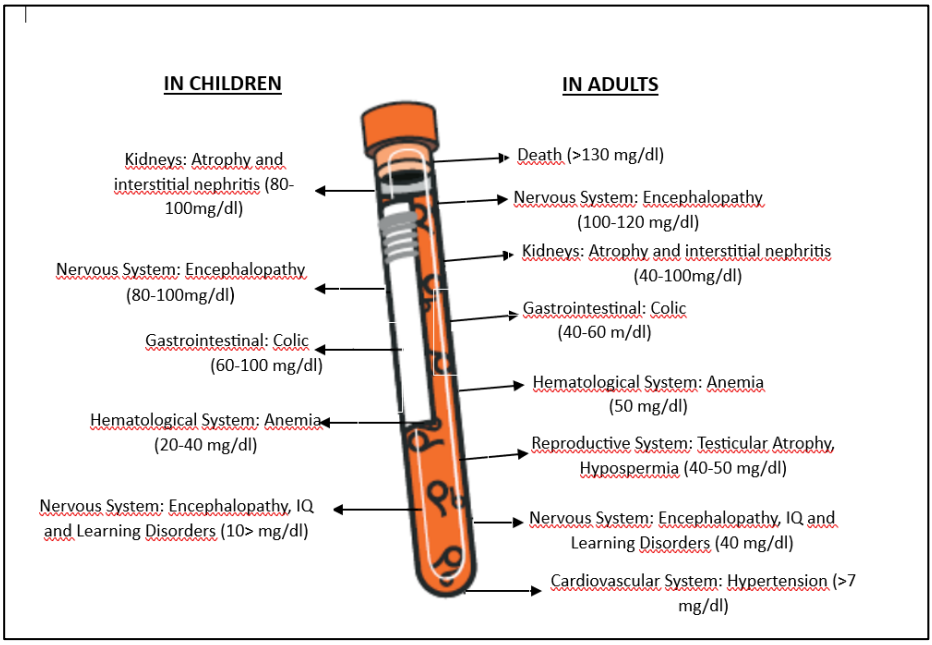


Figure 5: Effects of blood lead concentration (Özbolat and Tuli, 2016)

Copper (Cu)

In both human and animal beings, it is a necessary trace element. There are copper-containing enzymes that act as redox catalysts (cytochrome oxidase, nitrate reductase) or oxygen transporters (hemocyanin). Copper can exist in three forms with valences Cu^{o}, Cu^{+1}, and Cu^{+2} (Kiaune and Singhasemanon, 2011). Copper is important for body functions and is especially a key component of hair, skin, bones, and some internal organs. Under normal circumstances, copper—which an adult human's average daily intake is between 50 and 120 mg—is an essential component of metabolic processes involving vitamins, fatty acids, and amino acids. As a biocatalyst in human metabolism, copper serves a variety of purposes and is present in the structure of metalloenzymes. Among the most well-known copper metalloenzymes are tyrosinase, amine oxidase, ascorbic acid oxidase, urate oxidase, superoxide dismutase, cytochrome c oxidase, and dopamine β-hydroxylase. It is also essential for the body to properly use iron. Iron cannot attach to hemoglobin in the absence of copper. Every organ and tissue in the human body contains copper. Because it is both poisonous

and necessary, copper is absorbed from the small intestine and then dispersed throughout the body by loosely binding to amino acids and serum albumin. Ceruloplasmin is synthesized in parenchymal cells using copper that enters the liver as copper-albumin and copper-histidine complexes. According to Ranjan et al. (2006), ceruloplasmin and copper metalloprotein contain approximately 90% of copper is present in mammalian plasma. It builds up in algae and marine organisms with copper shells, where it enters the food chain. This metal has the ability to accumulate in the tissues of mammals and, when its concentration in the tissues reaches critical levels, exhibit hazardous consequences. It has been noted that exposure to this metal causes pathological alterations in a variety of tissues, most commonly the kidneys and liver. Hepatotoxicity, or liver injury, can result from copper's ability to harm liver cells. Additionally, it may result in liver tissue injury, inflammation, or edema (Linder, 2020).

Arsenic (As)

Since arsenic exhibits both metallic and non-metallic properties, it is chemically classified as a metalloid. Arsenic compounds can be classified as inorganic and organic. Arsenic compounds that do not contain carbon bonds are defined as inorganic arsenics, while those that do contain carbon bonds are known as organic arsenics. Inorganic arsenic species include arsenate and arsenite, while some of the organic species are methylated forms such as monomethyl arsenic acid and dimethylarsinic acid. Waters include four different forms of arsenic: dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), arsenite, and arsenate. The chemical structure of arsenic determines its toxicity; in general, soluble inorganic arsenic species are more harmful than organic ones (such MMA and DMA). Because organic arsenic is easily excreted from the body under normal conditions. In addition, arsenic types with large molecular structures, such as arsenobetaine and arsenocholine, are not toxic. Inorganic arsenic is 100 times more hazardous than organic arsenic, and arsenite is 60 times more deadly than arsenate (Jain and Ali, 2000; Mandal and Suzuki, 2002).

Arsenic enters the food chain through aquatic plants and plankton. It targets a wide range of functional groups in the body and exhibits different biological effects based on the tissue, dose, length of exposure, and metabolism. The most important mechanism in the occurrence of arsenic compound toxicity is the blockage of enzymes containing thiol groups in the organism. The highly reactive trivalent forms of arsenic bind to the sulfhydryl groups of many enzymes, such as DNA repair enzymes and antioxidant enzymes (thioredoxin reductase, glutathione peroxidase, etc.), thereby inhibiting these enzymes. Even at low doses, arsenic causes oxidative DNA damage and the formation of reactive oxygen and nitrogen species, resulting in lipid peroxidation. They combine with lipoic acid to inhibit pyruvic acid metabolism. However, an increasing number of experimental studies have shown that arsenic causes endocrine disorders, changes in cell cycle kinetics, epigenetic effects, and transcriptional changes (Muzaffar et al., 2023).

Cadmium (Cd)

Cadmium and its compounds can have various negative effects on human health. Mollusks, shellfish, and fish accumulate cadmium and enter the food chain. Because the human body is inadequate in excreting cadmium, the health effects of cadmium exposure are further increased. Cadmium is reabsorbed by the kidneys, which restricts its excretion. Short-term exposure to cadmium vapor can cause serious lung damage and irritation of the respiratory tract, while high doses of cadmium intake can affect the stomach, leading to vomiting and diarrhea. Long-term cadmium exposure leads to the accumulation of this metal in bones and lungs, which can cause damage to bones and lungs. Cadmium can disrupt bone mineralization, leading to health issues such as osteoporosis; studies conducted on animals and humans have shown that cadmium causes bone damage (Bernard, 2008).

In Japan, cadmium released as industrial waste has gradually begun to accumulate in the soil, algae, and river organisms. This situation has led to local phytoplankton and algal species absorbing cadmium in high amounts. Cadmium has passed into fish through the food chain. Additionally, fish have biologically accumulated cadmium in their gills

through passive transport. Cadmium has caused negative effects in fish, leading to endocrine system disorders, impairing reproductive ability, and sometimes resulting in death. The local people, unaware of the dangers of cadmium, used the water from the Jinzū River to irrigate their rice fields. The prolonged exposure of these fields to cadmium caused the rice to become rapidly contaminated and toxic. When the local people consumed this rice and drank the same water, high cadmium body burdens were formed, leading to the emergence of "Itai-itai" disease. In the cadmium toxicity associated with this disease, it has been observed that the risk of bone fractures in women increased, along with a loss of bone density and a decrease in height (Kobayashi et al., 2009).

Cadmium has high toxicity on the kidneys and can accumulate in the proximal tubules, leading to kidney dysfunction and kidney diseases. In addition, cadmium can cause imbalances in calcium metabolism, kidney stone formation, and hypercalciuria. The International Agency for Research on Cancer has designated cadmium as a Group 1 carcinogen in humans (Mudgal et al., 2010).

Cadmium-induced carcinogenicity is associated with DNA either directly or indirectly. The direct relationship requires covalent bonding between cadmium and DNA, while the indirect relationship causes oxidative damage in DNA, an increase in cellular oxidants, and consequently an increase in free radicals. It is suggested that the indirect relationship is also through DNA repair mechanisms, DNA-protein, and DNA-amino acid cross-link formation. Cadmium is a soft metal and preferentially binds to sulfhydryl groups in proteins and DNA bases rather than DNA phosphates. The cellular toxicity of cadmium causes the inhibition of sulfhydryl-containing proteins and the induction of reactive oxygen species through Cd-DNA binding. Additionally, cadmium indirectly reduces antioxidant levels and an increase in intracellular hydrogen peroxide is observed. The increase in hydrogen peroxide catalyzes iron/copper-mediated redox reactions, the resulting free radicals cause DNA cross-linking, and trigger lipid peroxidation. It has not been observed that cadmium produces free radicals, but lipid peroxidation in tissues increased immediately after application (Rojas et al., 1999; Fang et al., 2001).

Casalino et al. have summed up the impacts of cadmium as follows: 2. Interaction with antioxidant enzymes, 1. Modification of membrane structure or function, 3. Change in thiol proteins; 4. Energy metabolism inhibition; 5. DNA structure modification; and 6. Stress gene expression induction and important involvement in certain enzymatic activity impacts (Casalino et al., 2002; Hossain and Huq, 2002).

Nickel (Ni)

It is a hard metal with a silvery-white color. Nickel compounds are insoluble in water. Its water-soluble salts are chloride, sulfate, and nitrate. In biological systems, nickel combines with deoxyribonucleic acid, proteins, peptides, amino acids, and adenosine triphosphate to produce complexes (Zamble, 2017). Figure 6 illustrates the direct and indirect in vivo damage caused by nickel.

Among the main effects on human health are allergic contact dermatitis, respiratory system disorders, and carcinogenic effects. Long-term nickel exposure, especially in individuals exposed to nickel compounds in industrial settings, can increase the risk of nasal, sinus, and lung cancer. Additionally, high levels of nickel exposure can adversely affect cardiovascular and kidney functions. (Das et al., 2008).

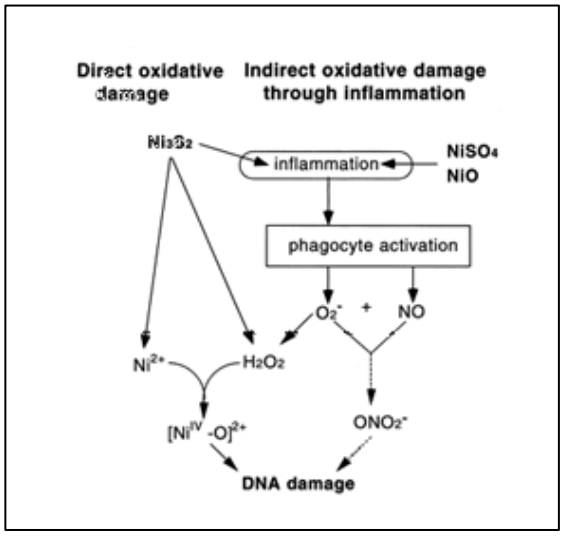


Figure 6: The direct and indirect in vivo damage caused by nickel (Boğa, 2007)

Zinc (Zn)

Zinc is a trace element that naturally occurs in soil, rocks, and aquatic environments. However, human activities such as mining, industrial operations, wastewater discharges, and fertilizer use can lead to increased zinc concentrations in aquatic ecosystems. This increase leads to the biological uptake of zinc by aquatic organisms. Especially aquatic organisms such as phytoplankton, zooplankton, crustaceans, and fish absorb zinc in its bioavailable forms. The transfer of zinc from aquatic organisms to humans occurs via the food chain. In this process, organisms at low trophic levels such as zinc, phytoplankton, and zooplankton are taken up. In this process, zinc is taken up by low trophic level organisms such as phytoplankton and zooplankton. Fish and shellfish at higher trophic levels accumulate the zinc load. The consumption of seafood leads to the transfer of zinc into the human body. Especially in areas with intense industrial pollution, this process can lead to zinc toxicity. One necessary trace element is zinc for the human body and plays an important role in processes such as the immune system, cell growth, wound healing, and DNA synthesis. However, when taken in excessive amounts, it can cause toxic effects. Symptoms such as nausea, vomiting, and diarrhea may be observed. Excessive zinc can weaken the immune system by inhibiting the absorption of other trace elements (such as copper). Prolonged high zinc exposure can lead to neurotoxic effects (Plum et al., 2010).

Cobalt (Co)

It is also crucial for living things' nourishment. The main component of vitamin B12 is cobalt. It is the most effective biocatalyst known to date. The transfer of cobalt from aquatic organisms to humans occurs through the consumption of these organisms. In its toxicity, it induces the cleavage of all bases in DNA, particularly inducing more Thymine instead of Guanine and Cytosine instead of Adenine. In the cobalt-oxygen complex, cobalt interacts with H_2O_2, O_2, and OH^-. When chelators are present, the formation of ROS is enhanced. Cobalt damages mitochondrial DNA in neuronally cells, according to recent research. (Plowman et al., 1991).

Chromium (Cr)

It can be found in plants, animals, rocks, and soil. It can be a gas, a liquid, or a solid. In aqueous sediments, chromium compounds are quite persistent. It exists in a variety of states, including hexavalent, pentavalent, tetravalent, and divalent forms. The most stable forms are Cr (VI) and Cr (III), and the only thing that is really interesting is how they relate to human exposure. Compounds containing chromium (VI), including lead, strontium, zinc, and calcium chromates, are extremely harmful and carcinogenic.

On the other hand, chromium (III), which is essential for glucose metabolism, is a dietary supplement that both humans and animals require. Humans exposed to high concentrations of chromium compounds may experience inhibition of the erythrocyte glutathione reductase enzyme, which lowers methemoglobin's ability to be converted to hemoglobin.

Chromate chemicals can cause DNA damage through a variety of mechanisms, resulting in sister chromatid exchanges, chromosomal abnormalities, DNA adducts, and modifications to transcription and replication, according to the results of numerous in vitro and in vivo research (Costa and Klein, 2006).

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CHAPTER 3

As, Cd, Cr, Pb, AND Hg CONTAMINATION IN FRESHWATER ECOSYSTEMS AND THEIR TOXIC EFFECTS ON AQUATIC ORGANISMS; A COMPREHENSIVE REVIEW ON RECENT STUDIES

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INTRODUCTION

Humanity has experienced two major phases of population expansion. The first phase occurred after the Paleolithic era, as the Neolithic agricultural revolution led to a dramatic population increase, reaching approximately 150 million by 1000 BCE. The second phase started with the Industrial Revolution when humanity stunned Malthusian factors (scarcity and diseases) by developments in industry, and due to that population growth rate is increased (Chu & Xu, 2024). This increment also led to an increase in the demands for resources to supply the needs of human populations. Consequently, the intervention of human in nature to meet the increasing demands is caused –and still causing- disruption to the ecological balance. Thereby, the self-purification mechanisms of nature have become insufficient, which in turn resulted in environmental contamination/pollution (Zhou et al., 2023a).

It is well documented that pollution has several detrimental effects on all compartments of nature and all living beings, and therefore making it a global concern (Ukaogo et al., 2020). In general, environmental pollution is considered the presence of toxic chemicals and/or energy forms that are released or discharged into ecosystems by human activities, and disturbing ecosystems through alterations in the physical, chemical, and biological features of ecosystems (Özkara & Akyıl, 2018). According to Holdgate (1980) environmental pollution is a situation that occurs by introducing materials or energy to the environment due to human activity. Singh et al. (1991) defined environmental pollution as a change of environment's condition from a stable (balanced) to unstable (imbalanced) system. Environmental pollution is associated with industrial and technological development that has magnified the release of pollutants, including toxic chemicals, heavy metals, nanoparticles, microplastic particles and exotic and invasive biological organisms, and physical agents such as heat and radiation into the environment (Ukaogo et al., 2020; Rai, 2016; Nassouhi et. al., 2018; Ergönül et al., 2021; Sazlı et al., 2022).

Pollution can be classified based on the environmental compartment of effected such as air, water, or soil pollution. Similarly,

pollution can be named according to the type of pollutant (i.e., noise, radioactive, or thermal pollution). Among these compartments, pollution of air, water sources, and soil has direct implications for human health and environmental sustainability and therefore admitted as most significant types of pollution (Münzel et al., 2023). Water is a very good solvent and several chemicals including toxic substances are readily dissolved in water, and therefore can be carried for long distances from terrestrial habitats (both from point or non-point sources) to oceans (Munafò et al., 2005). Thus, water pollution is considered an important concern for ecosystem sustainability and human health (Du Plessis, 2017; Hiranmai & Kamaraj, 2023).

Water pollutants originate from both natural sources (volcanic eruptions, floods, etc.) and human activities (sewage, agricultural runoff, industrial waste, etc.) (Nasr et al., 2007). These contaminants include organic pollutants, pathogens, nutrients, agricultural runoff, suspended solids, thermal wastes, radioactive waste, and inorganic pollutants (Wasewar et al., 2020). Among these pollutants inorganic pollutants include an extensive range of substances; trace elements, heavy metals, inorganic salts, metal complexes, cyanides, and sulfates (Borah et al., 2020). Heavy metals have several uses in various industries owing to their conductivity, catalytic activity, density, corrosion resistance, magnetic characteristics, biocidal effects, and alloy-forming capabilities (Briffa et al., 2020). However, heavy metals constitute a major hazard to all forms of living organisms and environmental quality (Nassouhi et al., 2018). They are naturally found on Earth generally as sulfates, hydroxides, oxides, sulfides, phosphates, and silicates (Masindi & Muedi, 2018). The amount of heavy metals released into environment have increased during the last decades through natural events (such as wildfires, erosion, and volcanic activities), and human activities (i.e. agriculture, mining, and industrial operations) (Singh et al., 2022). Eventually, there are several documented instances indicating that heavy metals are distributed to diverse habitats, particularly aquatic ecosystems, during their extraction, utilization and disposal (Ali et al., 2019; Vajargah, 2021). Unfortunately, heavy metals are persistent elements and they accumulate in the tissues of various organisms, and as

a result they have a tendency to increase in higher trophic levels through biomagnification (Ali et al., 2019).

Based on their functions in living beings, heavy metals have 2 broad groups; essential and non-essential heavy metals. Heavy metals such as iron, copper, cobalt, zinc, selenium, and manganese are required for the maintenance of an organism's physiological and biological functions and therefore named as essential heavy metals (FAO, 1996). Although, trace amounts of these metals have functions in living beings, they may exert toxic effects in higher concentrations (Das et al., 2019). For example, copper is a vital micronutrient for all organisms and has functions as a cofactor in numerous enzymes. However, at elevated concentrations in humans, it is associated with gastrointestinal disorders, Wilson's disease, as well as kidney and liver damage (Royer & Sharman, 2023). On the other hand, deficiency of copper results in symptoms similar to anemia, neutropenia, and other health complications (Wazir & Ghobrial, 2017). Similarly, cobalt is a crucial element for living organisms, functioning as a primary component of vitamin B12 (Russel, 2022). Excessive intake of cobalt is associated with peripheral neuropathy, cardiomyopathy, and hypothyroidism (Tower, 2010; Jelkmann, 2012).

Elements like Arsenic (As), Cadmium (Cd), Lead (Pb), and Mercury (Hg) have no known functions in living beings and they exhibit toxic effects even at trace amounts (Tchounwou et al., 2012) and therefore considered non-essential heavy metals. Toxic effects of these metals on several organisms have been well documented in several scientific reports (Prozialeck et al., 2002; Gogoi et al., 2024). Non-essential heavy metals can damage cell membrane integrity and alter the functions of organelles such as mitochondria, lysosome, endoplasmic reticulum, and nucleus. They may interact with DNA, enzymes, and proteins and lead to conformations that affect modulations in the cell cycle, ultimately leading to carcinogenesis and apoptosis (Gogoi et al., 2024). Some heavy metals including As, Cd, Pb and Hg are highly toxic since they lead to formation of reactive oxygen species (ROS) and therefore result in oxidative stress in organisms (Lee et al., 2012; Zhu & Costa, 2020; Gogoi et al., 2024); and based on this, these heavy metals

are considered carcinogenic agents in human beings and animals (IARC, 1993; USEPA, 2007).

Surface runoff and running waters carry several pollutants including heavy metals dissolved in water to aquatic habitats from terrestrial habitats (Pintilie et al., 2007; Brodie et al., 2012; D'Avignon et al., 2022). Several reports are available demonstrating the heavy metal concentration in sediments and water samples from rivers worldwide. The recent research on the concentration of heavy metals present in water and sediment samples of some large rivers have been summarized in Table 1.

Table1. Heavy metal concentrations in sediment and water samples from some large rivers worldwide.

River name/Country	Heavy metals	Concentration	References
Sediment (mg/kg)			
Congo River / Congo	As Cd Co Cr Cu Hg Ni Pb Zn	0.15-4.80 0.02-6.55 0.33-14.25 3.08-95.49 1.77-139.91 0.02-4.92 1.32-37.10 2.36-200.89 7.41-285.03	Mata et al., 2020
Euphrates River/ Iraq	Cd Cu Pb Zn	1.29 ± 0.96 67.52 ± 21.86 63.23 ± 27.88 156.15 ± 88.94	Kadhum et al., 2020
Pearl River / USA	As Co Cr Cu Pb Se U Zn	2.3 ± 0.7 4.0 ± 1.5 19.1 ± 5.7 14.0 ± 9.1 29.6 ± 19.6 0.2 ± 0.3 1.6 ± 0.4 49.0 ± 27.8	Paul et al.,2021
Yangtze River/ China	As Cd Cu Hg Mn Ni	8.97–33.86 0.33–0.89 13.94–37.00 0.01–0.09 724.7–1620.7 26.11–33.95	Li et al., 2020

	Pb Zn	16.89–41.76 71.92–130.93	
Nile River / Egypt	Cd Co Cr Cu Fe Mn Ni Pb Zn	0.2-2.6 31.7-79.1 ND-8.5 18.9-53.6 8398-14119 106.2-548.7 5.2-40 13.88-79.38 14.5-143.6	Goher et al., 2021
Arrecifes River / Argentina	As Cd Co Cr Cu Fe Mn Ni Pb Zn	4.93 0.14 10.4 15.5 19.1 29100 670 10.4 11.2 45.9	Peluso et al., 2022
Kızılırmak River / Türkiye	As Cd Cr Cu Fe Hg Mn Ni Pb Zn	15.8 0.86 212.3 18.5 30100 0.44 922 121.9 13.6 52.5	Cüce et al., 2022
Orange River / Namibia	As Cr Cu Fe Mn Ni Pb Zn	55-105 99-290 23-133 14787-95464 478-2211 23-65 4 44-205	Pitiya et al.,2022
Rhine River / Germany	Cd Cu Ni Pb Zn	1.3 ± 0.5 75 ± 38 55 ± 12 62 ± 10 430 ± 210	Klein et al., 2022
Danube River / Serbia	As Cd Co	15.05 ± 5.55 2.75 ± 1.00 18.05 ± 2.13	Kašanin-Grubin et al., 2023

	Cr Cu Ni Pb Zn	14.24 ± 2.31 98.79 ± 39.20 82.17 ± 19.58 91.45 ± 27.58 353.91 ± 93.97	
Chía River / Peru	Cu Cr Fe Mn Hg Mo Ni Pb V Zn	8.8 ± 0.04 11.57 ± 0.53 8922.33 ± 114.61 145.41 ± 0.55 0.16 ± 0.01 0.39 ± 0.03 9.39 ± 0.55 13.31 ± 0.05 15.94 ± 0.04 120.12 ± 1.86	Custodio et al., 2024
Ganges River / India	Cd Cr Cu Fe Ni Pb Zn	0.479 0.181 0.284 39.22 0.512 0.494 1.071	Debnath et al., 2024
Water (µg/L)			
Changjiang River/ China	Cd Cu Ni Pb Zn	0.89-1.21 2.31–4.53 0.85–1.44 2.03–15.03 1.79–13.67	Li et al., 2020
Mantaro River / Peru	As Cu Fe Pb Zn	21.10 ± 7.82 14.60 ± 7.37 1140 ± 1488.0 9.50 ± 9.10 58.30 ± 32.10	Custodio et al., 2020
Nyamwamba River / Uganda	Co Cu Pb Zn	10-250 140-800 20-50 20-80	Wilber et al., 2020
Nile River /Egypt	Cu Fe Mn Pb Zn	20-213 120-7290 10-1040 3-306 15-526	Ghannam, 2021
Arrecifes River / Argentina	As Cd Co Cr Cu	57.5 0.1 0.32 0.6 3.31	Peluso et al., 2022

	Fe Mn Ni Pb Zn	3.57 0.95 0.88 0.24 4.16	
Ganges River / India	Ba Cu Fe Li Mn Zn	44.3 7.78 151.4 4.08 16.7 20.4	Nazir et al., 2022
Grand River / Canada	Al Cd Co Cr Cu Fe Li Mn Mo Ni Pb U V Zn	1-24 0.01-0.8 0.1-3.6 0.08-2.7 0.6-4.7 5.4-39 0.1-5.3 1.2-17 0.1-14 0.2-9.3 0.2-2.2 0.5-0.8 0.3-5.9 1.6-19	Pinter, 2022
Kızılırmak River / Türkiye	Al Ag As B Ba Cd Cr Co Cu Fe Hg Mn Ni Pb Zn	837.6 0.6 3.1 342.5 71.5 0.014 3.9 0.8 5.2 788.9 0.032 47.2 5.2 0.7 10.14	Üstün Odabaşı & Ceylan, 2023
Niger River / Nigeria	Cd Ni Pb Zn	3-6 2-60 222-412 83-410	Ekpe et al., 2023
Volga River / Russia	Al As B	5.6 ± 3.4 1.9 ± 0.6 72 ± 29	Shinkareva et al., 2023

	Cu Li Mn Mo Ni Pb U V Zn	1.9 ± 2.4 8.5 ± 2.5 29 ± 43 0.8 ± 0.2 1.7 ± 0.8 0.3 ± 0.2 0.6 ± 0.4 1.4 ± 0.5 8.5 ± 4.9	
Tocantins River / Brazil	Al Cu Fe Hg	690 50 460 0.2	Acioly et al., 2024

Among heavy metals, As, Cd, Cr, Hg, and Pb are classified as non-threshold toxic elements and their concentrations in freshwaters have increased over the last decades (Kumar et al., 2019; Kumar et al., 2023; Soetan et al., 2024). Non-threshold elements have toxic effects on all forms of life even at trace amounts (Balali-Mood et al., 2021). As, Cd, Cr, Hg, and Pb are among the most hazardous elements and reported as the priority contaminants by ATSDR (2024). Similarly, according to WHO As, Cd, and Cr (VI) are Group 1 carcinogens, while Pb and Hg are considered Group 2A and 2B carcinogens, respectively (Briffa et al., 2020). These metals have a high capacity to accumulate in various tissues of several organisms and have a high tendency for biomagnification through the aquatic food webs, which in turn create a serious risk for global environmental quality and human health. Therefore, in this study we focused on the effects of As, Cd, Cr, Hg, and Pb exposure on aquatic organisms.

ARSENIC

Arsenic (As) is a non-metalic element (atomic number: 33) found in nature with a high mobility and toxicity (Jomova et al., 2011). Arsenic metalloids are divided into 2 groups; organic and inorganic forms. The arsenide form has a high solubility, and it is the most common form observed in aquatic habitats (Mudhoo et al., 2011).

Arsenic have several uses in agricultural activities (in pesticide formulations), wood industry (as a wood preservative), and metalworks

(as an alloying agent) and production of semiconductors. Arsenic emissions have increased due to improper disposal of wastes contaminated with As and also processing and combustion of fossil fuels like coal (Singh et al., 2022). Besides, natural processes such as volcanic activity, erosion, and geothermal activities have an important role in As pollution (Tchounwou et al., 2012). After its release into the environment, it quickly interacts with water, soil, and sediments. The mobility and bioavailability of this heavy metal is regulated by pH, redox conditions, and concentration of other ions present in the media (Bissen & Frimmel, 2003).

Arsenic pollution presents significant threats to human and environmental health. Prolonged exposure to arsenic in humans through consumption of water or food can result in carcinogenesis, including bladder, lung, liver, and skin cancers (Briffa et al., 2020). Exposure to arsenic may also lead to cardiovascular diseases, diabetes, neurotoxicity, and developmental complications (Singh & Sharma, 2022). Arsenic affects several biological activities of living organisms, probably due to oxidative stress and bioaccumulation (Byeon et al., 2021; Ghosh et al., 2022). The results of the recent experimental studies indicating the toxic effects of As exposure on several freshwater organisms are summarized in Table 2.

Table 2. Toxic effects of Arsenic (As) exposure on some aquatic organisms

Species	Exposure concentration and duration	Effects	References
Algae			
Chlorella pyrenoidosa	50 mg/L – 16 days	Decrease in biomass.	Podder & Majumder, 2016
Nostoc muscorum	50, 100 and 150 mM – 4 days	Reduction of chlorophyll a, carotenoids, and phycocyanin content. Increase in respiratory rate, SOD, CAT and GST activity.	Patel et al.,2018
Chlamydomonas acidophila	0.1 and 20 mM – 24 hours	Increase of hydroethidine fluorescence. Disruption in mitochondria, stigma and thylakoids. Overexpression	Díaz et al., 2020

		of *CaPCS2* gene.	
Invertebrates			
Asellus aquaticus	80 µg/L – 7 days	Increase in metallothionein level.	Bouskill et al., 2006
Daphnia magna	49 µg/L – 2 days	Alterations in alanine and lysine levels.	Nagato et al., 2013
Hyalella curvispina	0, 0.5, 1 or 1.5 mg/L – 4 days	Decrease in ChE and GST activity.	Kirilovsky et al., 2022
Fish			
Channa punctata	6.936 mg/L – 7 days	Induction of micronuclei frequency.	Yadav & Trivedi, 2009
Gobiocypris rarus	26-77 µg/(g.dm) – 30 days	Reduction in growth rate and feeding ratio, food conversion efficiency. Liver cell anomaly.	Erickson et al., 2010
Catla catla	20.41 and 2.041 – 4 and 35 days	Increase in hematocrit content, white blood cell counts, GPT and GOT level. Decrease in plasma protein level, and LDH activity.	Lavanya et al., 2011
Labeo rohita *Cirrhina mrigala* *Catla catla* *Ctenopharyngodon idella*	0.05 and 5 mg/L – 30 days	Induce DNA damage in erythrocytes.	Kousar & Javed, 2014
Danio rerio	50 µg/L – 90 days	Increase in ROS, MDA, and CD levels. Increased mRNA level of nuclear factor (erythroid-derived 2)-like 2 (*Nrf2*). mRNA expression of glutathione peroxidase (*Gpx1*), CAT, manganese superoxide dismutase, copper/zinc superoxide dismutase and cytochrome c oxidase1 (*Cox1*) were up regulated.	Sarkar et al., 2014
Heteropneustes fossilis	1,75 mg/L, 30 days	Decrease in glycogen concentration. Alteration in activities of glycogen metabolic enzymes and glycolytic enzymes.	Tariang et al., 2019

CAT: Catalase, CD: conjugated diene, ChE: Cholinesterase, GOT: Glutamic oxaloacetic transaminase, GPT: Glutamic-pyruvic transaminase, GST: Glutathione S-transferases, LDH: Lactate dehydrogenase, MDA: Malondialdehyde, ROS: Reactive Oxygen species, SOD: Superoxide dismutase

CADMIUM

Cadmium (atomic number: 48) is a smooth, light grey metal classified in Group XII of the periodic table, exhibiting physical and chemical similarities with zinc and mercury. It is pliable, ductile, and predominantly displays a 2^+-oxidation state in its compounds. It has a low water solubility. It rapidly oxidizes to cadmium oxide (CdO) when exposed to air. Cadmium can be found as cadmium chloride, cadmium sulfate, and cadmium nitrate through reactions with HCl, HNO_3 and H_2SO_4, respectively. This non-essential heavy metal has been categorized as a Group 1 carcinogen (IARC, 1993).

Cadmium is used in various factors as a stabilizer, ingredient, and pigment in PVC manufactures, Ni-Cd batteries, and paints. Cadmium's corrosion resistance characteristics offer advantages to its use in borosilicate glass, lamps, solar panels, and infrared optical devices (Karunakaran & Dhanalakshmi, 2009). Environmental cadmium concentrations have increased because of both natural processes (such as volcanic activities, erosion, and big forest fires) and anthropogenic activities (such as mining, fossil fuel burning, and use of phosphate fertilizers) (Casado et al., 2008). In addition, cadmium contaminated wastes are frequently associated to non-ferrous metal smelting and electronic waste recycling.

The discharge of cadmium into the environment presents considerable hazards to both people and environmental health. In human beings, cadmium exposure predominantly occurs through inhalation and, to a lesser degree, ingestion. Upon entering the body, it binds to white blood cells and albumin, accumulates in the intestines, kidneys, and, liver, with a gradual elimination by urine, and breast milk (Satarug, 2018, Tinkov et al., 2018). Prolonged exposure may lead to damage in kidney and liver cells, edema in lungs, alterations in testis functions, osteomalacia, and corruption in the adrenal glands and hematological systems. Cadmium can also affect epigenetic systems, including DNA methylation, histone modification, and microRNA production, which govern gene activity and facilitate carcinogenesis. It may result in oxidative damage in DNA (both cellular and mitochondrial), proteins, and lipids through ROS production and may alter ATP synthesis

(Amamou, 2015). The results of the recent experimental studies indicating the toxic effects of Cd exposure on several freshwater organisms are summarized in Table 3.

Table 3. Toxic effects of Cadmium (Cd) exposure on some aquatic organisms

Species	Exposure concentration and duration	Effects	References
Algae			
Chlorococcum sp.	0.1-200 mg/L – 10 days	Decrease in growth rate, chl-a and chl-b concentration. Thickening of the cell wall.	Qiu et al., 2006
Chlorella vulgaris	0.11 and 0.22 mg/L – 2 days	Decreases in growth rate, chlorophyll content, and mRNA expression of *psbA* and *rbcL* genes. Increase in ROS production and transcription of the *psbB* gene.	Qian et al., 2009
Scenedesmus obliquus	0.3 mg/L – 7 days	Decrease in the growth rate.	Butler, 2012
Microcystis aeruginosa	0.01-0.4 mg/L – 4 days	Decrease in phycocyanobilin and chlorophyll content, increase in malondialdehyde, superoxide dismutase, CAT, and peroxidase activities.	Qian et al., 2012
Invertebrates			
Potomida littoralis	0.08, 0.09, 0.1, 0,25 mg/L – 2 hours	Decrease in active filtration rate.	Mouabad et al., 2001
Procambarus clarkii	0.01 and 0.03 mg/L -21 days	Damage to gill epithelial cells and an increase in metallothionein levels.	Martín-Díaz et al., 2006
Daphnia magna	0,06, 18, 100 mg/L– 21 days	Downregulation of the expression of glucanase, peptidase, and fatty acid binding proteins, vitellogenin, lectin, and β-glucan binding proteins.	Poynton et al., 2008
Sinopotamon henanense	0.71, 1.43, 2.86 mg/L – 21 days	Decline in oxygen consumption, oxyhemocyanin levels, and	Xuan et al., 2013

		cytochrome c oxidase (*cco*) expression levels.	
Gammarus pulex	3.4, 6 µg/L- 10 days	Reduction in lipid and glycogen content, protein concentration. Increase MDA level, and GSH concentration.	Vellinger et al., 2013
Hyalella azteca	0,0012 mg/L – 1 day	Increase in the expression *Cnc*, heat shock protein (*Hsp90*), DNA repair protein (*Rad51*), and ABC transporter proteins (*Mrp4*).	Gott, 2016
Fish			
Carassius gibelio *Corydoras paleatus* *Cyprinus carpio*	0.005 and 0.1 mg/L – 21 days	Micronuclei formation in gill and liver cells	Cavas et al., 2005
Oreochromis niloticus	0.56, 1.12 and 2.24 mg/L – 14 days	Decrease in glutathione and metallothionein levels.	Atlı & Canlı 2008
Perca fluviatilis	0.2 mg/L – 14 days	Decline in leukocyte counts in the liver, kidneys, and spleen.	Zabotkina et al., 2009
Clarias lazera	0.07 and 0.17 mg/L	Decrease in the hepatosomatic index.	Habib & Samah, 2013
Gobiocypris rarus	0.002, 0.02, and 0.2 mg/L – 3 days	Increase in malformations, and transcription of *hsp70*, *vezf1*, *mt* and *cyp1a* genes. decrease in SOD, MDA, CAT, GSH and LDH activities.	Zhu et al., 2014

CAT: catalase, chl-a: Chlorophyll a ,chl-b: Chlorophyll b, *Cnc*: nuclear transcription factor, GSH: Glutathione ,LDH: Lactate dehydrogenase, MDA: Malondialdehyde ,SOD: Superoxide dismutase.

CHROMIUM

Chromium (Cr; atomic number 24) is a transition metal commonly used in alloy fabrication, electroplating, pigment synthesis, pharmaceuticals, and metal processing (Jeong et al., 2023). The wide scale use of Cr has induced considerable environmental pollution, particularly in freshwater habitats. Its primary sources are industrial wastes, electroplating, battery manufacturing, and uses in several fertilizer and pesticide formulations (Bakshi & Panigrahi, 2018).

Chromium can be found in different oxidation states, with Cr (III) and Cr (VI) being the most consistent structures. Cr (III) has a limited toxicity, but Cr (VI) (frequently found as chromate; CrO_4^{2-} and dichromate; $Cr_2O_7^{2-}$), is extremely mobile, reactive, and toxic. These characteristics enable Cr (VI) a significant environmental contaminant and an important problem for ecological systems (Bakshi, 2016). Inhalation, ingestion, and direct contact are the possible uptake mechanisms of chromium (VI) uptake in humans. The high accumulative capacity and bioavailability of Cr (VI) cause several alterations in living organisms, such as anemia, immunological suppression, DNA damage, malfunctions in osmoregulation, oxidative stress, and cellular dysfunction. In addition, long-term exposure to Cr (VI) may lead to damage in lung, kidney and liver cells, and ultimately leading to lung, liver, and kidney cancers (Briffa et al., 2020). In addition, Cr (VI) affects the microbial flora of aquatic sediments, decreasing microbial diversity and altering the general stability of the aquatic habitats (Velma et al., 2009; Bakshi & Panigrahi, 2018). The results of the recent experimental studies indicating the toxic effects of Cr exposure on several freshwater organisms are summarized in Table 4.

Table 4. Toxic effects of Chromium (Cr) exposure on some aquatic organisms

Species	Exposure concentration and duration	Effects	References
Algae			
Dictyosphaerium chlorelloides	0.1, 0.5, 1, 2, 5, and 10 mg/L – 3 days	Inhibition in growth and PSII activity.	Sánchez-f ortún et al., 2009
Monoraphidium convolutum	0.1, 0.5, 1, 5, and 10 mg/L	Increase in GR, and APX activity. Decrease in electron transfer rate.	Takami et al., 2012
Chlorella variabilis	446, and 942 µM – 12, 24, and 48 hours	Decrease in chl (a+b), ß carotenoid/chl (a+b) levels, and electron transport rate.	Zsiros et al., 2020
Chlamydomonas reinhardtii	20, 40, 60, and 80 µM – 6 days	Inhibition in growth, and PSII. Morphological changes. Increase in ROS level.	Zhang et al., 2021

Chlorella vulgaris	1, 3, 6, and 9 mg/L – 4 days	Inhibition of chl- a synthesis, and decrease in dry weight. Decrease in Fv/Fm, and PSII activity.	Zhou et al., 2023b
Invertebrates			
Barytelphusa guerini	11.3, and 15,62 mg/L, 30, 60, and 90 days	Decrease of glycogen, and glucose content in gill and hepatopancreas.	Sridevi & Reddy, 2000
Pseudosida ramosa	0.3, 0.6, 1, 3, and µg/L – 21 days	Decrease in fertility and fecundity rate.	Freitas & Rocha, 2014
Caenorhabditis elegans	1, 10, 100, and 1000 µM – 1 day	Increase in ROS level. Upregulation in expression of *actin*, *sod-3*, *hsp-16.2*, and *gst-4*.	Saikia et al., 2013
Geloina coaxans	4.34, 8.69, 17.38, and 34.76 mg/L – 1, 2, and 3 days	Histological disruption. Increase in the MDA content. Alterations in GST and CAT activity.	Guo et al., 2020
Daphnia carinata	0.05, 0.1, 0.2 mg/L – 21 days	Decrease in reproductive rate and body length.	Zhou et al., 2021
Fish			
Oncorhynchus mykiss *Ictalurus punctatus* *Lepomis macrochirus*	0.5, 1, 2, and 4 mg/L – 5, 7, and 10 days	Increase in DPX levels in erythrocytes.	Kuykendall et al., 2009
Channa punctatus	2, and 4 mg/L – 30, and 60 days	Lamellar fusion, edema, and hyperplasia in gill. Hypertrophy in epithelial, tissue in kidney. Vacuolization, cytolysis, and shrinkage of hepatocytes in liver. Decrease in cortisol level.	Mishra & Mohanty, 2009
Oreochromis niloticus	0.003 mg/L - 30 days	Increase in ALT and AST activities.	Öner et al., 2009
Danio rerio	100, 500, 1000, and 5000 mg/L – 1, and 2 days	Decrease in swimming activity.	Bichara et al., 2014

ALT: alanine transaminase, AST: aspartate aminotransferase, APX: Ascorbate peroxidase, CAT: catalase, chl: chlorophyll, DPX: DNA-protein cross-links, Fm: maximum fluorescence, Fv: variable fluorescence, GR: Glutathione reductase, GST: glutathione S-transferases, MDA: malondialdehyde, ROS: reactive oxygen species, ΦPSII: quantum yield of photosystem II

LEAD

Lead (Pb; atomic number 82) is an element with a distinctive blue-white color; and having a high corrosion resistance (Tchounwou et al., 2012). It is among the most ancient metals used by humanity and is predominantly found in the Earth's crust. Pb exists in several mineral forms, including lead carbonate ($PbCO_3$) and lead oxide (PbO). Lead mainly exists in two oxidation states: Pb^{2+} (lead (II)) and Pb^{4+} (lead (IV)), while Pb^{2+} being the more stable and widespread form in environmental pollution (Holecy ve Mousavi, 2012).

Historically, Pb was utilized in the manufacture of pipes, paints, and gasoline; however, its application in many of these products has been limited due to its harmful effects. But it is still widely used in the manufacture of lead containing batteries, and in radiation shielding. It is also utilized in soldering, and production of some alloys (Tchounwou et al., 2012).

Lead is considered a significant threat to environmental quality and to human health. Both natural events like volcanic explosions and rock weathering or tectonic activities, and several human activities (such as mining, extraction, metal plating, and the disposal of lead-containing goods) are responsible for the release of lead contaminated wastes into environment. The utilization of lead in battery production, plumbing, and electronics seems to be most important source of lead pollution. Lead pollution also arises from the incorrect disposal of lead-based products and emissions from leaded gasoline (Singh et al., 2022). Lead may remain in the environment for prolonged durations after discharge, especially in soil and sediments, where it forms a strong bond with both organic and inorganic substances, thereby limiting its mobility in certain circumstances.

In aquatic ecosystems, lead interferes with biochemical and physiological functions of aquatic organisms. Lead exposure may reduce enzyme activity, primarily by interacting with thiol groups, resulting in a series of metabolic disturbances. Lead exposure may also cause behavioral alterations, and reproductive deficiencies in fish and other aquatic creatures. Lead accumulates in the tissues of organisms, resulting in chronic toxicity and threaten animals at higher trophic levels (Lee et

al., 2019). Moreover, lead adversely affects microbial communities in sediments (George & Wan, 2019).

Lead uptake in humans occurs through consumption of contaminated food or water, inhalation or air emissions, or cutaneous exposure after direct contact. Upon absorption, lead accumulates in bones, kidneys, and soft tissues, where it imitates calcium and disrupts vital biological functions. Prolonged exposure to lead may end up with neurological deficits, renal injury, cardiovascular disorders, and reproduction toxicity (Collin et al., 2022). The results of the recent experimental studies indicating the toxic effects of Pb exposure on several freshwater organisms are summarized in Table 5.

Table 5. Toxic effects of Lead (Pb) exposure on some aquatic organisms

Species	Exposure concentration and duration	Effects	References
Algae			
Chlamydomonas reinhardtii	80 µmol/L – 1, 3, 5, and 7 days	Decrease in cell counts, photochemical effectiveness of PSII and total chlorophyll volume. Increase in CAT, POD, SOD activities, and MDA content.	Zheng et al., 2020
Scenedesmus sp. *Chlorella* sp.	100, 150, 200 mg/L – 10 days	Decline in chlorophyll content. Increase in SOD, MDA, and CAT activity.	Kashyap et al., 2021
Microcystis aeruginosa	0.5 mg/L – 2, 4, 6, and 8 days	Decrease in chlorophyll content. Increase in CAT and SOD activity, and total protein content.	Wang et al., 2021
Scenedesmus acutus Chlorella pyrenoidosa	300, 350, 400, 450, 500, 550, and 600 mg/L – 1 to 4 days	Decrease in electron transport rate, and photosynthetic pigment content.	Purushanahalli Shivagangaiah et al., 2021
Chlorella pyrenoidosa	2 mg/L – 36 hours	Increase in MDA and soluble sugar levels, and SOD activity. Decrease in soluble protein levels, CAT and POD activity. Decline in cell density and chl-a.	Yu et al.,2024
Invertebrates			

Gammarus fossarum	5 µg/L – 10 weeks	Inhibition of respiratory and digestive enzymes.	Lebrun & Gismondi, 2020
Proales similis	13, 25, 50, and 100 µg/L – 5 days	Decline in population density.	Rebolledo et al., 2021
Macrobrachium dayanum	29.12 mg/L – 10, 20, and 30	Alterations in haemocyte counts.	Tiwari et al., 2022
Daphnia magna Daphnia similis	50 µg/L – 4 days	Decrease in survival rates and decrease AChE activity.	De Araujo et al., 2024
Unio tigridis	100, 300, 500, 700, 900 mg/L – 1, 2, 3, and 4 days	Increase in GST, CAT activities and MDA levels.	Hanna & Shekha, 2024
Fish			
Oreochromis niloticus	100, 400, and 800 µg/g dry Weight – 60 days	Decrease in lipase, amylase, and trypsin activities.	Dai et al., 2009
Danio rerio Poecilia reticulata	500 µg/L – 1, and 3 days	Reduction of monoamine oxidase.	Senatori et al., 2009
Oreochromis niloticus	0.05 mg/L – 4, and 21 days	Increase in ALT and AST activities. Increase in the ALP and LDH activities.	Fırat et al., 2011
Cyprinus carpio	1.5 mg/L – 14 days	Reductions in GSH level in liver and brain cells. Increase GST and GSH-Px activities, and MDA level in liver.	Özkan-Yılmaz et al., 2014
Channa argus	50, 200, and 800 µg/L – 14 and 28 days	Decrease in CAT and GPX activities. Increase in PC and MDA contents, and relative expression of *HSP60*, *HSP70*, and *HSP90*.	Zhao et al., 2020

ALT: alanine transaminase, AST: aspartate aminotransferase, AChE: Acetylcholinesterase, CAT: catalase, GSH: Glutathione, GSH-Px: Glutathione peroxidase, GST: glutathione S-transferases, MDA: Malondialdehyde, POD: Peroxidase, PSII: Photosystem II, SOD: Superoxide dismutase

MERCURY

Mercury (Hg; atomic number: 80) is a hazardous heavy metal found in several chemical forms, each reflecting distinct toxicity levels. The predominant forms of mercury in the ecosystem are Hg(II) (Hg^{2+}),

Hg(I) (Hg^{+}), elemental mercury (Hg_0), and organic mercury compounds (such as methyl mercury). It is extensively used in mining, smelting, coal combustion, and the manufacture of batteries, thermometers, and electrical switches. Mercury is released into environment via industrial debris, sludge disposal, and the use of fungicides. A substantial fraction of mercury emissions originates from anthropogenic activities, comprising around one-third of total world discharges (Banjare & Markam, 2022).

Mercury lacks any recognized physiological functions in organisms and is considered a non-essential metal (Martinez-Finley & Aschner, 2014). Mercury (II) is a highly reactive and soluble variant that accumulates in higher plants and aquatic species, leading to considerable environmental problems and health issues (Ali et al., 2019). Mercury undergoes a variety of modifications when it enters the environment, including biomethylation and reduction to elemental mercury through bacterial activities (Gonzalez-Raymat et al., 2017). These mechanisms facilitate the accumulation and biomagnification of mercury along the food chain.

Mercury exposure impairs cellular functions by attaching to thiol groups, disrupting mitochondrial action, and eventually provoking oxidative stress (Wyatt et al., 2017). Mercury can also cause stomatal closure in plants, suppresses photosynthesis, and replaces magnesium in chlorophyll (Mei et al., 2021). Toxic effects of mercury in aquatic organisms includes inhibition of division in cells, chromosomal damage, reductions in growth rate and reproductive success (Crump & Trudeau, 2009; Nirchio et al., 2019). Moreover, mercury influences microbial populations, and reduces microbial diversity and alters nutrient cycling within ecosystems (Zheng et al., 2022).

Human beings are generally exposed to mercury through consumption of contaminated water sources and seafood or inhalation of contaminated air. Methyl mercury, the most dangerous variant of Hg, can easily pass through the blood-brain barrier and the placental barrier, and lead to impairment in nervous system, particularly in fetuses. Prolonged exposure to mercury may also cause to cognitive deficiencies, motor disorders, and developmental delays. Besides its neurotoxic

effects, mercury exposure is associated with renal impairment, cardiovascular disorders, and immune system malfunction (Bose-O'Reilly et al., 2010; Gao et al., 2022). Due to its extreme toxicity and environmental durability, mercury is considered a major threat for all forms of and for ecological integrity in all environmental compartments (Teixeira et al., 2018). The results of the recent experimental studies indicating the toxic effects of Hg exposure on several freshwater organisms are summarized in Table 6.

Table 6. Toxic effects of Mercury (Hg) exposure on several freshwater organisms

Species	**Exposure concentration and duration**	**Effects**	**References**
Algae			
Chlamydomonas reinhardtii	1, 2, 4, 6, and 8 μM – 4 days	Inhibition of cell growth and decrease in chlorophyll content. Increase in SOD, and CAT activities. Up-regulation in expression of the genes coding *Mn-SOD*, *CA*, *APX*, and *HO1*.	Elbaz et al., 2010
Chlorella vulgaris	2.6, 5.2, 10.4, 20.8, 41.7, and 83.4 mg/L – 2, and 7 days	Morphological transformations. Decrease in photosynthetic pigment and protein levels, and SOD activity. Increase in ROS content and CAT activity.	Ajitha et al., 2021
Scenedesmus quadricauda	0.1, 0.3, 0.5, 0.7, and 0.9 mg/L – 1, 3, 5, 7, and 9 days	Separation of cell wall, decrease in SOD, and POD activities. Increase in MDA content. Inhibition protein synthesis.	Ge et al., 2022
Microcystis aeruginosa	5, 10, 20 and 30 μg/L – 4 days	Cellular disruption. Decrease in photosynthetic activity. Increase in ROS content and SOD activity.	Tang et al., 2023
Invertebrates			

Brachionus patulus	0.675, 1.35, 2.7 and 5.4 µg/L – 24 days	Inhibition of population growth. Reduction in the length of posterior spines.	Sarma et al., 2008
Daphnia magna Euchlanis dilatata	5 µg/L - 1 day	DNA damage.	De León et al., 2021
Gammarus sp.	50, and 500 ng/L – 7, 21 days	Over-expression in genes involved in respiration, and apoptosis.	De Melo et al., 2021
Bellamya bengalensis	0.05, and 0.08 mg/L – 7, 14, 21, and 28	Decrease in total haemocyte count, protein content in hepatopancreas, and protein content in gonad.	Dhara et al., 2022
Daphnia magna	0.02, 0.04, 0.06, and 0.08 mg/L – 35 min	Inhibition of mobility.	Qin et al., 2024
Fish			
Cyprinus carpio	0.1 mg/L – 1, 15, and 30 days	Suppression of acetylcholinesterase activity.	Suresh et al., 1992
Brycon amazonicus	0.15 mg/L – 4 days	Increases in SOD, CAT, GST and GR activities in liver cells.	Monteiro et al., 2010
Oreochromis niloticus	0.5, 1, 2, and 5 - 3, 6, 9, 12, and 15 days	Lesions in renal tubule and intestinal epithelium.	Kaewamatawong et al., 2013
Oreochromis niloticus	0.08 mg/L – 3, 7, 10, and 14 days	Decrease in hemoglobin level and leukocytes counts.	Seriani et al., 2015
Danio rerio	10, and 100 µg/L – 1, 2, 3, and 4 days	Morphological malformations. Decrease in CAT, and ACH activity.	Henriques et al., 2023

ACH: Acetylcholinesterase, APX: Ascorbate peroxidase, CAT: catalase, GR: glutathione reductase, GST: glutathione S-transferase, MDA: Malondialdehyde, POD: Peroxidase, ROS: reactive oxygen species, SOD: Superoxide dismutase.

CONCLUSION

Arsenic, cadmium, chromium lead and mercury are among a number of persistent and hazardous contaminants that have a major impact on aquatic ecosystems. Discharge of these contaminants from industrial effluents, agricultural runoff, and mining activities, and natural

processes (such as erosion and volcanic activity) have been increased over the last decades. Heavy metals interact with biotic and abiotic components after entering the aquatic systems, and are absorbed in the tissues of several organisms and eventually result in biomagnification in greater trophic levels. Exposure to heavy metals causes oxidative stress, alteration of cellular and metabolic functioning, and genotoxicity, inhibition of growth, development, alteration of interactions among different trophic levels, and decrease survival rate of several species and inevitably declines in biodiversity worldwide.

In this chapter we presented an extensive review of the toxicological impacts of arsenic, cadmium, chromium, lead, and mercury on various freshwater organisms, based on the recent experimental studies. The findings underline the necessity for taking necessary precautions to mitigate heavy metal contamination and developing purification strategies for remediation of heavy metals from natural waterbodies.

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CHAPTER 4

EFFECTS OF NANOPARTICLES ON AQUATIC ORGANISMS

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INTRODUCTION

Nanoparticles (NPs) are particles of 100 nm and smaller. These particles are nanomaterials with very different shapes such as spherical and tube (Nowack and Bucheli, 2007; Cupaioli et al. 2014). NPs can be examined in two parts according to their formation as natural and anthropogenic. Natural particles are formed as a result of natural events such as volcanic eruptions. Anthropogenic particles are formed as a result of thermodegradation events such as simple combustion products and power plants caused by humans (Cupaioli et al. 2014). NPs derived from metals such as titanium dioxide (TiO_2) and silver can be given as examples of anthropogenic products. Another classification is the classification according to the elements contained in NPs. NPs containing carbon (C) are biogenic, geogenic, atmospheric and pyrogenic, while inorganic NPs are natural NPs such as organic acids (Nowack and Bucheli, 2007).

The use of metallic NP materials has also increased due to developments in the industrial field. NPs produced with different chemical compositions, sizes and shapes are used in many areas such as electronics, biomedical, textile. While natural NPs are heterogeneous, engineered NPs are homogeneous. This enables more intensive use of engineered NPs in the industrial field (Nowack and Bucheli, 2007; Saini et al. 2010; Cupaioli et al., 2014).

This intensive use of NPs and different formation mechanisms affect the abiotic and biotic environment in the ecosystem. In terms of the abiotic environment, they tend to bind to pollutants in the environment and increase their toxic effects (Cheng et al., 2004; Gilliland et al., 2004). Especially the very small particle size accelerates the penetration of these substances into the cell or body on the biota and they become carriers that enable the pollutants to be delivered to places they normally cannot reach (Lacava et al., 2003; Berry et al., 2004). Studies examining the effects of NPs on the environment have focused on revealing NP toxicology and demonstrating environmental health effects (Baranowska-Wójcik et al. 2019). It is known that NPs cause many negative effects such as activating cellular mechanisms, causing

oxidative stress due to the formation of reactive oxygen species (ROS), and causing inflammation at the organism level (Chowdhury and Saikia, 2020). The aim of this review is to show the effects of metallic NP substances mixed into aquatic ecosystems on aquatic biota.

CONTAMINATION OF METALLIC NANOPARTICLES INTO AQUATIC ECOSYSTEMS

Metallic NPs can mix into aquatic ecosystems through anthropogenically contaminated soil surface runoff, industrial wastes, and wastewater disposal. Natural mixing pathways are the result of the mixing of colloidal substances and mineral sediments (Batley et al., 2013) Organisms living in aquatic ecosystems take NPs into their bodies directly by ingestion, gills or skin (Moore, 1990; 2006). In filter-feeding organisms, NPs found together with sediment, water or other pollutants are taken into the organism (Ray et al. 2020) (Figure 1).

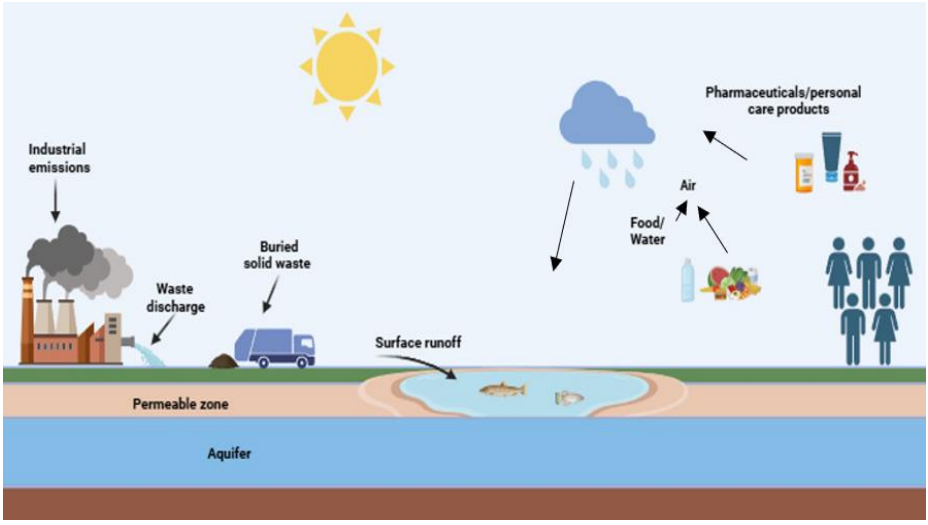

Figure 1. Formation of nanoparticles and their transport into the aquatic ecosystem (adapted from Kurwadkar et al. 2015)

LETHAL TOXIC EFFECTS OF NANOPARTICLES ON AQUATIC ORGANISMS

Xenobiotic substances mixed into aquatic and terrestrial ecosystems and the presence of these substances in the environment as a cocktail are of great importance in terms of environmental risk assessment (Persoone et al. 2009). There are many studies evaluating the possible toxic effects on organisms living in these ecosystems. The first step of these studies is to determine the lethal concentration values of xenobiotics in organisms. After determining these values, their adverse effects on organisms exposed to different routes such as oral and dermal are evaluated in acute toxicity studies (Saganuwan, 2016).

Acute toxicity tests are standard tests used to measure the effects of xenobiotics in aquatic ecosystems, and median lethal concentration values form the basis of toxicology studies (Brahma & Gupta, 2020). Studies on lethal concentration values have been standardized for organisms at different trophic levels (Persoone et al., 2009).

The mean lethal concentration values obtained in acute toxicity studies with aquatic organisms are given in Table 1.

Table 1. The median lethal toxicity (LC_{50}) values of NPs

Species	Nanoparticles (NPs)	Exposure time	LC_{50} (mg/L)	References
Daphnia magna	TiO_2	48h	5.5	Lovern and Klaper, 2006
Oncorhynchus mykiss	TiO_2	96h	>100	Warheit et al., 2007
Danio rerio (adult)	Ag Cu Al Co Ni TiO_2	48h	7.07 0.94 >10 >10 >10 >10	Griffith et al., 2008
Danio rerio (juvenile)	Ag Cu Al Co Ni TiO_2		7.20 0.71 >10 >10 >10 >10	
Daphnia pulex (adult)	Ag Cu Al Co Ni TiO_2		0.040 0.060 >10 >10 3.89 >10	

Table 1. The median lethal toxicity (LC_{50}) values of NPs (Continue)

Species	Nanoparticles (NPs)	Exposure time	LC_{50} (mg/L)	References
Ceriodaphnids dubia (neonates)	Ag Cu Al Co Ni TiO_2	48h	0.067 0.419 3.99 1.67 0.674 >10	Griffith et al., 2008
Pseudokirchneriela subcapitata	Ag Cu Al Co Ni TiO_2		0.19 0.54 8.30 Not measured 0.35 Not measured	
Daphnia magna	TiO_2 CuO ZnO	48h	~ 20 000 3.2 ± 1.6 3.2 ± 1.3	Heinlaan et al., 2008
Thamnocephalus platyurus	ZnO CuO	24 h	0.18 ± 0.03 2.1 ± 0.5	
Daphnia magna	TiO_2 ZnO	48h	143.387 1.511	Zhu et al., 2009
Daphnia magna	TiO_2	48h	> 10	Kim et al., 2010
Danio rerio (adults)	TiO_2	96h	124,5	Xiong et al., 2011

BIOCHEMICAL EFFECTS OF NANOPARTICLES ON AQUATIC ORGANISMS

NPs easily overcome biological barriers due to their small size. Thus, they accumulate at cell and tissue levels and are transported in increasing concentrations in the food chain through bioaccumulation and biomagnification (Sreya and Chitra, 2021).

One of the biochemical effects of NP toxicity in aquatic organisms is the formation of oxidative stress. Oxidative stress causes ROS, which are formed as a result of the increase in free radicals formed in the cell, to have negative effects on organic biomolecules (Chowdhury and Saikia, 2020; Wang et al., 2020). The cell defense mechanism against these events activates antioxidants. While examples of antioxidant enzyme systems are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), there are also small non-protein antioxidants such as ascorbic acid, reduced glutathione and vitamin A (Liu et al., 2018). One of the examples of lipid peroxidation (LPO) is the biomarker malondialdehyde (MDA) (Wojtczyk-Miaskowska and Schlichtholz, 2018).

Examples of biochemical effects obtained from NP toxicity studies with aquatic organisms are given in Table 2.

Table 2. Toxic effects of NPs

Species	Nanoparticles	Toxic effects	References
Oncorhynchus mykiss	TiO_2	Liver tissues: ↓GSH Gill tissues: ↑GSH; ↑LPO Brain tissues: ↑LPO Intestine tissues: ↑LPO	Federici et al. 2007
Danio rerio	Ag	↑LPO; ↑GSH; ↓CAT; ↓GPx	Choi et al. 2010
Daphnia magna	TiO_2	Antioxidant enzyme activities were significantly increased. CAT, GPX and GST showed a concentration-dependent increase.	Kim et al., 2010
Artemia salina	Pb	Oxidative stress has occurred.	Cornejo-Garrido et al.,2011
Artemia salina	C60 and TiO_2	Mortality values were observed at different levels with the change in nanoparticle size.	Rajasree et al.,2011
Danio rerio (adults)	TiO_2	In liver tissues, SOD, CAT, GSH levels decreased while protein carbonyl levels increased. Increased SOD and MDA levels were observed in intestinal tissues.	Xiong et al., 2011
Lymnaea luteola	ZnO	↑ LPO; ↑ CAT; ↓ GPx; ↓ GST; ↓ GSH; ↑DNA Damage	Ali et al., 2012
Cyprinus carpio	Ag	Brain: ↓GST	Lee et al. 2012
Chlorella vulgaris	Ag	↑ROS; ↑LPO	Oukarroum et al. 2012
Chlamydomonas reinhardtii	CuO	↑ROS	Perreault et al.,2012
Biomphalaria alexandrina	ZnO	For hemolymph ↑ LPO; ↓ GSH; ↓ GST; For tissues ↑ LPO; ↓ GSH; ↓ GST;	Fahmy et al., 2014

Table 2. Toxic effects of NPs (Continue)

Species	Nanoparticles	Toxic effects	References
Lemna gibba	CuO	↑ROS	Perreault et al., 2014
Daphnia magna	TiO_2	No significant effects on ROS	Tan and Wang, 2014
Oreochromis niloticus	TiO_2	Acute exposure results in decreased SOD, CAT and GPx activity, while chronic exposure results in increased CAT, GPx, GST and GR activity.	Fırat and Bozat, 2019
Labeo rohita	CuO	There was a decrease in tissue CAT activity and an increase in TBARS levels. The micronucleus ratio in erythrocytes increased.	Aziz and Abdullah, 2023
Pomaceae paludosa	ZnO	Esterase and alkaline phosphatase activities decreased.	Jeyavani and Vaseeharan, 2023
Unio delicatus	Ag	Gill: ↓MDA, ↑GSH Digestive gland: ↑MDA, ↓GSH	Şimşek et al., 2024

CONCLUSION

NP substances are among the materials used in many areas of daily life. The entry of these substances, which are used so widely, into ecosystems and their effects on organisms are quite alarming. The fact that their average lethal concentration values on aquatic organisms are quite low, they easily pass through biological barriers such as the blood-brain barrier and cause biochemical effects in the organism. For these reasons, it is very important that the studies on NPs in the coming years are more comprehensive and examined with more than one biomarker.

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CHAPTER 5

NANOPLASTICS AND THEIR EFFECTS ON AQUATIC ORGANISMS

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INTRODUCTION

The plastic age began after the discovery of first synthetic polymer named bakelite which is derived from formaldehyde and phenolic compounds (Frias and Nash, 2019; Williams and Rangel-Buitrago, 2022). Plastics are defined as "a polymeric material includes chemical substances, used to reduce cost and to enhance performance." (Rai et al., 2021). Synthetic polymers such as high- and low-density polyethylene, polyethylene terephthalate, polypropylene polystyrene, polyvinyl chloride are commonly used in the production of daily life goods (Boyle and Örmeci, 2020). Due to their lightweight, low cost, and ease of processing, they are widely used and produced polymers (PlasticsEurope, 2016). Nowadays, plastics are widely used in several industries including agriculture, electrical-electronics, packaging, construction and healthcare (Rai et al., 2021; PlasticsEurope, 2023).

The widespread use of plastics created a huge demand for plastic material production which inevitably resulted in the accumulation of plastic wastes almost in every compartment of the nature (Borrelle et al., 2020). The worldwide plastic load has exceeded 9.2 billion tons during the last few decades. For example, plastic production reached to approximately 400 million tons globally in 2022 (PlasticsEurope, 2023). Unfortunately, approximately 6.5 billion tons of plastic waste have been released into environment (Pilapitiya and Ratnayake, 2024), which resulted in a 20-fold increase in plastic pollution; (Walker and Fequet, 2023). Although, there is a great effort to eliminate plastic pollution by developing rules and policies to support plastic recycling (Knoblauch and Mederake, 2021) there are several studies indicating that recycling of plastic materials is relatively problematic (Roy et al., 2023). Similarly, most of the plastic waste needs a very long time to degrade in nature mainly due to the strong covalent bonds between monomers of synthetic plastic polymers. For example, approximately 117 years is required for single a plastic bottle made from high-density polyethylene to completely decompose in saltwater, or even up to 500 years in soil (Chamas et al., 2020).

There is no doubt that plastic pollution (mainly originating from exposure to micro or nano-sized particles) exerts as a substantial risk to

all forms of life (Beaumont et al., 2019; Kasavan et al., 2021). In addition, several additives added into plastics during their production phases in order to increase stability, flexibility or for an easy further processing. However, most of these chemicals are considered potent endocrine disruptors and they are not strongly bond to plastics so they can easily leach in water (Maddela et al., 2023; Ullah et al., 2023). For instance, bisphenol A (BPA) is a common plastic additive incorporated into polycarbonate plastic polymers (Chakraborty et al., 2022). However, the use of BPA in the production of food grade plastic is associated with a high health risk (Plasania et al., 2024). Furthermore, BPA may alter fertility, may lead to allergic skin reactions and irritation in respiratory track or even cancer in in humans (Vogel, 2009). Another common additive is phthalate which is used to decrease fragility in plastic materials particularly in the production of PVC. They are widely used in the production of food and beverage boxes/packages. However, phthalates alter the production of the sex hormone, androgen (Arrigo et al., 2023). Exposure to phthalates may induce cancer in human beings (Kumar, 2018). Similarly, another group of plastic additives is brominated flame retardants used to produce flame resistant plastic materials (Hennebert, 2020). But, polybrominated diphenyl ethers (PBDEs) are also considered potent EDCs (Ullah et al., 2023). Exposure to PBDEs are known to alter glucose metabolism and some hormone functions and to increase cancer risk in humans (Renzelli et al., 2023). The above mentioned additives also effect aquatic life. For example, PBDEs are found to be associated with apoptosis and arrhythmia in *Danio rerio* (Feiteiro et al., 2021). Therefore, since plastics may contain toxic additives, or adsorb toxic chemicals (such as heavy metals) on their surfaces (Coşkun et al., 2024), and are capable of freely circulating in nature, it is anticipated that the risks of exposure to micro- or nano-sized plastic particles are underestimated (Yurtsever, 2015; Coşkun et al., 2024).

Approximately 60-80% of the wastes found in oceans is made up of plastics (Shaikh and Shaikh, 2021; Vivekanand et al., 2021). Furthermore, up to 14 million tons of plastic debris flow into marine habitats each year (Haward, 2018). Most of the plastic wastes in the

oceans is generally linked to terrestrial activities. For instance, settlements, urbanization, and industrial activities along the coastal areas are primary sources of plastic pollution in terrestrial habitats (Sazlı et al., 2023).

The available research indicates that plastic particles -particularly micro- and nano-sized plastic particles- freely floating in water column are ingested by aquatic organisms which in turn lead to reduced stomach capacity, intestinal blockage, internal injuries, or even death in aquatic organisms (Sigler, 2014; Shaikh and Shaikh, 2021). Unfortunately, it has been reported that more than 140,000 marine organisms, including whales, dolphins, sea turtles, seals, and many fish species have died since 1990's as a result of ingesting plastics (Hidalgo-Ruz et al., 2012). Therefore, assessing the current status of plastic pollution in aquatic habitats and revealing their impacts on aquatic life is crucial to protect aquatic biodiversity. Thus, in this study, we reviewed the recent literature demonstrating i. the presence of nanoplastic particles, their size range and their distribution in aquatic ecosystems ii. and the impacts of Np exposure on some aquatic organisms, and biochemical responses of these organisms to nanoplastic particles.

Plastic particles: size does matter

Plastic wastes accumulated in aquatic and terrestrial ecosystems is quite likely to break down into fine particles through biotic and abiotic degradation processes (Choi et al., 2024). The resulting plastic particles differ in size (Li et al., 2016; Cai et al., 2018) and have been categorized into 4 subcategories; macroplastics (>5 mm), mesoplastics (5 mm to 2.5 cm), microplastic, and nanoplastic particles (Dhaka et al., 2022; Allen et al., 2022). Although, there is a debate on the size range of micro- and nano-sized plastic particles, microplastics are generally defined as particles <5 mm, and nanoplastics are 1 to 100 nm in at least one dimension with a colloidal behavior (Mattsson et al., 2018; Allen et al., 2022). Generally, nanoplastic particles are considered more hazardous to organisms due to their relatively small size, large surface area and higher persistence in nature (Oliveira and Almeida, 2019; Liang et al., 2023).

Nanoplastics are categorized into 2 main classes based on their sources. Primary nanoplastic materials are produced industrially to be used in the manufacturing of cosmetics, paints, pharmaceuticals and medical applications (Mattsson et al., 2018). Secondary nanoplastics are formed from larger plastics due to physico-chemical breakdown, photodegradation, biological activities and meteorological events (Mattsson et al., 2018; Sazlı et al., 2023). Micro and nanoplastic particles are regarded as the final phase of plastic degradation (Zhao et al., 2023). For example, it was found that microplastics present in facial cleansers can break down into nano-sized polyethylene particles (Hernandez et al., 2017). The researchers stated that polyethylene nanoparticles (24 to 52 nm) were found in 3 different facial cleansing products containing plastic microbeads (~0.2 mm).

NANOPLASTICS IN AQUATIC ENVIRONMENT

The extensive use of plastic polymers in several industries inevitably gave rise to an increase in the amount of plastic wastes deposited in environment (Sazlı et al., 2023). Improper plastic waste disposal, incomplete or poor recycling of plastics, or poor regulations on the use of disposable plastic materials, seems to be responsible for elevated nanoplastic concentrations in all compartments of nature (Bläsing and Amelung, 2018; Shi et al., 2024). Nanoplastic particles can disperse from the equator to the polar regions, or even in the deep ocean layers (Materić et al., 2022a; b). Nano-sized plastic particles can be observed in surface waters or sediments depending particularly on their density. Generally, nanoplastic polymers with a high density such as polyvinyl chloride, polyethylene terephthalate, and polystyrene have a tendency to accumulate in sediments. On the other hand, polymers such as low-density polyethylene, and polypropylene floats at the surface layers (Haegerbaeumer et al., 2019).

The initial phase of the transportation of plastic debris to aquatic ecosystems (i.e. rivers, lakes, and particularly oceans) takes place in terrestrial habitats in which plastic wastes carried to these water bodies through several natural events such as surface runoff, wind, and erosion (Sazlı et al., 2023; Coşkun et al., 2024). As a result, micro- and nano-

plastic particle concentrations increase particularly in large rivers and then carried to the oceans (Yee et al., 2021). For instance, the concentration of polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and polymethyl methacrylate (PMMA) nanoplastic particles in the surface water samples from in Fuhe River (China), ranged between 0.28 and 0.79 ppb (Xu et al., 2022). Sullivan et al. (2020) reported similar results from Tawe River (United Kingdom) with an average polystyrene nanoplastic particle concentration of 241.8 ppb. In an another study, the average microplastic and nanoplastic particle concentration in Chi River (Thailand) were found as >204 particles/L; >22 particles/L in tap water; and upto 73 particles/L in bottled water (Wibuloutai et al., 2023). Similar reports are available from marine habitats. For example; polystyrene nanoplastic particle concentrations were reported as 3.8 to 4.5 ppb in the surface layers of Wadden Sea (Netherlands) (Materić et al., 2022b). Various polymer types, including polyethylene (6.5 ng/mL), polyethylene terephthalate (2.7 ng/mL), polystyrene (0.11 ng/mL), polyvinyl chloride (0.11 ng/mL), and polypropylene (0.57 ng/mL) were observed in Greenland ice cores with an average nanoplastic concentration of 13.2 ng/mL (Materić et al., 2022a). In addition, there are also reports available indicating that nanoplastics are observed in water treatment facilities (Atugoda et al., 2022). But, removal techniques applied in drinking water treatment facilities such as filtration alone fails to remove those particles (Devi et al., 2022).

THE EFFECTS OF NANOPLASTIC PARTICLES ON AQUATIC ORGANISMS

Recent experimental research on the impacts of nanoplastic particles on aquatic organisms suggests that these particles have significant and complex toxic effects (i.e. on reproductive and growth rates, enzyme activities, oxidative stress, cellular functions and behavioral traits) (Besseling et al., 2019; Auclair et al., 2020; Liang et al., 2023) and therefore considered a significant threat to aquatic biodiversity (Mattsson et al., 2018).

Aquatic organisms ingest nanoplastic particles either voluntarily or involuntarily. They can also be internalized through dermal absorption, or special mechanisms such as endocytosis depending on the level of organization of the organism (Li et al., 2020; Trevisan et al., 2022). Upon ingestion these particles may accumulate particularly in alimentary tract for a particular period of time before defecation. For example, it was observed that 90% of the particles were eliminated after 6 hours of starvation in *Daphnia magna* exposed to polystyrene nanoplastic particles (1000nm) while reduction rate was 40% when exposed to 20 nm particles (Rosenkranz et al., 2009). These findings indicate that smaller particles tend to remain in *Daphnia* for a longer period of time. In a similar study where *Danio rerio* larvae was exposed to or injected with 70 nm polystyrene nanoplastic particles, researchers found that nanoplastics mainly accumulated in the yolk sac and intestines after injection based tests, while accumulation mainly occurred in eyes and brain after exposure tests (Zhang et al., 2020). Thus, it is possible that nanoplastic particles may pass through cell membranes and can be translocated into other organs such as brain and eyes (Li et al., 2020; Zhou et al., 2023). Similar reports are also available for algae. For example, nanoplastic particles were found to have more adverse effects on reproductive output and growth rates of *Chlamydomonas reinhardtii*, (Yan et al., 2021).

Nanoplastic particles may accumulate in the aquatic food chains and reach higher concentrations in higher trophic levels (Zhang et al., 2020). For instance, in a report, researchers initially exposed the algae (*Scenedesmus* sp.) to nanoplastic particles. Secondly *Daphnia* were fed with the nanoplastic exposed algae. And in the last step fish (*Carassius carassius*) were fed with nanoplastic contaminated *Daphnia*. Results indicated that the experimental fish showed clear signs of alterations in their feeding and hunting behavior. They also reported alterations in lipid metabolism of the fish.

In order to achieve a better understating on the effects nanoplastic pollution on aquatic organisms we presented a review of the experimental studies predominantly from the last 10 years that are focusing on nanoplastic pollution is supplied in this study (Table 1). Any

details on the chemical structure, size and concentration of the nanoplastic particles, exposure duration are also given where possible.

Table 1. Experimental studies on the toxic effects of nanoplastic particles on some aquatic organisms.

Organism	Polymer type	Size (nm)	Concentration	Exposure duration	Effects	References
Plankton						
Chlorella sp., *Scenedesmus* sp.	PS-NH_2, PS-COOH	20	0,08-0,8 mg/mL	2 h	-Decrease in photosynthesis rate -ROS production	Bhattacharya et al., 2010
Raphidocelis subcapitata	PS-COOH	87.17-106	0.5, 1, 2.5, 5, 10 and 50 ppm	72 h	-Decrease in photosynthesis rate -Increase in cell volume -Reproductive anomalies and mitotic phase disturbances -Increase in oxidative stress and neurotoxic effects	Bellingeri et al., 2019
Chaetoceros neogracile	PS-NH_2	50	0.05 and 5 μg/mL	96 h	-Deformation of cell morphology -Decrease in cell growth rate -Reduction in photosynthetic pigments and decreased photosynthesis efficiency -Increase in ROS levels	González-Fernández et al., 2019

					-Decrease in esterase activity	
Daphnia pulex	PS	75	10, 50, 100, 150, 200 and 400 ppm	24, 48 h and 21 days	-Decrease in growth rate -Alterations in SOD, GST, and CAT activities. -Increased levels of heat shock proteins	Liu et al., 2019
Tetraselmis chuii *Nannochloropsis gaditana* *Isochrysis galbana* *Thalasiosira weissflogii*	PMMA	40	0 -304.1 ppm (*T. chuii, N. gaditana, I. galbana*) 0 -293.0 ppm (*T. weissflogii*)	96 h	-Alterations in growth rate depending on exposure concentration	Venâncio et al., 2019
Rhodomonas baltica	PMMA	50	0.5 and 100 µg/mL	72 h	-Increase in cell volume -Decrease in DNA content -Disruptions in the cell cycle -Along with oxidative stress, increase in ROS and LPO levels. -Decrease in photosynthesis rate	Gomes et al., 2020

Chlorella vulgaris	PS, PS-COOH	20 and 50	250 ppm	38 days	-Along with oxidative stress, increase in ROS level -Deformation of cell wall and cell membrane -Inhibition of algal growth -Reduction in photosynthetic pigment and photosynthetic efficiency	Hazeem et al., 2020
Platymonas helgolandica	PS	70	0 to 2000 ppb	6 days	-Increase in heterocyst frequency -Increase in mitochondrial membrane potential -Reduction in photosynthetic efficiency -Deformation of cell morphology	Wang et al., 2020
Scenedesmus obliquus	PS	100	10 to 100 ppm	72 h	-Decreased growth rate -Decrease in photosynthesis rate -Increase in ROS level.	Yang et al., 2020
Chlorella vulgaris	PS-NH_2	90	0 to 200 ppm	72 h	-Decreased growth rate -Decrease in photosynthetic pigment concentration	Khoshnamvand et al., 2021

					-Deformation of cell morphology	
Daphnia pulex	PS	71.18	0.1 to 2 ppm	21 days	-Decrease in growth and reproduction rate -Alterations in protein and lipid metabolic pathways -Along with oxidative stress, increase in ROS, GSH and GST	Liu et al., 2021
Chlamydomonas reinhardtii	PS	100	50, 250 and 500 ppm	96 h	-Reduction in photosynthetic pigment photosynthetic efficiency -Increase in cell volume and cell membrane permeability -Lipid peroxidation rates increased -Increased levels of POD, SOD, and CAT -Increase in EPS production	Yan et al., 2021
Chlorella pyrenoidosa	PS	80	0 to 50 ppm	96 h	-Decreased growth rate -Reduction in photosynthesis rate -Increased levels of ROS, MDA, SOD, and CAT	Yang et al., 2021

					-Changes in gene expression related to stress response, DNA repair, and protein synthesis -Altered enzymatic activities, leading to oxidative stress	
Microcystis aeruginosa	PS	60	25, 50 and 100 ppm	30 days	-Decreased growth rate -Decreased photosynthesis rate -Deformation of cell morphology -Increase in lipid peroxidation	Zheng et al., 2021
Invertebrates						
Mytilus edulis	PS	27.6	0 to 0.3 g/L	8 h	-Reduction in filtration activity -Decrease in food intake -Production of pseudofeces -Disruption in energy balance	Wegner et al., 2012
Paracentrotus lividus	PS-NH_2	50	1 to 25 µg/mL	24 h	-Reduction in the stability of lysosomal membrane in coelomocytes -Apoptic-like nuclear alterations	Marques-Santos et al., 2018
Sterechinus neumayeri	PS-NH_2,	40 and 50	1 and 5 µg/L	24 h	-Decreased phagocytic capacity -Increased immune response and apoptosis	Bergami et al., 2019

	PS-COOH				-Oxidative stress -Increase in antioxidant enzyme activities.	
Hydra attenuata	PS	50 and 100	1.25, 2.5, 5, 10, 20, 40 and 80 ppm	96 h	-Formation of lipid-like liquid crystals -Along with oxidative stress, decrease in lipid peroxidation at high concentrations -Morphological changes	Auclair et al., 2020
Mytilus galloprovincialis	PS-NH_2	50	10 µg/L	24 and 72 h	-Reduction in mitochondrial membrane potential and lysosomal membrane stability -Downregulation cellular stress genes -Increased immune response	Auguste et al., 2020
Macrobrachium nipponense	PS	75	0, 5, 10, 20 and 40 ppm	28 days	-Increase in SOD activity -Increase in CAT activity -Increased levels of GSH-Px and GSH-ST	Li et al., 2020
Mytilus galloprovincialis	PS	50 and 100	10 ppm	24 h	-Decreased ROS concentration -Reduction in phagocytic capacity -Increase in apoptosis	Sendra et al., 2020

Crassostrea gigas	PS-NH_2, PS-COOH	50	0.1 to 25 mg/mL	1 h	-Reduction in spermatozoa motility	Tallec et al., 2020
Hydra viridissima	PMMA	40.8	1, 5, 10, 20, 40, 80, 160, 320 and 640 ppm	96 h	-Mortality -Morphological and physiological changes	Venâncio et al., 2021
Procambarus clarkii	PS	100	1.4 x 10^{11} particles/L	72 h	-Increase in total hemocyte counts -Oxidative stress -Increased gene expression levels in hepatopancreas, food metabolism and detoxification mechanism	Capanni et al., 2021
Vertebrates						
Carassius carassius	PS	24.7 and 27.5	9.3 × 10^{12} particles/mL	61 days	-Increased ethanol, leucine, phenylalanine and tyrosine concentration in liver -Decreased ethanol concentration in muscle -Increased inosine/adenosine and lysine concentration in muscle	Mattsson et al., 2015

					-Prolonged feeding duration and reduced activity -Structural changes in brain and muscle tissue -Increase in group behaviour	
Pimephales promelas	PS	41	0.025, 005, 0.1, and 0.2 µg/µL	1 h	-Increase in oxidative burst -Activation of phagocytosis -Increase in neutrophil activity	Greven et al., 2016
Danio rerio	PS	50	1 ppm	48-72 h	-Hypoactivity -Decrease in body length -Upregulation of mRNA expression for the zfrho and zfblue genes	Chen et al., 2017
Carassius carassius	PS	53	0.029 and 0.1 g/L	67 days	-Decrease in feeding rates -Decrease in exploration and locomotor activity -Reduced brain water content	Mattsson et al., 2017
Dicentrarchus labrax	PMMA	45	0.02, to 20 ppm	96 h	-Decrease in esterase and alkaline phosphatase enzyme levels -Increase in pparα and pparγ levels	Brandts et al., 2018

Oryzias sinensis, Zacco temminckii	PS	51	5 ppm	7 days	-Decrease in locomotor activity -Increase in level of cholesterol -Alterations in liver tissue morphology	Chae et al., 2018
Danio rerio (larvae)	PS	51	0.1, to 10 ppm	6-120 h	-Bradycardia -Hypoactivity	Pitt et al., 2018
Ctenopharyngodon idella	PS	23	0.04-34 ng/L and 34 ppb	21 days	-Oxidative stress -Increase in micronucleus frequency -DNA damage -Morphological changes in erythrocyte	Guimarães et al., 2021
Larimichthys crocea	PS	80	1-100 ppm, and 5-80 ppm	24 h	-Along with oxidative stress, increase in ALP, AST, ALT, MDA levels -Decreased levels of SOD and GSH activity -Disruption of lipid metabolism -Reduction in growth rate and survival rate	Lai et al., 2021
Poecilia reticulata	PS	23	50 ppb	30 days	-Reduction in the number of embryos and pregnancy rate	Malafaia et al., 2022

					-Decrease in protein and carbohydrate levels -Increase in triglyceride levels -Along with oxidative stress, increase in ROS and H_2O_2 levels. -Increased levels of SOD and CAT activity	
Danio rerio (juveniles)	PS	20-80	0.1, 1, 10 and 100 µg/mL	45 days	-Oxidative stress -Increase in CAT and GSH levels -Inhibition of Ache activity -Neuronal damage in brain tissue -Decrease in alpha-ketoglutarate dehydrogenase activity	Aliakbarzadeh et al., 2023

(PS: Polystyrene, PMMA: Polymethyl Methacrylate, ALP: Alkaline Phosphatase, ASP: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, CAT: Catalase, EPS: Extracellular Polymeric Substances, GFAP: Glial Fibrillary Acidic Protein, GPx: Glutathione Peroxidase, GSH: Glutathione, GST: Glutathione S-Transferase, H_2O_2: Hydrogen Peroxide, LDH: Lactate dehydrogenase, LPO: Lactoperoxidase, MDA: Malondialdehyde, POD: Peroxidase, ROS: Reactive Oxygen Species, SOD: Superoxide Dismutase)

CONCLUSION

Plastic wastes deposited in the environment degrades into smaller particles and transformed into micro- or nano-sized plastic particles through physical, chemical or biological processes and meteorological events. Recent studies reveal that micro and nanoplastic particles are spreading in all water bodies and have a world-wide distribution through environmental transport mechanisms. Several studies indicate that micro or nanoplastic particles can be internalized through ingestion, dermal absorption, or endocytosis and can exert various negative effects on aquatic organisms. Since they have a capacity to accumulate, more severe toxic effects can be observed in species in higher trophic levels. Furthermore, the toxic additives added during their production and other pollutants (such as heavy metals) adsorbed on their surfaces increase risks associated with plastic nanoparticles. Nanoplastics are considered more hazardous because of their relatively small size, large surface area and higher persistence in nature.

In this study, a comprehensive review on experimental studies focusing on the effects of nanoplastic particles on aquatic organisms is given. We also focused on the experimental conditions such as physico-chemical characteristics and size of the nanoplastics particles. The results presented here clearly reveal that the observed responses are dependent upon experimental parameters, including the shape, size, polymer type, concentration of nanoplastics, exposure conditions, and the species. In addition, results also indicate that nanoplastic particles can cross cellular membranes and accumulate within cells.

It should be noted that findings reported here are based on controlled laboratory conditions, and significant uncertainty remains regarding how real environmental conditions effect the toxic effects of these particles. In order to achieve a better understanding on the effects of nanoplastic particles there is a need for i. long-term studies that incorporate natural environmental conditions and ii. development of standardized experimental protocols. Future studies should also focus on the internalization mechanisms of nanoplastic particles through cell membranes.

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CHAPTER 6

ANTIMICROBIAL RESISTANCE IN AQUACULTURE-A GROWING RISK TO HUMAN HEALTH

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INTRODUCTION

Aquaculture, also called aquaculture farming, is the controlled cultivation of fish, shellfish, aquatic plants (seaweed, etc.) and other aquatic organisms. This concept involves the breeding, growing and harvesting of these organisms in a variety of aquatic environments, including freshwater systems (ponds, rivers and lakes), marine systems (open ocean and coastal waters) and artificial systems (tanks, channels and circulation systems) (FAO, 2024a).

Aquaculture is one of the fastest growing food industries in the world today. The production of aquaculture worldwide in 2022 totaled around 130.9 million tons or approximately one-third of the world's total aquatic animal production (185.4 million tons). It has also expanded owing to rising demand for seafood as people grow, urbanize and lifestyles change. Aquaculture development and expansion contributes to food security around the world by providing protein and livelihoods for millions of people (FAO, 2024b).

But the industry has likewise endured a considerable transformation. Nowadays, it's a more than 50% of global aquatic animal production that's held by China, India, Indonesia and Vietnam, with 90% of the world's aqua products being supplied by these countries (FAO, 2024b). The industry will produce 205 million tons per year by 2032 (FAO, 2024a). Such expansion is essential to satisfy the world's food needs, but it comes with a set of environmental and health problems.

Aquaculture operations usually demanding many inputs for high productivity and control of diseases. Those contributions are antibiotics for preventing and treating infections. But the vast adsorption and abuse of these drugs has caused serious public health issues around antimicrobial resistance (AMR) (Shahabuddin et al., 2024). World Health Organization (WHO) characterizes AMR as a worldwide epidemic that could undo decades of medical advances in infectious disease treatment (WHO, 2017).

Antimicrobial resistance can be defined as the process by which the microorganisms including viruses, fungi and bacteria, parasites become resistant to the effects of drugs after some time. Some of the ways through which this occurs include genetic variations or horizontal

transfer of genes (WHO, 2023). In aquaculture, antimicrobial use in the management of fish diseases accelerates the development of resistance thus the pathogens released into the environment through consumption of seafood or environmental pathways (Chintagari et al., 2018).

The consequences of antimicrobial resistance are various. The pathogens that are resistant to treatment are harder to cure and may require the use of more aggressive drugs with adverse effects. It was noted that the number of deaths from diseases caused by antimicrobial resistant organisms is about 5 million per year and AMR might lead to 10 million deaths per year and will cost $100 trillion to the global economy by 2050 (WHO, 2019). Also, since aquaculture products are usually eaten raw or undercooked, the chances of getting infected by resistant pathogens is also high (Chintagari et al., 2018). Studies have shown that the level of antimicrobial resistance to many clinically significant antimicrobials in the bacterial pathogens in aquaculture environments is more than 50% (Schar et al., 2021). This resistance not only has an impact on human health but also on animal health and welfare particularly in the production systems. It is important to note that the presence of resistant bacteria in water bodies is dangerous to the environment as well as can pose a challenge to disease control efforts (Schar et al., 2020).

Furthermore, the international trade in the global supply chain of seafood and maritime transport also lead to the dissemination of resistant bacteria across the borders. This is especially so because such countries have weak regulatory systems as regards the use of antimicrobials in agriculture, and have scarce health resources (WHO, 2019). The fight against AMR in aquaculture cannot be achieved through a single approach and this includes enforcing proper regulations on use of antimicrobials, encouraging the practice of other disease control measures such as vaccination and improved biosecurity and establishing surveillance systems that will help in the identification of patterns of antimicrobial resistance. It is also important to conduct awareness sessions for farmers on the proper use of the antimicrobials to help minimize this threat (Preena et al., 2020). In this section, the use of antimicrobials in aquaculture will be assessed and the situation of

antimicrobial resistance, as well as the present and future potentials of health threats, the authors will make an assessment on how sustainable farming is likely to be with the measures that can be adapted.

ANTIMICROBIALS USED IN AQUACULTURE

The application of antimicrobials in aquaculture is deemed as an essential aspect in the management of diseases and promotion of health of aquatic animals to facilitate sustainable production. Since it is expected that the use of antibiotics will rise in conformity with the growth of the aquaculture sector, it is crucial to know which antibiotics are applied in what context and how often to address the problems related to AMR in the first place.

It is reported that antimicrobials are used for 3 purposes in aquaculture. These are the prevention and treatment of diseases and the promotion of growth. An effective antimicrobial treatment can reduce production losses and ensure the sustainability of the system (Schar et al., 2020).

In farming conditions where high-density production is carried out and stress levels are accordingly high, metaphylactic and prophylactic antimicrobial applications are made. These applications aim to protect the health of farm animals and minimize economic losses associated with disease outbreaks. Prophylactic application is the continuous application of a certain amount of antimicrobial regardless of the disease outbreak. Antimicrobial application, especially when some individuals in the herd show signs of disease or when an outbreak is expected depending on the rearing conditions, is called metaphylactic application (Bondad-Reantaso et al., 2023). Apart from these, it has been observed that some antimicrobials increase feed utilization and stimulate growth by affecting the intestinal microbiota.

WHO reports that incorrect and/or excessive use of antimicrobials can lead to the emergence of resistant strains that pose significant risks not only in aquaculture but also to other living organisms in the ecosystem and human health through the food chain (WHO, 2017).

However, it is stated that these applications, which are not used as treatment in many countries today, are prohibited or restricted due to their effects on the development of AMR (He et al., 2017).

Antimicrobials Consumption

The choice of antimicrobial is generally considered to depend on the pathogens present, the type of aquaculture system (freshwater or marine) and local regulations governing the use of antimicrobials (Ferri et al., 2022). Although they vary, the antimicrobials used in practice are concentrated on a few main groups. The most used are reported to be quinolones, tetracyclines, phenicols and sulfonamides (WHO, 2019).

It has been reported that quinolones are the most commonly used antimicrobial class globally with a rate of 27% (WHO, 2019). Antimicrobials in this class show their effects by inhibiting bacterial deoxyribonucleic acide (DNA) gyrase and protein synthesis. On the basis of the findings of this study, enrofloxacin and difloxacin have been identified as the most commonly applied antimicrobials in this class in aquaculture (Miranda et al., 2013). The use of these antibiotics has been widely documented to be common in the treatment and prevention of infections from Gram negative bacteria for instance *Aeromonas spp.*, *Vibrio spp.*, *Escherichia coli* (*E. coli*) and *Edwardsiella spp.* As the use of these antibiotics has increased, it was discovered that the quantity of genes that are transferable such as the *qnr* gene which is a plasmid-mediated quinolone resistance gene has also increased in the environment (Yan et al., 2017; Miranda et al., 2022).

Tetracyclines are the 2nd most used antimicrobial class across the globe with 20% (WHO, 2019). It has been noted that the antibiotics in this class are referred to as broad spectrum and they have activity against many Gram positive and Gram negative bacteria. It has also been mentioned that they have a simple application method of adding it into the feed or water and that is why they are popular in aquaculture (Cabello, 2006). It has been mentioned that oxytetracycline and doxycycline are the most commonly used in this class and they are used in bacterial infections including *Aeromonas spp.*, *Vibrio spp.* and *Flavobacterium spp.* (Ferri et al., 2022).

Phenicols have been identified as the third most frequently applied antibacterial class in the world with the usage share of 18% (WHO, 2019). In aquaculture, one of the antimicrobials in this class, florfenicol is used to prevent deaths caused by bacterial infections including *Aeromonas hydrophila*, *Vibrio anguillarum* and *Edwardsiella ictaluri*. Florfenicol acts by binding to the 50S ribosomal subunit thus blocking the protein synthesis in the bacteria. This antibiotic is safer as it does not cause human aplastic anemia as is the case with chloramphenicol. The presence of genes that encode for this group of antibiotics for example *floR* in the aquaculture systems raises concerns on the spread of resistance (Miranda et al., 2013). Also, the application of this class of antibiotics in aquaculture is restricted due to the residues which may be hazardous to human health, therefore strict regulations are imposed on its usage (Ferri et al., 2022).

Sulfonamides are 4th on the list of most often applied antibacterial agents with the usage rate of 14% (WHO, 2019). It has been stated that sulfadimethoxine and ormethoprim of this class are used in combination therapy since it is believed to be more effective. These antimicrobials work by suppressing the synthesis of folic acid which is imperative to bacterial DNA synthesis. They are often administered along with trimethoprim with an aim of enhancing the bacterial killing. However, it was seen that the aquaculture environments are prone to the increase in the amount of *sul1* and *sul2* genes which are resistance genes to sulfonamides (İbrahim et al., 2020).

Macrolide antibiotics which include erythromycin and azithromycin have been used mainly to cure *Flavobacterium spp*. And Gram positive organisms such as *Streptococcus spp.* infections (Sarmah et al., 2006). These antimicrobials have a wide spectrum activity and, like the phenicol antimicrobials, act through binding to the 50S ribosomal subunit thereby blocking protein synthesis. Cesare et al. (2013) and Miranda et al. (2013) noticed that the frequency of detection of the *erm* and *mef* genes that confer resistance to antibiotics of this class has been on the rise in aquatic environments.

It is reported that frequently used compounds such as neomycin, lincomycin and formalin can be used as disinfectants especially for the treatment of fungal infections (Lee et al., 2021).

Amount and Frequency of Antimicrobial Use

According to research, the global antimicrobials use in the year 2013 was 162,000 tons (Zhang et al., 2015). It has been estimated that the majority of it was applied in animal production; the majority of these antimicrobials used were used in aquaculture. It has been noted that aquaculture may intensity differ considerably depending on usage in the area and the production system used. In some countries where there are no legal found. rules In and a regulations study and conducted no in check Bangladesh, is it done, was excessive observed use that of 71 antimicrobials % can of the fish farms applied antimicrobials at least once during the production cycle. It was also observed that the usage of antimicrobials was significantly higher in the freshwater systems as compared to the brackish water the systems current (Chowdhury et al., 2022). Usage in animal is production believed in that China is about half of the global total and most of it is used in aquaculture. It was also highlighted that accounted in 57.9% China of the global consumption, India accounted for 11. 3% and other countries made up the remaining 30.8% in 2017 (Mulchandani et al., 2023). by These the two-year countries 2030 are with expected China's to market remain share the projected biggest to consumers drop of to 55.9% while India's market share is expected to be the same (Tiseo et al., 2020).

A research study done in Chile revealed that there has been an increasing trend of the use of antimicrobials in the salmon farming sector and the most commonly used antimicrobial in the seawater farming zones was identified to be florfenicol (Ibrahim et al., 2020).

RESISTANCE MECHANISMS

It is therefore necessary to identify the mechanisms of antimicrobial resistance to assess its effects in aquaculture and the environment. Several mechanisms are involved in the evolution and dissemination of antimicrobial resistance which include genetic

mutations, horizontal gene transfer and selection pressure. The factors that cause selection play a significant role in determining the type of microbial communities that are present as well as their rate of evolution with regards to antimicrobial resistance. In aquaculture, antimicrobial use in the prevention and treatment of diseases as well as promotion of growth ensures that only the resistant strains survive and propagate the susceptible ones (Gullberg et al, 2011).

Genetic Mutations

Genetic mutations are considered as one of the major causes of AMR. These mutations can happen naturally during DNA replication or can be caused by other factors such as antimicrobial exposure. When bacteria are subjected to antimicrobials, only those that have mutations on their genetic make up which confers resistance to the antimicrobials used will reproduce and multiply in the population. Hence the frequency of resistant bacteria increases in the population over a period (Levy and Marshall, 2004).

Out of the genetic mutations that take place there is the variation in the target site which is affected by the antimicrobials. Antimicrobials work by attaching themselves to specific targets in the bacterial cell and once these targets are modified by mutations for instance; the affinity for the antimicrobial is reduced and thus the antimicrobial is no longer effective. For instance, mutations in the *gyrA* gene of E. coli can lead to alteration of the DNA gyrase enzyme thus conferring resistance to fluoroquinolones (Harms et al., 2016). Some of the bacteria have efflux pumps which eject antimicrobial agents out of the bacterial cell. Alterations in the genes that regulate the expression and function of these pumps can cause the development of resistance in the bacteria. For instance, the upregulation of the *acrAB* efflux pump in *E. coli* leads to the development of resistance to a number of antibiotics (Piddock, 2006). Biofilms are another way through which mutations can occur in bacteria since they form a protective shelter for the bacterial communities. Biofilms may also block the action of antimicrobials, shielding bacteria from the host defense mechanisms and therefore allowing the persistence of resistant strains as seen (Donlan and Costerton (2002).

Horizontal Gene Transfer

Other mechanisms that also involve in the dissemination of antimicrobial resistance in the bacterial population include Horizontal gene transfer (HGT). Vertical gene transfer is where genes are passed from parent to offspring while in HGT genes are exchanged between bacterial species or strains. This process can cause the rapid spread of resistance genes within microbial communities, especially in aquaculture environments where different bacterial populations coexist (Villa et al., 2019).

HGT occurs in three main ways. The first of these is transformation. In transformation, competent bacteria take up free DNA that causes resistance in the environment. Thus, they can become resistant to antimicrobials (Lorenz & Wackernagel, 1994). Another method, transduction, involves bacteriophages (viruses that infect bacteria) transferring genetic material between bacterial cells. During this process, a bacteriophage may unintentionally package a piece of bacterial DNA containing resistance genes and transfer it to another bacterium (Hatfull, 2008).

In the third method, conjugation, bacteria directly acquire genetic material from another bacterium through physical contact. The acquired genetic material is usually plasmids. A bacterium forms a conjugation pili to conjugate with another bacterium. Once this is done, plasmids carrying multiple and different antimicrobial resistance genes can be transferred (Grohmann et al., 2003).

The ability of bacteria to acquire resistance genes through HGT is more readily available in aquaculture environments where antimicrobial use is widespread. The presence of resistant bacterial strains in livestock on production farms can lead to the spread of their genes to wild populations or other aquatic environments through water exchange or sediment interactions (Fu et al., 2022).

SPREAD OF ANTIMICROBIAL RESISTANT PATHOGENS

The use of antimicrobials in aquaculture for the management of antimicrobial resistant bacteria (ARB) poses a risk to human health. It is

hence important to know how these pathogens can be transmitted to humans to minimize the risks that are associated with the use of antimicrobials in aquaculture.

Direct Transmission

ARBs are released into the environment in different ways such as; waste water discharges from farms, improper disposal of antimicrobial residues and antimicrobial waste from aquaculture facilities, agricultural runoff and surface water drainage from fish farms amongst others. Thus, there is direct transmission of antimicrobial-resistant bacteria to humans from aquaculture through the following mechanisms. The major route of ARB transmission is through the consumption of aquaculture products infected with bacteria. When fish or shellfish are produced using production systems that are subject to antimicrobial usage, it makes fish or shellfish imported from such systems potentially contaminated with antimicrobial residues or resistant bacteria. It was established that bacteria including *Vibrio spp.*, Salmonella and *E. coli* were detected in aquaculture products which pose a direct threat to consumers (Ferri et al., 2022).

A study conducted in China by Wu et al. (2023), focused on ARB and antibiotic resistance genes (ARG) in aquaculture. In this study, 136 possible ARB were identified in 6 water samples taken from the Zhejiang province. It was determined that 80% of ARB consisted of *Aeromonas spp.*, *Shewanella spp.*, *Acinetobacter spp.*, *Myroides spp.*, *Pseudomonas spp.*, and *Citrobacter spp.* However, it was determined that 80.15% of the isolates were resistant to at least one antibiotic, and most isolates were resistant to more than one antibiotic. Another result that is different from these results and more important is that genotypic and phenotypic resistance data did not fully overlap with each other. It was reported that ARGs were more diverse (Wu et al., 2023).

In another study conducted in China by Ye et al. (2013), 100% of the bacteria isolated from 10 different seafood products purchased from markets were found to be commensal ARBs and 505 were multidrug resistant (MDR). *Acinetobacter spp.*, *Morganella spp.* were commonly found in these samples. and *Pseudomonas spp.* were isolated, and the

most resistant isolates were *Aeromonas spp.* and *Enterobacteriaceae* (Ye et al., 2013).

Also, the techniques that are used in preparation of food are also vital in the risk of contamination. If proper hygiene measures are not taken, those who handle the raw seafood may contract the disease or transfer the bacteria to their hands or kitchen utensils. These bacteria may then be spread to other foods or ingested directly and cause infections. The Centers for Disease Control and Prevention (CDC) (2024) has stated that casual contact with raw seafood is a means of ARBs' spread.

Other contact with the environment can also contribute to the contamination as well. People who are engaged in recreational activities in the water within the proximity of the aquaculture farms may have a potential of getting in touch with ARB's which are present in such farms. These include activities such as swimming or fishing among others. Research have also indicated that exposure to polluted water during recreation may result in stomach upsets due to bacterial infection by resistant strains (Pepy and Focardi, 2021).

Indirect Transmission

Besides direct contact there are also the indirect methods of ARB transmission to human being. The first of these is environmental. The antimicrobials used in aquaculture release the antibiotics into the environment through wastewater discharge from the farms. These wastewater-borne antimicrobials and ARB are reported to survive in the aquatic environments for some time. These pollutants let loose into rivers or coastal waters will definitely affect the quality of water and may help in the dissemination of resistance genes among the environmental bacteria. The presence of resistant strains in environmental waters poses a danger to the aquatic life as well as to human beings who may come in direct or indirect contact with the waters or consume seafood products from the waters (Heuer et al., 2009; Edebuani et al., 2021).

The use of antimicrobial residues and resistant bacteria in aquatic environment pose a threat to the food chain. Plankton and other small organisms can consume these pollutants. Other larger animals that consume these creatures also accumulate antimicrobial residues and

ARBs in their tissues since they consume the creatures (Heuer et al., 2009).

Besides direct transmission, there are also indirect methods that ARB can be transmitted to humans. The first of these is environmental. Antimicrobials in aquaculture can be released into the environment through wastewater outputs from the fish farms. This wastewater usually contains antimicrobial residues and ARB which can be found in aquatic environments for some time. These pollutants released into rivers or coastal waters can affect local microbial communities and therefore may promote the dissemination of the resistance genes among the environmental bacteria. The presence of the strains in the environmental waters poses a threat not only to the aquatic life but also to human being who may come in contact with these waters or consume sea foods (Heuer et al., 2009; Edebuani et al., 2021).

Wastewater from aquaculture can be used as fertilizer in agricultural areas. This practice can lead to contamination of soil and products with antimicrobial residues and ARBs. When these produced foods are consumed by humans or fed to farm animals, the risk of ARB spreading to the human population increases (O'Neill, 2016).

HTG is also an important factor which leads to the emergence of antimicrobial resistance. The ability of the bacteria to transfer genes across different species of bacteria is through HGT which can be done through many ways. One of these is biofilms. Structures like nets, tanks and sediments enable the formation of biofilms in aquaculture systems. Biofilm is capable of accommodating many different types of bacteria and the transfer of genes between the bacteria is enhanced due to the fact that the bacteria are located in close proximity. The biofilm mediates the transfer of the resistance genes in the aquatic bacteria as well as those in the human pathogens since plasmids are present in the biofilm (Heuer et al., 2009; Pepi and Focardi, 2021). The waterbodies in the vicinity of the aquaculture sites are also the depots of resistant bacteria. These environments are characterized by the presence of human, animal and environmental pathogens that can share genes through HGT process. Therefore, the genes originating from aquatic bacteria can be passed to the human pathogens, including *Salmonella* or *Shigella*, thus reducing

the effectiveness of the antimicrobial treatments used for infections caused by these bacteria (Fastl et al., 2023).

EFFECTS ON HUMAN HEALTH

The increasing antimicrobial resistance in aquaculture has many effects on human health.

Rise in Infection Rates

The infections due to ARBs are on the rise, which is a direct risk to public health. There are more and more ARB strains that appear in the human population with the food chain or with the use of recreational services and seafood products, which may lead to the development of the infections that are hard to treat (Longo & York, 2024). Analyzing the levels of ARBs in seafood products from different areas of the world has revealed that seafood produced in areas with high intensities of aquaculture contains higher levels of ARBs than those produced in areas with low intensities of aquaculture (Founou et al., 2016). The infections caused by the resistant strains lead to a number of days in the hospital, high expenses on medication and enhanced mortality (O'Neill, 2016). The mortality rate of patients affected by carbapenem-resistant Enterobacteriaceae has been estimated to be greater than 50% (WHO, 2023).

Restricted Treatment Alternatives

The availability of antibacterial agents reduces the efficacy of the following antimicrobials that are commonly used in treating infections. The spread of antimicrobial resistance is making the healthcare providers to struggle in managing infections that could have been cured with standard drugs. This therefore means that there is need to use the last line antimicrobials which may have severe side effects and may not even work (WHO, 2023).

Epidemics

There is a high chance of large scale outbreaks of public health concern where ARBs are concerned and are coming from aquaculture.

Some of the ARBs have been isolated from wild aquatic animals that act as their natural host. These bacteria may spread to other farm animals or to humans directly or indirectly through exposure to the environment and food (Abia et al., 2016). Outbreaks of ARB species *Salmonella* and *Vibrio* have been known to be related to seafood products and this includes outbreaks originating from contaminated seafood. Such outbreaks can cause a lot of pressure on the health care systems and pose a very dangerous level of morbidity and mortality (Boeckel et al., 2015).

Besides, some categories of people including the elderly, immunocompromised, and those with implantable devices are more prone to infections from resistant pathogens from aquaculture settings (Boeckel et al., 2015).

Surgical Complications

Patients who are undergoing a surgical procedure are at higher risk of acquiring infection from ARBs. Surgical side infections (SSI) are of interest as they can lead to other complications and will increase the duration of the recovery period (Iwu et al., 2020).

Financial Drawbacks

Thus, the costs of AMR are not only the expenses of the healthcare sector. The expected future increase in the number of admissions and failures of treatment may negatively affect the availability of the public health funds (Laxminarayan et al., 2016).

ECOLOGIC IMPACTS

The consequences of AMR in aquaculture are not confined to the aquaculture farms alone. It affects the whole aquatic system and there is a decline in species diversities. Research has indicated that the antibiotic residues from aquaculture are able to remain bound to sediments or water columns for relatively long time and enhance the growth of resistant bacteria. All these change the microbial population with a huge risk of affecting the farmed species as well as the wild ones in the same area (Zhang et al., 2024).

Spread of Resistance in Aquatic Environments

Antimicrobial resistance in aquatic environments is a multifaceted issue which is directly linked with aquaculture. The application of antimicrobials in aquaculture is as a result of attempts by farmers to manage diseases. But this remains a major factor in the formation and dissemination of the strains of resistance (FAO, 2023). When antimicrobials are added to aquaculture farms, it creates a selective pressure that enables the proliferation of the bacteria that are resistant to the antimicrobials while eliminating the sensitive ones. Furthermore, it has been evidenced that ARBs originating from aquaculture can remain in sediments for a certain period even after the antimicrobial use has been stopped (Zhang et al., 2024). This sustained presence indicates that the resistant strains can still impact the surrounding environments even after the use of antimicrobials in aquaculture and may play a role in the total ARB burden in aquatic systems (Iwu et al., 2020).

Impacts on Biodiversity

AMR's effect on biodiversity is said to be a major concern which needs to be addressed. Biodiversity is important as it aids in the ability of ecosystems to bounce back from any form of change. Different ecosystems have different abilities in coping with changes in environment and fighting diseases. But as AMR advances further into the aquaculture environment, the robustness of marine ecosystems declines (Ahmad et al., 2022).

Among the causes of the reduction of biodiversity, one can identify the effect of AMR on keystone species that are very numerous and very well positioned in the ecosystem. The loss of keystone species or the decline of keystone species due to competition with AMR species will have chains of effects on ecosystems (FAO, 2023). When a specific fish that is crucial in the nutrient cycle is less abundant due to the competition with the resistant farmed fish, this can have an impact on other organisms that require these nutrients (Villéger et al., 2017).

Furthermore, the declines in the fish stocks due to AMR can also affect the provision of habitat and nutrient cycling. Fish are important in balancing the food chain and supporting a rich and diverse marine life.

But when antibiotics reach the aquatic ecosystems because of aquaculture, for instance, such functions may be affected (Tičina et al., 2020). Loss of biodiversity also generates a number of ethical questions regarding conservatory measures and our duty of preserving natural habitats. With more and more man-made interventions taking place in the aquatic systems including the aquaculture without an adequate understanding of the ecological consequences, we may be losing the genetic resource that will be needed for the management of future changes (Lagerstrom et al., 2021).

ANTIMICROBIAL RESISTANCE MANAGEMENT STRATEGIES

Since the risk of AMR in aquaculture is on the rise, it is crucial to design and put in place efficient and suitable measures to address the problem. These strategies should concentrate on disease control mechanisms, optimization of antimicrobial usage, legal measures, and right practices in aquaculture (FAO, 2024b).

Alternative Methods for Striving Diseases

In aquaculture, other measures are vital in reducing the usage of antibiotics. These methods include several measures that are used in order to avoid diseases as well as to enhance the health of aquatic animals not using antimicrobial treatment.

Vaccination

One of the most promising alternatives to antimicrobial use is vaccination. Vaccination can provide effective protection against certain pathogens by reducing the incidence of disease in farmed fish. The first record of vaccination dates to 1938. Snieszko et al. (1938) reported that vaccinated carp became resistant to *Aeromonas punctata*. Vaccination against *Aeromonas salmonicida* in rainbow trout dates to 1942 (Duff, 1942). The first vaccination to protect against the disease was applied in 1949 (Snieszko and Friddle, 1949). It has been stated that vaccination has been applied against various agents for many fish species since the first vaccination (Su et al., 2021).

It is reported that vaccination is generally done in 3 ways. These are oral, injection and immersion. The vaccines used can be inactivated, attenuated, recombinant, or synthetic peptides, DNA, or nanomolecular (Mondal and Thomas, 2022).

Probiotics and prebiotics use

As in many other farming systems, they are used in aquaculture to activate both humoral and cellular immunity and to control disease. Probiotics are beneficial microorganisms that support health. These microorganisms that settle in the gastrointestinal system change the microflora. Thus, they help control infection by preventing the colonization or proliferation of pathogenic bacteria (Pereira et al., 2022). For this purpose, in addition to algae and yeast, some specific microorganisms such as *Bacillus sp.*, *Lactococcus sp.*, *Micrococcus sp.*, *Carnobacterium sp.*, *Enterococcus sp.*, *Lactobacillus sp.*, *Streptococcus*, and *Weissella sp.* are also used (Gheziel et al., 2019). Studies have shown that certain probiotic bacteria such as *Enterococcus casseliflavus* can effectively reduce fish mortality rates by preventing Streptococcus iniae infection encountered in rainbow trout (*Orcorhynchus mykiss*) farming (Safari et al., 2016). It has been reported that prebiotics are the feed additives which are not digested by the organisms and enhance the activity and number of the beneficial bacteria in the gastrointestinal tract and can contribute to the improvement for of this fish purpose, health short-chain and fructooligosaccharides, performance oligofructose, (Merrifield & Carnavali, 2014). For this purpose, mannanoligo saccharides, transgalactooligosaccharides, inulin and galactooligo saccharides are utilized (Ringo et al., 2016). Research studies have also shown that the oil added to the feeds used can also have an effect on the intestinal micro flora. In a study performed by Huang (2008), it was observed that *Proteobacterium microflora* of grew grass more carp while that *Clostridium* was maritinum-like fed bacteria with reduced diet in containing the feed intestinal oil containing phospholipid:rice bran at the ratio of 2:1 at the rate of 1.23% for 8 weeks (Huang, 2008).

Enhanced biosecurity measures

It is therefore important to put in place proper biosecurity measures so that diseases do not occur in the aquaculture facilities. Some of the measures include control of access to the farms, proper hygiene in equipment and constant monitoring of water. The following practices can therefore be adopted to reduce the use of antimicrobials as they will minimize the chances of introducing and are spreading therefore pathogens important (Bera et al., 2018).

Controlled environmental conditions

The environment is favorable for the fish as this enhances their health. Some of the factors that include water temperature, salinity, and oxygen concentration levels are known to affect disease susceptibility. The management of these parameters can therefore help in preventing stress related diseases which in most cases will involve the use of antimicrobials (Baker-Austin et al., 2006).

Reduced Antimicrobial Use

AMR is one of the biggest threats in aquaculture as use of antimicrobials in aquaculture contributes to it. It has been suggested that the use of antimicrobials can be reduced through this way so that the selection pressure can be reduced. It is highlighted that such programs should be developed that will establish the principles of reducing antimicrobial use and the guidelines on when and how to use them should be laid down (Laxminarayan et al., 2016).

However, the use of antimicrobials and the monitoring of antimicrobial resistance should be done routinely to assess the efficacy of measures that have been put in place to reduce antimicrobial use. Surveillance programs assist in recognizing the patterns of resistance and thus offer the information needed in decision making when it comes to antimicrobial use (WHO, 2015).

Establishing treatment guidelines where other means of intervention are given a higher priority than the use of antimicrobials is deemed to help in the fight against the unnecessary use of antimicrobials. The following guidelines should be developed based on current best

practices and be appropriate to the specific aquaculture systems and species (Laxminarayan et al., 2016).

It is reported that governments should implement stricter regulations regulating the use of antimicrobials in aquaculture. It is also stated that it is important to include limiting the types of antimicrobials that can be used, prohibiting their use for growth promotion, and requiring veterinary prescriptions for therapeutic applications (WHO, 2015).

On the other hand, it is reported that introducing reporting requirements for antimicrobial use and emerging resistance among aquaculture producers can increase transparency and accountability in this sector. Thus, the information obtained can be used to improve public health by informing policy makers and the public (FAO, 2023).

In addition, it is suggested that the regulatory legislation to be prepared should include incentives for aquaculture producers who minimize antimicrobial use and prefer sustainable production. Thanks to the financial support programs to be implemented, producers will be encouraged to invest in alternative disease management strategies and improve sustainability in production (FAO, 2023).

Sustainable Aquaculture Practices

Implementing sustainable aquaculture practices is important to reduce AMR risks while ensuring food safety and environmental protection. Today, we encounter different examples of this.

Integrated multi-trophic aquaculture (IMTA)

Integrated multi-trophic aquaculture (IMTA) is the growing of several organisms of different trophic levels in the same culture system. This approach minimizes waste production by ensuring that nutrients are reused hence enhancing the system's stability. A suitable example is the businesses in which other species for instance shellfish or seaweed are included in fin fish farming; this helps in reducing the social and environmental effects while increasing the production output (García-Poza et al., 2020).

Organic aquaculture

When organic aquaculture is mentioned, the first production method that comes to mind is the environmentally friendly production systems in which antimicrobials and other chemicals are used in minimal amounts. It has been suggested that the organic aquaculture systems that are developed according to the production standards that protect the environment and the biodiversity of the area and use natural feed resources can produce safe and high-quality seafood with the reduced risk of AMR (FAO, 2023).

Research and innovation

Today, it is stressed that the understanding that supports and embraces new approaches and research into the topic is required in order to build sustainable systems in aquaculture. It is stated that it is important to consider methods including new feed additives, new antibacterial strategies (bacteriophages or antimicrobial peptides), and new varieties of disease resistant strains in the management of challenges arising from AMR (Baker-Austin et al., 2006).

CONCLUSION

The issues of AMR in aquaculture system cannot be overemphasized given the fact that there is need to appreciate the mechanisms of AMR emergence and its effects on the environment and measures that can be taken to curb it. AMR is a serious threat to aquatic life as well as human health through consumption of infected or infected products.

Genetic mutations allow the bacteria to acquire resistance as well as horizontal gene transfer that enables the spread of resistance genes fall under the AMR mechanisms. Some of the ecological impacts of AMR are 1) alters marine ecosystems through habitat degradation, 2) poses a threat to biodiversity through nutrient enrichment from aquaculture activities that result in blooms and 3) threats competition with indigenous species. The spread of ARBs into the environment makes these issues even more complex by increasing the likelihood of antibiotic resistant infections. Other measures which have been used in the fight against the

disease include AMR reduction strategies, vaccination, probiotic and prebiotic usage, biosecurity improvements, establishment of surveillance and monitoring activities and regulations that control the use of antimicrobial agents and encouragement of sustainable aquaculture practices such as IMTA.

To this end, the following should be the focus of future research: Identification of resistance mechanisms; New therapies including bacteriophages or immunostimulants; Analysis of AMR development over time in various aquaculture systems through resistance modelling.

To tackle this global challenge of AMR in aquaculture, intensive research, international cooperation, public awareness, and more especially the improvement of food safety policies will be of great impact in reducing risks.

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CHAPTER 7

ECOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF PESTICIDE EXPOSURE IN FRESHWATER MUSSELS

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INTRODUCTION

Human activities have pressed hard on freshwater systems being polluted. In fact, various human-induced effects impinge directly and indirectly on freshwater ecosystems, with the dominant role of agricultural activities, followed by industrial and domestic influences (Turgut & Özgül, 2009). There is great awareness that pesticides, extensively used in agriculture, reach freshwater systems via numerous pathways and are acting destructively on these systems.

Pesticides are a general category of chemicals that can be subdivided based on their target organisms (Table 1) and chemical structures (Table 2). They are classified into distinct groups, each with specialized mechanisms of action. In addition, it is known that each of them has different effects on aquatic organisms in aquatic environments where they are contaminated.

Table 1. Pesticide Groups Based on Target Organism

Pesticide Groups	Target Organisms
Insecticides	Insects
Fungicides	Fungi
Herbicides	Weeds
Molluscicides	Molluscs
Rodenticides	Rodents
Acaricides	Mites, ticks
Bactericides	Bacteria
Avicides	Birds

Table 2. Pesticide Groups Based on Chemical Structure

Pesticide Group	Chemical Structure	Mechanism of Action	Examples
Organochlorines	Contains chlorine atoms	It affects the nervous system, causing paralysis in insects' nervous systems	DDT, BHC, Aldrin
Organophosphates	Contains phosphorus atoms	Inhibits acetylcholinesterase enzyme, affecting the nervous system	Malathion, Parathion, Diazinon
Carbamates	It contains carbamic acid esters	Inhibits acetylcholinesterase enzyme, similar to organophosphates	Carbaryl, Aldicarb
Pyrethroids	Derived from natural pyrethrins or synthetically produced compounds	It affects the nervous system, causing paralysis in insects' nervous systems	Permethrin, Deltamethrin
Neonicotinoids	It has a nicotine-like structure	Binds to nicotinic acetylcholine receptors in insects' nervous systems, causing paralysis	Imidacloprid, Thiamethoxam
Triazoles	It contains an azole ring	Inhibits ergosterol synthesis in fungal cell membranes, killing fungi	Propiconazole, Tebuconazole
Strobilurins	It contains a strobilurin core	Inhibits the respiration chain in fungi, leading to fungal death	Azoxystrobin, Kresoxim-methyl

Pesticides disrupt vital activities of aquatic organisms and hence cause ecosystem disequilibrium; this might bring up adverse ecological effects in the long run. The influence of pesticides within aquatic ecosystems extends beyond acute toxic effects to non-target organisms; such substances can result in long-lasting changes at levels up to subpopulations (Günal et al., 2021; 2022). Among the most important

biotic components of an aquatic ecosystem, freshwater mussels have ecological importance. They are contributory to water filtration, helping to maintain water quality. However, the mussel is highly sensitive to environmental stressors, and pesticide exposure often results in severe perturbations in their physiological and biochemical processes. These disruptions may appear in various forms of biochemical response, such as oxidative stress, cytotoxicity, loss of enzyme activity, and genotoxicity. These disruptors can further cause low mussel reproduction that will further affect population dynamics, with cascading effects on the ecosystem (Arslan, 2022). Roberts (1972) furthers those pesticides also disrupt the reproductive systems of mussels.

Recent studies indicate that pesticides have caused serious physiological and biochemical damage in freshwater mussels and pose a serious threat to ecosystem health (Moulton et al., 1996; Robillard et al., 2003; Kumar et al., 2011; Bellas et al., 2014; Bolognesi et al., 2014; Machado et al., 2014; Arslan, 2022). In light of studies conducted so far in the literature, this review evaluates the impacts of pesticides in the respiration, digestion, reproduction, antioxidant defense, and immune system of mussels. It also aims to further address the biochemical responses of mussels and related implications at the ecosystem level.

PHYSIOLOGICAL EFFECTS

Effects on Respiratory Functions

Exposure to pesticides can induce specific changes in the respiratory functions of freshwater mussels (Yancheva et al., 2017). Organophosphate pesticides have been shown to inflict adverse effects on the respiration apparatus of freshwater mussels and mitochondrial energy production in organisms. This has been further reported to disturb cellular energy production processes and reduce oxygen transport (Weis, 2014). Moreover, pesticides have been proven acting as inhibitors to respiration enzymes, which reduce the ability of oxygen transportation within the cells (Yancheva et al., 2017).

Ion Balance and Electrolyte Homeostasis Effects

Freshwater mussels are highly sensitive to osmoregulation, and environmental stressors may easily tip this balance (Deaton et al., 1989;

Freitas et al., 2017). In this context, pesticide exposure has been a huge stressor that influences mussel ion balance at the cellular level by disturbing electrolyte homeostasis. The regulation of the concentration of ions intra- and extracellularly is highly important for the determination of cell functionality. The ion balance, according to the ions Na^+, K^+, Ca^{2+}, and Cl^-, has been maintained through the ion channels and carrier proteins found at the cell membrane. However, pesticide damage to these ion channels might lead to disruption in the ion balance, leading to osmotic stress and failure of cellular function (Martem'yanov, 2000).

One of the outcomes of pesticide exposure in freshwater mussels is defects in the homeostasis of the two important ions, Na^+ and K^+. Intra- and extracellular concentrations of sodium and potassium stabilize the electrical aspects leading to normal metabolic functions within the cells. Pesticides increase the permeability of the cell membrane to allow intracellular ions to escape from the cell, hence disrupting cellular homeostasis (Yoloğlu, 2019).

Moreover, Ca^{2+} ions have also been implicated in other biological functions such as cellular signal transduction and muscular contraction. Pesticides may cause a perturbation in these cell signals by interfering with the chemical assembly of calcium within the cell. Normally, the intracellular concentration of free Ca^{2+} ions are low inside the cell and high outside the cell. However, pesticide exposure can increase cell membrane permeability to calcium; intracellular Ca^{2+} levels rise and disrupt many biological processes in the cells, such as muscle contraction, protein phosphorylation, and energy production (Ermak & Davies, 2002; Pinto, 2015).

Because pesticides disrupt this ion balance, they are known to affect cellular functions by compromising the structural integrity of the cell membranes. Ion imbalances can create excess membrane permeability with the accompanying fluid loss at a cellular level, which may lead to cell death (Yoloğlu, 2019). It is thought that it can have detrimental effects on mussel ecology and the general ecosystem for a long duration.

Any disruption of ion balance may cause an increase in ATP consumption. Cells need to upregulate the Na^+/K^+ ATPase metabolism

to compensate for this disturbance. Pesticides have been reported to reduce the efficiency of Na^+/K^+ ATPase in mussels, thereby leading to an energy crisis in cells. The metabolism of Na^+/K^+ ATPase is crucial in maintaining low sodium and high potassium levels in cells. However, this process is metabolically energy-intensive (Palecz et al., 2005).

Pesticide exposure disrupts the balance of ions and electrolytes, essential for general biological processes in mussels. This action reduces survival rates in mussels. An imbalance in electrolytes makes mussel health problematic at an individual level and reduces their filtration capacity, negatively affecting ecosystem functioning (Yang et al., 2017).

Digestive Functions and Histopathological Changes

Pesticide contamination in aquatic ecosystems can greatly harm the digestive systems of freshwater mussels, causing both tissue damage and disruption of essential digestive enzymes (Donkin et al., 1997). Exposure to pesticides, particularly organophosphates, has been linked to notable cellular changes in the digestive glands, such as degeneration, atrophy, and inflammation (El-Shenawy et al., 2009).

Structural issues negatively affect nutrient digestion and energy metabolism, as changes in the digestive glands decrease the production of enzymes essential for nutrient absorption. Furthermore, research has indicated that pesticide exposure can lead to excessive cellular growth and inflammatory responses in the digestive tissues of mussels (Stara et al., 2020).

Structural changes in the digestive glands may become permanent with chronic pesticide exposure. Pesticides are reported to cause permanent fibrosis in the digestive gland tissues of mussels, resulting in a loss of structural cellular integrity. This condition is likely to impair mussels' digestive functions irreversibly (Benjamin et al., 2016).

BIOCHEMICAL EFFECTS

Freshwater mussels have been one of those groups of organisms that most readily show biochemical effects from various water pollutants (Moulton et al., 1996). Among these, pesticides give rise to several biochemical processes, due to acting on their antioxidant mechanisms of

defense and lipid peroxidation, then leading to DNA and genetic toxicity (Arslan & Günal, 2023).

Oxidative Stress and Antioxidant Defense Mechanisms

Pesticide exposure in mussels causes a significant biochemical perturbation manifesting as oxidative stress. Pesticide exposure enhances intracellular levels of ROS, thus inducing oxidative stress. This destroys the integrity of basic biomolecular structures, usually manifested as lipid peroxidation, protein oxidation, and deterioration of DNA (Arslan & Günal, 2023).

Antioxidant defenses against oxidative stress exist in the mussels, involving a number of enzymatic antioxidant defense systems that neutralize ROS—such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The antioxidant enzymes themselves may generally be inhibited by pesticides. Indeed, some evidence has reported possible changes linked to pesticide exposure in SOD, CAT, and GPx activities in mussels (Paçal et al., 2022).

It was recorded that by inhibiting the production of such enzymes in antioxidant defense mechanisms, pesticides increased oxidative stress and caused cell damage among mussels (Günal et al., 2021).

Effects on Superoxide Dismutase (SOD) Activity

SOD is the defense enzyme neutralizing ROS thus preventing oxidative cellular damage. Inhibition of the SOD activity by pesticides results in intracellular accumulation of ROS in mussels and further rise of oxidative damage (Arslankoç et al., 2019). Various researchers reported that pesticides suppress SOD activity in mussel tissues, leading to accumulation of ROS in the cell (Al-Fanharawi et al., 2019; Serdar et al., 2023).

Mitochondria have a high sensitivity to oxidative damage. Cellular energy production is negatively affected by reduced SOD activities since the superoxide radicals produced during mitochondrial respiration cannot be effectively intercepted. Therefore, increased intracellular levels of ROS initiate the mitochondrial detoxification process by triggering mitoptosis and mitophagy. Several studies have exhibited reduced SOD activities and the development of mitochondrial

dysfunction in mussels exposed to pesticides. This impairment can disrupt the balance and production of cellular energy in mussels (Melov, 2000; Storz, 2007; Venditti et al., 2013).

Effects on Catalase (CAT) and Glutathione Peroxidase (GPx)

Other antioxidant enzymes that take part in the serious detoxification of ROS include CAT and GPx. CAT protects the cell from oxidative damage by converting injurious ROS into water and oxygen, such as hydrogen peroxide (H_2O_2) (Kurama et al., 2002). It was reported, on the other hand, that pesticide exposure inhibited the activity of CAT in mussels, leading to metabolic accumulation of hydrogen peroxide (Serdar, 2021). GPx, together with glutathione, reduces toxic peroxides, which are products of lipid peroxidation. Pesticides have been reported to inhibit the activity of GPx, thus increasing the rate of lipid peroxidation within the cell membranes and making the structure of the cell membrane vulnerable (Liu et al., 2024). The imbalance between GPx and ROS can lead to membrane damage, which, in turn, might amplify the cellular effects of oxidative stress.

Moreover, de Almeida et al. (2004) reported increased GPx enzyme activity in mussels exposed to pesticides, leading to enhancement of lipid peroxidation. Lipid peroxidation interferes with the cell membrane structure, disturbing the intracellular functions.

DNA disruption is among the possible side effects of pesticide exposure. Many exposures directly cause DNA strand breaks and increase further the chances of mutation (Bolognesi, 2003). Especially, organochlorine pesticides disrupt DNA strand breaks and induce chromosomal anomalies in mussels and give rise to a genotoxic impact that congests normal cell division, thus making cells senesce or mortality (Bolognesi & Cirillo, 2014).

According to La Vecchia (2022) and Fallet (2023), pesticide exposure interferes with DNA methylation in mussels, leading to epigenetic changes. These epigenetic changes then negatively impact gene expression or cellular response and hence result in long-lasting and irreversible harm. It is also reported in the same research that inhibiting

the DNA repair mechanism through pesticide exposure restricts the cells' power to repair genetic material, raising the probability of mutation.

Lipid Peroxidation and Cell Membrane Damage

Lipid peroxidation is considered a biochemical process induced by various environmental and endogenous stressors that impairs the structural integrity of cellular membranes, thus confronting cellular functions with danger (Abdollahi et al., 2004). This oxidation of the cell membrane polyunsaturated fatty acids increases membrane permeability, disrupting intracellular homeostasis (Pamplona et al., 2002). Exposures to environmental pesticides is also known to accelerate the process of lipid peroxidation in mussels, leading to cell membrane damage (Liu et al., 2024).

This increase in lipid peroxidation impairs cellular membrane functions, which, in turn, disrupt ion balance and intracellular energy production processes for the worse (Freitas et al., 2017). Various studies have reported increased ion permeability of cell membranes by pesticides through lipid peroxidation, which results in an electrolyte imbalance at the cellular level (Martem'yanov, 2000; Santos, 2001; Yaman & Ayhanci, 2021).

Glutathione (GSH) and Detoxification Enzymes

GSH is an important constituent of intracellular detoxification processes and has an important role in the regulation of oxidative stress. Intracellularly, glutathione scavenges for ROS, thus decreasing cellular destruction and maintaining intracellular homeostasis. However, pesticide exposure decreases glutathione levels, further debilitating detoxification processes in mussels (Arslan et al., 2023). Pesticides suppress levels of GSH, further enhancing oxidative stress, which results in aggravated cellular destruction in mussels (Canesi et al., 1999).

Metallothioneins (MT) and Heavy Metal Binding Capacity

Another biochemical effect of environmental stressors on mussels is related to MT (metallothioneins). MTs are considered heavy metal-binding proteins, which protect cells from metal toxicity. Some pesticides can induce changes in MT levels in mussels, hence affecting

their defense mechanisms against environmental pollutants such as heavy metals (Žurga et al., 2024). According to Dondero et al. (2011), exposure to organophosphate pesticide chlorpyrifos suppresses the MT levels of mussels, leading to heavy metal accumulation followed by alterations in cellular MT response. Glyphosate, a well-known herbicide, increased the thiol levels of MT in freshwater mussels without the typical action of binding Zn, Cu, and Cd. These disturbances in the biochemical mechanism are regarded to increase the susceptibility of mussels to heavy metal toxicity (Dondero et al., 2011; Khoma et al., 2021).

Pesticide contamination in aquatic ecosystems can greatly harm the digestive systems of freshwater mussels, causing both tissue damage and disruption of essential digestive enzymes (Donkin et al., 1997). Exposure to pesticides, particularly organophosphates, has been linked to notable cellular changes in the digestive glands, such as degeneration, atrophy, and inflammation (El-Shenawy et al., 2009). Structural issues negatively affect nutrient digestion and energy metabolism, such as changes in the digestive gland tissues decrease the production of enzymes. It is stated in the latest researches has indicated that pesticide exposure can lead to excessive cellular growth and inflammatory responses in the digestive tissues of mussels (Stara et al., 2020).

Structural changes in the digestive glands may become permanent with chronic pesticide exposure. Pesticides are reported to cause permanent fibrosis in the digestive gland tissues of mussels, resulting in a loss of structural cellular integrity. This condition is likely to impair mussels' digestive functions irreversibly (Benjamin et al., 2016).

EFFECTS ON POPULATION DYNAMICS

Freshwater mussels serve important organisms in aquatic ecosystems due to their filter-feeding nature. Considering the ability of pesticides to affect these organisms, effects beyond just individual health issues have arisen because they may considerably influence population dynamics. Exposure to pesticides reduces reproductive capacity (Aldridge et al., 2023), suppresses growth rates (Perry & Lynn, 2009),

induces behavioral changes (Chmist et al., 2019), and increases mortality rates severe at the population level (Moulton et al., 1996).

One of the striking effects pesticide exposures has on freshwater mussels comes from the impacts on the reproductive system. Pesticides can suppress reproductive function and thereby, as a consequence, reduce renewal capacity in such populations (Aldridge et al., 2023). Whereas spermiotoxicity testing initially started with sea urchins (Dinnel et al., 1987), its use with other organisms was later approved by USEPA (2009) for ecotoxicological research. Adequate motility and viability, mitochondrial activity, and production of ROS are some critical constituents of reproductive competence (Rolton et al., 2022).

Triclosan, a pesticide-like chemical, as indicated by Rolton et al. (2022), suppressed gametogenesis and caused significant reductions in sperm and oocyte number. This research emphasized that pesticides lead to reproductive failures as a result of the inhibition of the maturation of the gamete cells. Furthermore, research conducted by Canesi et al. (2011) showed that pesticides may act as endocrine disruptors with estrogenic effects, thereby disrupting the work of the endocrine system and hormonal balance. Pesticides disrupt the synthesis and/or regulation of sex hormones within mussels and lead to structural changes in reproductive organs that may culminate in reproductive failures, resulting in a reduction in offspring numbers.

Pesticide exposure limits the population's renewal capacity, mainly by reducing the growth rate of juvenile mussels. Perry and Lynn (2009) studied the effects of Baylucide insecticides on apoptosis rates in freshwater mussels. They stated that as a result of high-dose-mediated suppression of apoptosis, cellular abnormalities and embryonic deformities occurred. Furthermore, Lindsay et al. (2010) found that various pesticides, such as hexazinone, 2,4-D, and phosmet, impaired shell growth and overall development in veliger-stage juvenile mussels, leading to lower growth rates and body weights. Among these, 2,4-D was particularly damaging, significantly reducing survival and reproductive rates.

Mussels are very energetic species with regard to growth, and pesticide exposures may affect energy metabolism processes. He et al.

(2020) reported that some pesticides (DDT, diazinon, imidacloprid, chlorpyrifos, and malathion) lowered energy absorption in the gut and disturbed energy storage mechanisms within the liver, fat tissues, and muscles, leading eventually to a decline in growth rates and increased population-level stress. All these pesticides also disrupted immune system and pancreas functions by disturbing energy homeostasis and inhibiting growth hormone secretion, interfering with physiological development processes of mussels.

The presence of pesticides interferes with physiological and biochemical processes, which characterize the living conditions of mussels; therefore, their populations are more likely to decline with time. Results showed that exposure to the hexazinone and phosmet significantly caused high mortality rates and growth retardation during the veliger stage of larvae in juvenile mussels, which resulted in reduction in population size (Lindsay et al., 2010). In addition, exposure to different groups of pesticides was reported to suppress cellular functions of mussels; with enhanced oxidative stress, too, the cell death rate is getting higher. This contributes to higher cellular damage, which degrades the tissues and consequently impairs the functioning of organs, thereby further increasing mussel mortality. Besides, pesticides are reported to have the ability to induce lipid peroxidation, disturbing the integrity of the cell membrane, which may subsequently lead to premature cell death (Paçal et al., 2022).

EFFECTS ON THE IMMUNE SYSTEM

Pesticide is a notable environmental stressor, and the immune system — which responds to environmental stressors — can be directly affected by pesticides. In mussels, immune mechanisms such as phagocytosis, cytotoxicity, and antioxidant defence is controlled by hemocytes (Günal et al., 2018). But pesticides contaminations can deactivate these physiologic pathways and cause immunosuppression in mussels (Renault, 2015). Acute exposures to the agricultural insecticides, fipronil and cyphenothrin, have been found to reduce total hemocyte counts (THCs) in freshwater mussels, while chronic exposures have been shown to increase THC. Contrarily, short-term exposure

activates this response and has been mentioned together with an immune system activation (Arslan, 2022; Arslan & Günal, 2023).

ECOSYSTEM-LEVEL EFFECTS

Among the roles of mussels in ecosystems, there are water filtration, food web, and habitat functions achieved through reproductive symbiosis (Gül, 2023). When pesticides contaminate by various ways such as flood, to aquatic environments, they can disrupt these vital functions in mussels and placing the entire ecosystem at risk.

Mussels are critical organisms for water quality by removing both organic and inorganic particles from aquatic environments. Nevertheless, the ability to filter aquatic environments is impaired under pesticide contaminations. This loss of filtration may lead to negative changes in water quality. Therefore, there is a high probability of a higher concentration of suspended and dissolved matter in water, may resulting in the disruption of the plankton balance and indirectly in the food web disruption (Schultze et al., 2023). Exposure to glyphosate-containing herbicides such as Roundup has been shown to reduce water flow capacity in mussels.

It is reported that pesticides can be passed to higher trophic level predators via mussels and their toxic effects can be further amplified during the transfer. This indirect effect can be detrimental to the health of other aquatic organisms and to the equilibrium of the ecosystem (Katagi, 2010; Tulcan et al., 2021).

CONCLUSION AND RECOMMENDATIONS

Pesticide effects on mussels may lead to physiological, and biochemical changes. Pesticides can disrupt respiratory, gastrointestinal and reproductive physiology, interrupting the life cycle of mussels, and leading to population declines. In addition, oxidative stress and DNA damage are increased by antioxidant defence mechanism inhibition, which is impairs cellular functions. The immunosuppressive effect of pesticides on the immune system elevates the negative health risks of mussels to pathogens. It is predicted that difficulties experienced by mussels will result in broad ecosystem trouble in the future. Careful management of pesticide application to minimise those effects is of

paramount importance together with long-term monitoring studies to elucidate their ecological impacts.

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CHAPTER 8

BIOACTIVE SUBSTANCES IN AQUATIC ECOSYSTEM

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INTRODUCTION

Aquatic environments are crucial ecosystems, rich in potential pharmaceutical resources and biologically active substances (Chen, 2024). The biological resources produced and isolated from these environments have garnered significant research interest due to their pharmacological relevance (Supanekar et al., 2024). These bioactive substances encompass a range of compounds with properties such as anticancer, antibacterial, anti-inflammatory, and immunomodulatory effects (Xiao et al., 2022). Organisms like seaweed, sponges, corals, and microorganisms are valuable sources for drug discovery and development, offering therapeutic potential for treating various diseases (Chen, 2024). Their properties hold promise for developing treatments for diseases like cancer, infections, and neurodegenerative disorders. In this article, some bioactive compounds found in aquatic environments were aimed to be understood in terms of their biological effects and evaluated for their therapeutic potential in the field of health.

SOURCES, CLASSIFICATION AND PHARMACOLOGIC EFFECTS OF BIOACTIVE SUBSTANCES

Marine ecosystems consist of a rich variety of life forms, including microorganisms, plankton, benthic organisms, seaweed, corals, invertebrates, and vertebrates. This diversity is shaped by various ecological processes and environmental factors that affect the distribution and abundance of these organisms (Al-Sodany and Diab, 2023; Biswas et al., 2023). Microorganisms in ocean environments play a critical role in various ecological processes, including the carbon and nitrogen cycles, organic matter decomposition, and the maintenance of ecological balance. They significantly contribute to the nutrient cycle and waste management necessary for the sustainability of marine ecosystems (Haripriyaa and Suthindhiran, 2024; Raina and Seymour 2024; Shilky et al., 2023). Furthermore, marine life positively contributes to the global economy and human society by providing food, medicine, and other resources (Chen, 2024).

Bioactive substances are compounds that interact with biomolecules in living organisms, causing physiological or biochemical

reactions. These substances, which can be obtained from various biological sources such as plants, animals, microorganisms, and marine organisms, play significant roles in health and disease management, particularly in the food industry, pharmaceuticals, and agriculture (Kang and Kim, 2023).

Aquatic bioactive substances are compounds obtained from marine organisms, including microorganisms, algae, invertebrates, and fish (Senadheera et al., 2023). Aquatic bioactive substances can be classified based on various characteristics such as chemical composition, effectiveness, and roles (Ismail, et al., 2023). The marine biosphere is rich in components such as bioactive peptides, polysaccharides, polyunsaturated lipids, carotenoids, polyphenolic compounds, minerals, saponins, and phytosterols (Hosseini, et al., 2022).

Bioactive peptides derived from marine organisms exhibit a wide range of biological functions, including antioxidant, antimicrobial, anti-diabetic, antihypertensive, anti-inflammatory, immunomodulatory, and anticancer properties. These functions are influenced by the molecular characteristics of the peptides, such as size, shape, charge, and hydrophobicity. Various production methods, including enzymatic hydrolysis, microbial fermentation and chemical hydrolysis, contribute to the extraction of these peptides from sources like shellfish, mollusks, and algae (Hosseini et al., 2017; Ramezanzade et al., 2017; Kim and Wijesekara, 2010). The investigation of bioactive peptides as α-glucosidase, α-amylase, and dipeptidyl peptidase-4 (DPP-IV) inhibitors offers a promising approach for managing type II diabetes. Specific peptides derived from various sources, including fish and shellfish, have shown significant inhibitory effects on DPP-IV, an enzyme crucial for regulating blood sugar levels. A peptide derived from oysters has demonstrated strong DPP-IV inhibition (Chen et al., 2024) while salmon hydrolysates have also exhibited strong anti-diabetic activity due to hydrophobic amino acid residues (Guo et al., 2024).

Antimicrobial peptides derived from fish and other aquatic organisms are emerging as effective agents against bacterial pathogens, including the species of *Listeria* and *Staphylococcus*. These peptides exhibit unique antimicrobial properties and mechanisms of action,

making them promising alternatives to traditional antibiotics, especially in the context of increasing antibiotic resistance (Asif et al., 2024; Cervera et al., 2024; Lee et al., 2024).

Omega-3 polyunsaturated fatty acids, which can be isolated and purified from marine sources through methods such as crystallization, distillation, and solvent extraction, are recognized for their significant health benefits. These benefits are largely attributed to long-chain n-3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid which play important roles in cardiovascular health and inflammation modulation (Boyer et al., 2022; Tang et al., 2023). Studies have also demonstrated the oxidative stress regulation and anti-aging effects of fish oil and polyunsaturated fatty acids (Zhang et al., 2016).

However, in addition to the potential therapeutic effects of these bioactive components, there are some potential disadvantages. For example, persistent organic pollutants that may accumulate in fish can negatively impact the health benefits of omega-3 long-chain PUFAs derived from fish by accumulating high levels of xenobiotics (Cui et al., 2018).

Algae-derived polysaccharides have biological activities such as antioxidant/antibacterial, antidiabetic, immunomodulatory, anti-inflammatory, and anticancer effects (Gomaa 2024; Jagtap et al., 2024; Matin et al., 2024). These biological activities depend on properties such as molecular weight, branching structures, charge densities, hydrophobicity, monomer types, and bond types (Fernando et al., 2019).

Sulfated polysaccharides (SPs) derived from marine organisms exhibit a broad spectrum of biological activities, offering significant benefits for various therapeutic and nutritional purposes. These polysaccharides show anticancer properties by modulating critical intracellular signaling pathways and enhancing apoptosis in cancer cells (Bhuyan et al., 2023). They are also recognized for their antioxidant, anticoagulant, anti-inflammatory, immunostimulatory, and antitumor properties, which make them valuable in various health-related fields (Abdelhedi et al., 2024; Lakhrem et al 2024; Wang et al., 2024). Sulfated polysaccharides extracted from *Sargassum duplicatum* effectively suppress colony formation in HCT-116 human colon cancer cells at

defined concentrations (Gomaa, 2024). Similarly, polysaccharides obtained from *Sargassum fusiforme* exhibit potent *in vitro* cytotoxicity against HepG2 human liver cancer cells and significantly inhibit tumor growth *in vivo* (Sadeghi et al., 2024).

Fucoidans, a class of sulfated polysaccharides rich in fucose derived from brown seaweeds, exhibit a wide range of biological activities, making them significant for therapeutic applications. Their unique chemical structures allow them to interact with various biological targets. They have been shown to reduce inflammation by modulating immune responses in periodontal diseases, inhibit tumor growth and metastasis by regulating cell signaling pathways and promoting apoptosis in cancer cells (Zahariev et al., 2023). Additionally, fucoidans have the potential to protect against oxidative stress and neuroinflammation, making them promising agents in the treatment of brain disorders (Batista et al., 2023) Moreover, fucoidans can form biopolymer nanoparticles with cationic chitosan, enhancing drug delivery efficiency in oncology (Zahariev et al., 2023).

Carrageenan, a sulfated polysaccharide obtained from red seaweed, showcases notable biological properties that support its applications in pharmaceuticals and nutrition. As a plant-based alternative to gelatin, it demonstrates anticoagulant, anticancer, immunomodulatory, anti-hyperlipidemic, and antioxidant activities, contributing to advancements in both dietary and biomedical fields. Biomedical applications include drug delivery systems and tissue engineering, which benefit from its immunomodulatory and antioxidant properties. These polysaccharides can be extracted using water or alkaline solutions, and their efficiency and sustainability can be achieved through environmentally friendly methods, such as hydrothermal extraction (Álvarez-Viñas et al., 2024)

Alginate is a polysaccharide present in significant quantities within various species of brown macroalgae (Lone et al., 2016). The ability of alginate to form hydrogels enhances therapeutic efficacy by allowing controlled drug release (Wawszczak and Kołodyńska, 2024). With its moisture-retaining properties, it is ideal for wound care, promoting healing while preventing infection. It is widely used as a thickening and

gelling agent that improves the texture and shelf life of food products (Pournaki et al., 2024).

Chitin, a polymer derived from marine waste such as shrimp, crab, and lobster shells, as well as some green algae, can also be produced from the cell walls of certain fungi and protozoa (Duan et al., 2018; Hu et al., 2016). Chitin and its derivatives can be used in the production of various functional materials such as membranes, films, gels, nanoparticles, and nanofibers. For example, chitin-based fiber materials have been shown to increase collagen growth, thereby accelerating wound healing processes (Hassen et al., 2024; Yanat and Schroën, 2023). Chitosan, a derivative of chitin, serves as a carrier for active pharmaceutical ingredients (Sarjadi et al., 2020). It has also been shown that chitin nanoparticles can strengthen biodegradable plastics, providing a sustainable alternative to traditional materials used in food packaging (Yanat and Schroën, 2023). In the cosmetic industry, they are being researched as eco-friendly substitutes for synthetic polymers (Hassen et al., 2024; Sarjadi et al., 2020).

Chitosan is derived from chitin, one of the most abundant biopolymers in nature. It is produced through the alkaline deacetylation of chitin and possesses important properties such as biocompatibility and biodegradability. These features make chitosan a versatile material with a wide range of applications across various fields, including biomedical, environmental, and industrial sectors. Chitosan's non-toxic properties enable its safe use in medical applications such as drug delivery, wound healing, and antibacterial treatments (Thakare et al., 2024; Lingait et al., 2023). It is also used in drug delivery systems and tissue engineering (Thakare et al., 2024; Hemmami et al., 2023). Chitosan is also effective in wastewater treatment and removal of metal ions due to its adsorption capability (Amor et al., 2024; Hemmami et al., 2023).

Chito-oligosaccharides, derived from chitosan, have become promising candidates for therapeutic agents due to their good water solubility, easy absorption properties, and antibacterial, antifungal, and antiviral activities (Li et al., 2024; Struszczyk-Świta, 2024). They have been reported to show significant antitumor properties, particularly against cervical and pancreatic cancer cells, by inducing apoptosis and

inhibiting cell migration through mechanisms such as regulating epithelial-mesenchymal transition. Due to their biocompatibility and ability to enhance drug solubility, they are used in drug delivery systems (Li et al., 2024). Additionally, they are also described as a calcium-binding agent that can potentially increase calcium solubility (Yu et al., 2024).

Carotenoids, natural pigments produced by photosynthetic organisms, exhibit various biological functions that contribute to human health. These functions are due to their antioxidant, anti-diabetic, anti-proliferative, and wound-healing properties, which lead to significant effects such as improved eye health and reduced risks of chronic diseases like coronary heart disease and cancer (Beltrán and Wurtzel, 2024; Sharma et al., 2024). For example, carotenoids like lutein and zeaxanthin have been reported to provide protection against age-related macular degeneration, and regular consumption of carotenoid-rich foods has been associated with a lower risk of heart disease (Sharma et al., 2024).

Fucoxanthin, a carotenoid derived from various brown algae, exhibits bioactive properties that make it a promising candidate, particularly in the management of diabetes and neurodegenerative diseases. It has been shown to effectively inhibit critical enzymes in carbohydrate metabolism, α-glucosidase and α-amylase, while stimulating insulin secretion. For example, studies on *Sargassum wightii* demonstrate that fucoxanthin can significantly reduce hyperglycemia in diabetic models and improve enzyme activity (Raji et al., 2023). *In vitro* studies show that fucoxanthin and its metabolite, fucoxanthinol, exhibit antioxidant properties, protecting neuronal cells from oxidative stress and neurotoxicity associated with neurodegenerative conditions (Kumarasinghe and Gunathilaka, 2024; Pruccoli et al., 2024).

Astaxanthin, a powerful xanthophyll carotenoid obtained from *Haematococcus pluvialis* and various shellfish, is renowned for its antioxidant properties. Its versatile health benefits include preventing lipid peroxidation, scavenging reactive oxygen species, and reducing the expression of matrix metalloproteinases (MMPs), which play a crucial role in cancer progression (Xie et al., 2024). Notably, when combined with carbendazim, astaxanthin has shown enhanced anti-proliferative

effects against MCF-7 breast cancer cells, highlighting its potential in cancer treatment (Atalay et al., 2019; Kim et al., 2024). Astaxanthin is a carotenoid that can cross the blood-brain barrier, and therefore, it has been reported to possess various neuroprotective properties (Zhou et al., 2019; Kusak et al., 2024).

Marine polyphenols, especially those extracted from macroalgae and seaweeds, exhibit significant antioxidant properties and potential therapeutic effects. Phlorotannin compounds, primarily found in brown algae, have shown strong antioxidant and anti-inflammatory effects that contribute to their anticancer potential (Goya and Mateos, 2024). Flavonoids such as quercetin and kaempferol, found in various seaweed species, stand out for their superior antioxidant abilities compared to traditional antioxidants like alpha-tocopherol (Esmaeili 2024). Bromophenols are recognized for their antimicrobial and anticancer properties and hold potential for use in functional foods (Goya and Mateos, 2024). Recently, bromophenol derivatives isolated from the red alga *Symphyocladia latiuscula* have shown promising effects as a D4R agonist (Paudel et al., 2019).

Seaweeds are valued for their abundance of B vitamins, including A, C, D, E, riboflavin, niacin, pantothenic acid, folic acid, and folate derivatives. Fish oils provide vitamins A, D, and E in significant amounts (Kim et al., 2011). Seaweeds also enhance dietary iodine levels, serving as a key contributor for many populations (Ficheux et al., 2023). Additionally, varieties like *Laminaria* contain calcium and potassium, which are crucial for various bodily functions (Blikra et al., 2024.). The minerals in seaweed are generally more bioavailable than those in land plants, increasing their nutritional benefits and potential applications in promoting human health)

Calcium phosphate extraction from fish bones and scales through thermal treatment results in the formation of materials such as hydroxyapatite and biphasic calcium phosphate (Alshemary et al., 2024). Hydroxyapatite derived from fish scales exhibits excellent biocompatibility and osteogenic potential (Thomas et al., 2024). Hydroxyapatite is a bioceramic material very similar to the chemical composition and crystal structure of human bone, making it a prime

candidate for medical applications, especially in bone defect repair. Its osteo-inductive and osteo-conductive properties facilitate bone regeneration. It demonstrates excellent biocompatibility, promoting cellular adhesion and osseointegration, which are essential for successful implants (Mondal et al., 2023), and is used in applications for bone grafts and dental implants (Mysore et al., 2024).

Fish processing by-products (scales, bones, and skin) are rich sources of collagen. Collagen obtained from aquatic sources offers a viable substitute for mammalian collagen in diverse applications, including wound dressings. Collagen peptides have demonstrated potential in managing osteoporosis and supporting bone health over time (Lara Juache et al., 2024). They also exhibit antioxidant properties that can reduce oxidative stress and inflammation (Makgobole et al., 2024).

Due to their moisturizing and rejuvenating effects, collagen peptides are increasingly used in skincare products and provide protection against ultraviyole damage (Makgobole et al., 2024). Additionally, they serve as stabilizers and emulsifiers in various food products, enhancing texture and nutritional value (Farooq et al. 2024).

Marine gelatin, predominantly derived from collagen-rich fish skins, bones, and jellyfish, exhibits promising bioactive properties, making it suitable for functional food products (Farooq et al., 2024; Joy et al., 2024).

It demonstrates gel strength and viscosity essential for stabilizing emulsions and improving the texture of food products (Silviwanda and Naenum, 2024). With antioxidant and antimicrobial properties, marine gelatin is also applied in health-focused food innovations (Shaik et al., 2024).

Saponins found in certain marine animals and sea cucumbers have been reported to act as pancreatic lipase inhibitors in *in vitro* tests. Specifically, echinoside A (EA) and holothurin A (HA), saponins derived from the sea cucumber *Pearsonothuria graeffei*, have been shown to suppress lipase activity and exhibit anti-obesity effects (Lin et al., 2022; Yue et al., 2022; Wang et al., 2018).

Red seaweeds contain phycoerythrin, a reddish-pink pigment that plays a key role in photosynthesis by transferring energy to chlorophyll α (Sudhakar et al., 2019).

Phycoerythrin and other pigments extracted from red seaweed exhibit various biological activities, including antioxidant, anti-inflammatory, and anti-diabetic effects. Additionally, they hold significant potential as natural colorants in nutraceutical applications (Carpena et al., 2023; Ramu Ganesan et al., 2023).

Fucosterol is the primary sterol found in brown seaweeds, whereas cholesterol is present in red seaweeds. Phytosterols offer numerous health benefits, including cholesterol-lowering effects, as well as antioxidant, anti-diabetic, anti-obesity, anti-atherosclerosis, anti-cancer, and anti-Alzheimer activities. Fucosterol has been reported to enhance the activity of antioxidant enzymes, such as superoxide dismutase and catalase (El Omari et al., 2024). It also upregulates heme oxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor 2 (Nrf2) pathways (El Omari et al., 2024; Meinita et al., 2021).Furthermore, it reduces neuroinflammation associated with Alzheimer's disease by inhibiting pro-inflammatory cytokines such as Interleukin-6 and Tumor Necrosis Factor- α in microglial cells. Additionally, by lowering adipocyte marker proteins, fucosterol inhibits adipogenesis and contributes to its anti-obesity effects (Meinita et al., 2021).

OPPORTUNITIES AND CHALLENGES

The pharmacological properties of marine bioactive substances are crucial for understanding their mechanisms of action, physiological effects, and interactions within living organisms. These compounds demonstrate their pharmacological activities through various mechanisms, including enzyme inhibition, receptor modulation, and disruption of cellular processes. Understanding the metabolic pathways of marine bioactive substances also helps determine their bioavailability, pharmacokinetics, and drug interactions, thereby ensuring their efficacy and safety for drug development and clinical applications.

Compounds derived from various marine organisms are increasingly recognized for their ability to address diverse health issues,

from cancer treatment to the management of autoimmune diseases (Selvaraj, 2024). They are being explored for their potential in developing new pharmaceutical agents (Supanekar et al., 2024).

This researchs involve complex, multi-step, interdisciplinary processes, including isolating, identifying, and studying marine bioactive compounds from marine organisms. These processes aim to identify their biological activities, such as antioxidant, anti-inflammatory, anticancer, and antibacterial effects, while further exploring their potential applications in advanced drug development, clinical trials, industrial applications, biotechnology, cosmetics, or ecological research. Despite their exciting potential, marine bioactive substances face challenges such as sustainability and cost, which restrict their broader applications. Addressing these challenges is essential for achieving sustainable resource management and expanding their use (Chen, 2024).

CONCLUSION

Marine environments are a rich and largely untapped source of bioactive substances with unique chemical structures and promising therapeutic potential. These natural compounds have demonstrated diverse biological activities, ranging from antimicrobial and anticancer effects to anti-inflammatory and neuroprotective properties. Despite significant advancements in the discovery and characterization of these substances, their clinical translation remains challenging due to complex extraction processes, limited scalability, and regulatory hurdles. Addressing these challenges is essential to harness the full potential of marine bioactive compounds for innovative medical applications.

The potential medical applications of marine bioactive substances offer new hope for global health and sustainable development. Realizing this potential and creating opportunities for future research and applications will require dedicated efforts. Investment in marine bioresearch and biotechnological innovation is vital to overcoming barriers in drug development and ensuring the sustainability of these compounds.

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CHAPTER 9

OMICS TECHNOLOGIES IN AQUATIC TOXICOLOGY

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INTRODUCTION

Rapidly developing technology in the 20th century has provided a better understanding of organisms and the ecosystem-organism relationship (Sundaray et al., 2022). In this period, called the "omics revolution" in science, studies can be carried out with organisms with special equipment (Lokman and Symonds, 2014). By determining the genome sequences of organisms, it has become possible to examine the only structure of that organism that remains unchanged throughout its life. By determining the expression levels of the products (such as protein, transcriptome, metabolite) coming out of the genes of this organism, it is revealed what kind of changes occur in the instantaneous state of the organism or in a certain life stage (such as larva, juvenile) (Sundaray et al., 2022). Thus, genetic markers have become one of the methods that can be used to determine the living conditions of organisms in ecosystems or their responses to ecosystem changes (Rise et al., 2019).

Towards the end of the 20th century, at the beginning of the millennium, –omics technologies, which began to be used in aquatic ecosystems in the field of aquaculture, have now begun to be used in the field of aquatic toxicology. Thus, this field has acquired a new name: ecotoxigenomics. These methods are very important indicators in monitoring pollution in aquatic ecosystems. Changes occurring in the community, population, individual, cell, molecule, or genome levels are identified and allow the effects of toxic substances on environmental health, especially in the aquatic ecosystem, to be estimated (Revel et al., 2017).

"omics" defines scientific studies that provide the highest yield in biological studies. These studies provide a holistic approach by combining individual omics technologies such as genomics, proteomics, transcriptomics, and metabolomics. Genomics examines the entire genome, while proteomics performs a comprehensive analysis of all proteins. Transcriptomics examines mRNAs formed from genes, while metabolomics covers studies involving interactions of chemical molecules and chemical reactions between small or large biological macromolecules (Nam et al., 2023). Some omics fields and targets applicable to environmental pollution assessment are given in Figure 1.

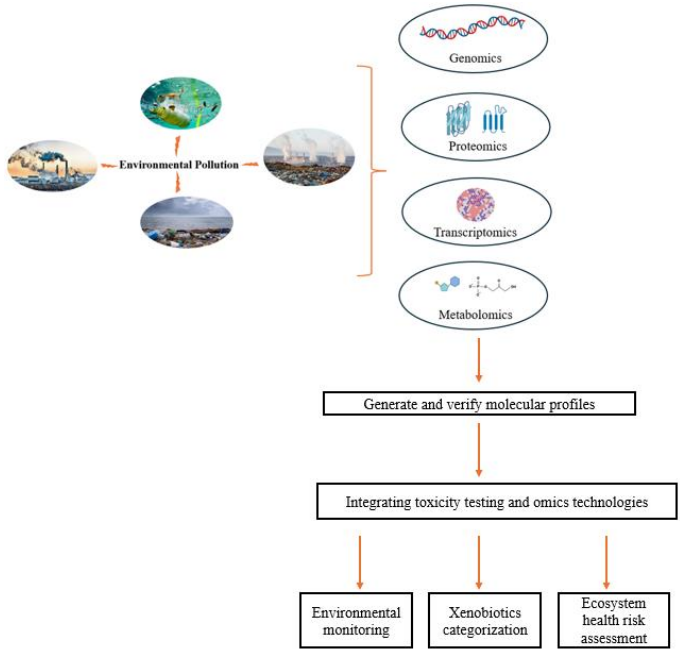

Figure 1. The use of omics in environmental toxicology (adapted from Ge et al., 2013)

There are many different threats that aquatic ecosystems face today. The most important of these is the stress that occurs in aquatic organisms due to climate change and global warming, which cause changes in water quality parameters. In addition to this stress in aquatic ecosystems, many factors such as pesticides used in agricultural production, domestic and industrial areas, industrial wastes, pharmaceuticals, personal care products, heavy metals, plastic wastes cause pollution of aquatic ecosystems and affect aquatic organisms. In ecosystem monitoring studies, the examination of water quality parameters and aquatic biota are the basic factors that indicate ecosystem health (Machuca-Sepúlveda et al., 2023).

These omics technologies used in environmental pollution studies are also used to examine pollution occurring in aquatic ecosystems. The primary goal of monitoring aquatic organisms as bioindicator model organisms is to understand the health status of the ecosystem in which they live, to detect early warning signals, and to determine reliable ranges of environmental changes or xenobiotics. Aquatic ecosystems are

under great threat due to direct or indirect anthropogenic activities. Although characterizing xenobiotics in samples taken from these ecosystems is important for environmental risk assessment, it is still insufficient. The effects of xenobiotics are examined from the cellular level to the population level using aquatic organisms (Nam et al., 2023). In this review, -omics technologies used in aquatic toxicology studies will be described.

GENOMICS

Scientific developments in the field of genetics have created the field of genomics. Genetics is the branch of science that studies genes and gene functions. Genomics is a field where genome sequences are documented (Solanke and Kanika, 2015).

Studies in the field of genomics are older than other omics technologies in terms of environmental toxicology and therefore aquatic toxicology. Gene expression data allow us to examine the toxicity mechanisms developed by the organism and to determine their biological responses to xenobiotics or environmental stressors and to make inferences about the conditions in which the organism is located (Solanke and Kanika, 2015; Baettig et al., 2024).

Although the history of genomics began in the 1800s, modern genomics began in the 1970s. Following the sequencing of the first genome strand by Frederick Sanger, genomic studies progressed at an increasing pace with the development of automated DNA sequencing and polymerase chain reaction techniques. The completion of the genomes of the Human Genome Project and model organisms (*Escherichia coli*, *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Danio rerio*, *Arabidopsis thaliana*, *Mus musculus*) constituted important parts of genomic studies (Solanke and Kanika, 2015). With the development of technology, the genetic codes of many aquatic organisms have been studied using high-throughput next-generation sequencing (NGS) in the field of genomics (Nam et al., 2023).

Genomics studies on aquatic organisms taken from aquatic ecosystems or tested under controlled laboratory conditions are shown in Table 1.

Table 1. The genomics studies in the aquatic toxicology

Organisms	**Condition**	**Key results**	**References**
Padina pavonia *Laurencia pap*illosa *Colpomenia sinuosa* Cuttlebone	Textile dye (CI Reactive Red 66)	Various genes have been identified in the biotransformation of textile dyes.	Chaieb et al., 2023
Danio rerio	Cadmium	It has been determined that DNA methylation occurs in cadmium exposure and epigenetic changes occur in estrogen-sensitive genes.	Pierron et al., 2023
Danio rerio	Antimony	Antimony exposure showed increased non-synonymous single nucleotide polymorphisms (SNPs) in coding gene regions.	Yao et al., 2023
Perna canaliculus	Copper Benzo[a]pyrene	As a result of substance exposures, detoxification mechanisms, cell membrane transport events, endocrine system disruption and genotoxicity were determined.	Baettig et al., 2024
Danio rerio	Dibutyl phthalate	Increased oxidative stress due to substance exposure disrupted mitochondrial functions, resulting in oxidative stress in mitochondria.	Fan et al., 2024
Mytilus galloprovincialis	Polyethylene terephthalate microparticles	Increased DNA methylation and increased toll-like receptor gene expression were observed in mussels with microplastic exposure.	Park et al., 2024

PROTEOMICS

Proteins, one of the most studied biological macromolecules in aquatic organisms, are particularly effective on the taste and nutritional values of the organism in aquatic living culture. In traditional protein studies conducted with living organisms in aquatic ecosystems, aquatic living culture or aquatic toxicology, the amount of protein contained in the organism, amino acid composition, the role of enzymes, and the content of antioxidant substances are determined. A new perspective has developed in protein studies with proteomics studies. Proteomics, which allows the examination of post-transcriptional modification systems, reveals how living organisms respond under the influence of xenobiotics (Wang et al., 2023b). Therefore, proteomics is the measurement of whole protein in a cell or tissue and is used to analyze the biological responses of aquatic organisms to various xenobiotics (Rise et al., 2019).

Proteomic studies include three main technologies: electrophoresis, chromatography, and mass spectrometry. The results obtained from these three technologies are interpreted through bioinformatics. Following sampling from aquatic organisms, the resulting protein mixtures are separated by electrophoresis or chromatographic means and prepared for mass spectrometry. Using mass spectrometry, the protein content of the organism is identified (Andreeva, 2023). Apart from these three methods, isobaric methods that provide label-free quantitative relative and absolute protein identification have also been developed in recent years. With these methods, proteins can be identified, and the relative concentration of proteins can be measured (Meng et al., 2023).

Proteomics studies on aquatic organisms taken from aquatic ecosystems or tested under controlled laboratory conditions are shown in Table 2.

Table 2. The proteomics studies in the aquatic toxicology

Organisms	Condition	Key results	References
Thalassiosira pseudonana	Low and high CO_2	Low CO_2 environment resulted in fewer carbon acquisition enzymes, high nitrogen metabolism enzymes and only one of the chloroplast target proteins. Increased Calvin Cycle proteins were observed in high CO_2 environment.	Clement et al., 2017
Chlorella sp.	Alpha-cypermethrin	53 proteins were identified that showed differential accumulation with substance exposure in important cellular metabolic events such as photosynthesis, carbohydrate metabolism, cell division, and lipid metabolism.	Chanu et al., 2023
Danio rerio (embryo)	Benzyl benzoate	A total of 83 differentially expressed proteins (49 up-regulated and 34 down-regulated) were identified due to substance exposure. These proteins were found to be involved in different biological activities including translation, amide biosynthetic process, lipid transport, stress response and cytoskeletal activity.	Kwon et al., 2023
Alosa pseudoharengus, Myoxocephalus thompsonii, Salvelinus namaycush	Per- and polyfluoroalkyl substances (PFAS)	Effects of PFOS on Proteins Obtained from fish serum proteins. PFOS-exposure was found to contain similar serum proteins in all three fish species. Albumin was found to be observed only in *Salvelinus namaycush* protein for the other two species.	Point et al., 2023

Table 2. The proteomics studies in the aquatic toxicology (continue)

Organisms	Condition	Key results	References
Danio rerio (embryo)	Copper	The increase in the duration of copper exposure was observed to cause higher proteome differentiation in fish embryos. Apart from oxidative stress, cell respiratory events and neurotransmission, proline, glycine and alanine amino acids were shown to cause differentially expressed proteins.	Green et al., 2024
Danio rerio	Glyphosate and its metabolits aminomethylphosphonic acid	Proteome changes were observed in cellular respiration events, carbohydrate and lipid metabolism reactions to substance exposure.	Morozov and Yurchenko, 2024
Rhamdia quelen	Low and high temperature levels	It was observed that temperature change caused changes in 42 proteins in female fish and 62 in male fish.	Vicentini et al., 2024

TRANSCRIPTOMICS

Messenger RNA, ribosomal RNA, transfer RNA, and other non-coding RNA molecules within the cell all make up the transcriptome of a cell (Solanke and Kanika, 2015). Transcripts are biomarkers used as an early diagnostic test in that they generate sensitive responses to any substance exposure or environmental conditions, unlike the genome (Joseph, 2017).

Transcriptomics is a technology that studies transcriptomes that occur during the transcription process within the protein synthesis process within the normal metabolism of the cell. Transcriptomic responses obtained from organisms taken from natural habitats or from organisms in controlled experiments in the laboratory environment are the responses of the cells and therefore the organism to these stress factors (Jeffrey et al., 2023).

Transcriptomic analysis emerged with the development of large-scale and high-throughput methods. It allows the expression of all genes in a sample and the detection of genes that are expressed differently between different samples. The methods used in transcriptomic analysis include quantitative real-time PCR and microarray analysis (Solanke and Kanika, 2015). High-throughput RNA sequencing technology enables comprehensive analysis of genes differentially expressed under different environmental conditions, revealing the entire transcriptome of organisms (Liu et al., 2023).

Transcriptomics studies on aquatic organisms taken from aquatic ecosystems or tested under controlled laboratory conditions are shown in Table 3.

Table 3. The transcriptomics studies in the aquatic toxicology

Organisms	**Condition**	**Key results**	**References**
Dicentrarchus labrax	Polystyrene nanoplastics	It has been determined that the substance exposure has effects on biological events such as membrane receptors and fish immunity in fish.	Cuesta et al., 2023
Siniperca chuatsi	Hypoxic stress	Under hypoxic conditions, there were changes in the expression of certain genes in endoplasmic reticulum activities and cell signaling metabolism.	Ding et al., 2023
Mytilus edulis	Hypoxic stress	Changes in transcriptomes responsible for organelle activities were observed during the early stages of hypoxic conditions.	Hall et al., 2023
Sander vitreus	Hypoxic stress	Hypoxic conditions have been shown to be associated with transcriptomes related to protein catabolism, DNA repair, molecular chaperones, and ion regulation.	Jeffrey et al., 2023
Platax teira	Heat stress	Differentially expressed genes in cell division and metabolism events in fish under heat stress were identified.	Liu et al., 2023
Mytilus galloprovincialis	Decabromodiphenyl ethane	It was observed that genes related to cholesterol homeostasis were changed in female and male individuals under the effect of the substance. In addition, effects occurred on reproductive genes.	Wang et al., 2023
Mytilus trossulus	Norfluoxetine	In females, serotonin synthesis and transport were stimulated, accelerating gamete formation. In males, serotonin levels decreased, delaying sperm maturation. Thus, transcriptomic analyses revealed that the substance had effects on gametogenesis.	Goździk et al., 2024

METABOLOMICS

Metabolomics is a method based on the qualitative and quantitative investigation of metabolites obtained from all samples in a biological system from cell to organism (Bedia, 2022). Metabolomics is an omics technology that studies endogenous low molecular weight (<1000 Da) metabolites including carbohydrates, fatty acids, organic acids present in a biological sample (Olesti et al., 2021; Tian et al., 2024). Unlike other omics technologies, it is a method close to the cellular phenotype (Olesti et al., 2021).

In environmental science research, metabolomics is an ecotoxicological tool used to characterize the effects of abiotic stress and xenobiotic substances on ecosystem health (Bedia, 2022). The European Centre for Chemical Ecotoxicology and Toxicology showed in 2016 that omics technologies are not widely used in hazard assessment. Subsequently, thanks to the risk assessment research derived from metabolomics studies, the draft Metabolomics Standards Initiative in Toxicology was published as a standard facilitating the use of metabolomics (Olesti et al., 2021).

Measurements of metabolites are performed using nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography–mass spectrometry (LC-MS) instruments. In the analyses performed with these instruments, data obtained from GC-MS have higher throughput (Nguyen and Alfaro 2020; Huang et al., 2021).

Metabolomics studies on aquatic organisms taken from aquatic ecosystems or tested under controlled laboratory conditions are shown in Table 4.

Table 4. The metabolomics studies in the aquatic toxicology

Organisms	**Condition**	**Key results**	**References**
Pimephales promelas	Spironolactone	Changes in hormone concentrations were observed in females and males. Metabolites involved in osmoregulation, cell membrane transport, carnitine and vitellogenin synthesis were found to be altered.	Davis et al., 2017
Pagrus major, Verasper variegatus, Pleuronectes yokohamae	Dithiocarbamate	Fifty-three different metabolites were obtained from fish species, and reduced glutathione was increased in all of them.	Hano et al., 2017
Daphnia magna	Heat stress Salinity stress Hypoxia stress	It has been found that carbohydrates, organic acids, amino acids and fatty acid metabolites in the organism change under stress conditions.	Garreta-Lara et al., 2018
Daphnia magna	Fenoxycarb	It has been determined that metabolites occurring during the reproductive cycle change.	Jeong and Simpson, 2020
Danio rerio	Bisphenol A	In the study evaluating the relationship between substance effect and obesity, it was determined that lipid derivatives (triglyceride, diglyceride, phosphatidylcholine and phosphatidylinositol) concentrations increased.	Martinez et al., 2020
Danio rerio	Polystyrene microplastics	Substance exposure has been found to have effects on lipid metabolism.	Dimitriadi et al., 2021
Danio rerio	Heat stress Polystyrene microplastics	Substance exposure has been found to have effects on lipid metabolism including arachidonic, oleic and stearidonic acid.	Sulukan et al., 2022

MULTIOMICS

Multi-omics approaches are high-throughput methods for studying biological cellular events developed by an aquatic organism against xenobiotic or physicochemical parameters (Zhou et al., 2023).

Environmental factors and xenobiotic substances affect the genetic and epigenetic mechanisms of an organism, causing changes in the global gene expression profile. Environmental factors and xenobiotic substances first interact with the genetic material DNA in the living being and cause DNA damage through DNA adduct formation or mutation. Epigenetic mechanisms affect the gene expression profile through effects such as DNA methylation and histone modification (Joseph, 2017). The detection of peptides and proteins and the acquisition of transcripts form the basis of other omics technologies. The response of the organism to any stress exposure can be examined with single omics technologies as well as with multiple omics technologies, allowing more comprehensive results to be obtained (Canzler et al., 2020).

Data from different omics technologies are used to comprehensively analyze complex biological interactions (John Martin et al., 2024). The goals of toxicity studies are to prevent or manage adverse health effects on the organism by comprehensively studying the basic mechanisms, especially in xenobiotic-induced toxicity (Joseph, 2017).

Multiomics studies on aquatic organisms taken from aquatic ecosystems or tested under controlled laboratory conditions are shown in Table 5.

Table 5. The multiomics studies in the aquatic toxicology

Organisms	Condition	Omics types	Key results	References
Oncorhynchus mykiss	Zinc	Genomics Proteomics	It has been observed that substance exposure affects energy production and protein synthesis processes.	Hogstrand et al., 2002
Triplophysa siluroides	Heat stress	Metabolomics Transcriptomics	Differentially expressed transcriptomes (2372 in total) were identified, with 1360 up-regulated and 1013 down-regulated due to heat stress. Heat shock proteins were observed to affect the protein synthesis process in the endoplasmic reticulum. 155 differentially regulated metabolites (118 up-regulated and 37 down-regulated) were identified in terms of metabolism.	Chen et al., 2023
Fenneropenaeus chinensis	Different carbonate alkalinity and pH level	Proteomics Metabolomics	Important proteins and metabolites in carbohydrate and lipid metabolism were found to be up-regulated. In addition, it was observed that the inflammatory response was due to the oxidative stress caused by the changing abiotic conditions.	Gao et al., 2024
Litopenaeus vannamei	Salinity stress	Transcriptomics Proteomics	In low salinity stress, 754 and 649 expressed genes were identified in the hepatopancreas tissue caused by rapid adaptive and acute stimulatory responses, respectively. 206 and 66 differentially expressed proteins were identified in rapid adaptive and acute stimulatory responses, respectively.	Lu et al., 2023

Table 5. The multiomics studies in the aquatic toxicology (continue)

Organisms	Condition	Omics types	Key results	References
Eriocheir sinensis	Heat stress	Transcriptomics Proteomics	Heat stress was observed to have an effect on heat shock proteins and glutathione-s-transferase.	Shen et al., 2023
Oryzias sinensis	Estrone, 17β-Estradiol, Estriol, 17β-Estradiol-D2	Genomics Transcriptomics	In the study conducted with laboratory species, the effects of the substances were determined by specific genes and transcriptomes. In species taken from the wild environment, it was shown that environmental estrogens and estrogenicity of surface water caused adverse results.	Wang et al., 2023
Mactra veneriformis	Norgestrel	Transcriptomics Metabolomics	The xenobiotic was supported by transcriptomic and metabolomic results that it caused detoxification system, antioxidant defense system against oxidative stress, carbohydrate, protein and lipid metabolism to be disrupted.	Zhao et al., 2023
Acipenser schrenckii	Titanium dioxide nanoparticles	Proteomics Metabolomics	It was observed that 9, 19 and 25 proteins and 35, 73 and 158 metabolites were differentially expressed in different concentrations (low, medium and high) of substance exposure. It was determined that variable proteins were involved in intracellular substance transport (endocytosis) and protein synthesis functions.	Zhou et al., 2024

CONCLUSION

The two most worrying problems encountered are the changes in physicochemical parameters that occur due to changing environmental conditions in aquatic ecosystems, exposure to xenobiotic substances by anthropogenic activities, and the effects on abiotic and biotic elements of water. For these reasons, aquatic ecosystem health needs to be examined comprehensively. Basic studies in the field of aquatic toxicology constitute biomarkers used in the examination of aquatic ecosystem health. Depending on the development of technology, the effects of parameters that threaten aquatic ecosystem health on aquatic organisms in toxicological studies are explained with the applications of omics technologies. In the studies, it has been shown that the use of single-omics technology and multi-omics technologies yield comprehensive results on aquatic organisms and therefore on the aquatic ecosystem. In the future, it is foreseen that individual-omics technologies and especially multi-omics technologies will replace standard procedures in environmental risk assessment studies.

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CHAPTER 10

SANGER AND NEXT-GENERATION DNA SEQUENCING TECHNOLOGIES: USE ON AQUATIC ORGANISMS

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INTRODUCTION

Many scientists have worked hard for the last 50 years to develop the necessary tools and methods for sequencing deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules, and they are continuing their research. There is much research to identify nucleic acid residues in biological samples. Uncovering the DNA sequence of any species has been an important step towards understanding the molecular origin of the organism throughout life. Any living thing has a specific order that defines its characteristics, such as who it is, how it should behave, and how it adapts to changing environmental conditions. Two of these methods are Sanger and Next Generation Sequencing (NGS), respectively. After sequence analysis was introduced in 1975, many changes have occurred with large-scale parallel sequencing and *de novo* sequencing (Liu et al., 2012). Sanger sequencing has not only formed the basis of new approaches, but has also supported the verification of sequences, experimental monitoring, and many phylogenetic analyses. In this sequencing, amplified or complementary DNA is first ligated to an oligonucleotide primer and then extended by the enzyme DNA polymerase with a mixture of four deoxynucleotide triphosphates (dNTPs) or a chain-terminating dideoxynucleotide triphosphate (ddNTP). The extension reaction is stopped by adding the rate-limiting ddNTP and DNA fragments of varying lengths are then obtained (Sanger 1975; Crossley et al., 2020). With the discovery of NGS, it has enabled significant steps to be taken, especially in genomic research, and the analysis of RNA and DNA molecules in a cost-effective manner and highly efficient. NGS has become a fundamental tool in many interdisciplinary studies ranging from basic biology to clinical diagnosis. With the advancement of this technology, it has accelerated genomic advances in many different fields. NGS provides extensive information on genetic variations, genome structure, gene expression profiles, and epigenetic modifications by rapidly sequencing millions of DNA fragments simultaneously. In addition, the increase in genomic research areas with the advancement of NGS technologies has facilitated studies in different areas such as the detection of cancer genomics and rare genetic diseases. This technology has provided great convenience for

epigenomics, metagenomics and transcriptomics studies and has provided new opportunities for understanding topics such as genetic diversity, epigenetic modifications and microbial diversity (Vaser et al., 2017; Satam et al., 2023). It will also be important for future research to identify disease-causing variants, uncover new drugs, and shed light on the developmental processes of tumors.

This book chapter will briefly introduce the basis, history, and principles of Sanger sequencing and the principles and platforms available for NGS, and will include case studies on aquatic organisms.

SANGER SEQUENCING

In 1953, the structure of DNA was discovered and it became clear that the genetic code is part of our life. This discovery led to new ideas that DNA should be sequenced. It took an average of a quarter of a century to build on these ideas and develop first-generation DNA sequencing techniques. The first DNA sequencing (1968) was performed 15 years after the discovery of the DNA double helix. DNA sequencing outputs have been increasing, and molecules of 200 kb or larger (human cytomegalovirus) have been sequenced, resulting in bioinformatics and computational analysis. Sequencing efforts have increased and reached new heights with the launch of the Human Genome Project, which led to the establishment of the first "sequencing factory" in 1992. In 1995, the first bacterial genome was sequenced, followed by other sub-bacterial, archaebacterial and eukaryotic genomes and human genome sequence was published in 2001. However, more innovation is needed before many can reach what many call the “thousand-dollar genome.” Sanger's introduction of the "pros and cons" of DNA sequencing later pioneered the next generation of methods (Sanger and Coulson, 1975, Liu et al., 2012). The color-coded sequencing technologies and the timeline of their development processes are given in Figure 1. Polyacrylamide gels have been used to separate the products of DNA polymerase synthesis to increase chain length, thus providing the key to progress (Hutchison, 2007).

Sanger sequencing is important in many fields, such as medicine and the environment, due to its sensitivity, reliability, and ability to

sequence even tiny amounts of DNA. It is also used to identify genetic disorders, establish biological connections between individuals, and investigate drug metabolism in areas such as forensic medicine (Verma et al., 2017).

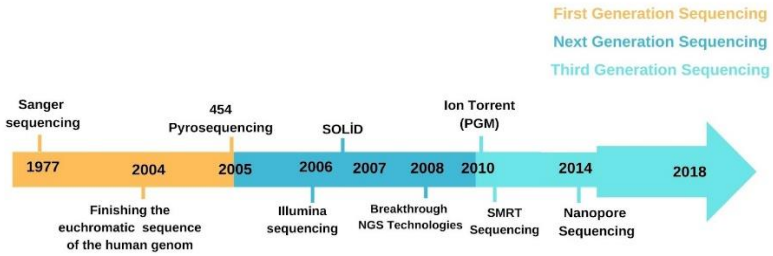

Figure 1. Timeline of sequencing technologies and their development processes coded in different colors according to generations (Sun and Zhu, 2022).

It was developed and automated by Applied Biosystems over the years (1987), thus paving the way for the Human Genome Project. Despite costing $10 million and additional advances allowing for human genome sequencing, the cost, time, and technology have increased considerably (Hu et al., 2021). It was obvious that new technologies needed to be developed and used to improve sequencing methods. NGS technologies, which have serious importance in sequencing data outputs and for the continuation of biomedical research, were introduced between 2004 and 2006 (Mardis, 2013). This was performed with an important distinguishing feature of NGS, regardless of sequencing properties and high-throughput sequencing platform.

Sanger sequencing data analysis

Sanger sequencing is also very important in the life sciences, which, together with data on nucleic acids, provides a better understanding of cellular mechanisms and some diseases. The first Sanger sequencing projects focused on sequencing very small genomes (~5000 base pairs) or single genes. A computational package developed by Sanger's laboratory at the Medical Research Council (MRC) was developed by Rodger Staden (Staden et al., 1999). This package allowed for the random sequencing of smaller DNA fragments from a much larger DNA source. It allows the reconstruction of the entire sequence, which allows computational overlaying of larger input sources. The Staden package has been compiled and made more widely available, especially for easy use on Unix operating systems. With the combination of the Staden Package, approximately 10 times larger genomes were sequenced than was possible in the first phase (Jarvie, 2005; McCombie et al., 2019). Sequencing projects have focused on much larger genomes and much longer DNA sequences. The staden package has been replaced by the phred-phrap-consed package. Phred provides basic statistics for Sanger reads, phrap is used for read assembly, and consed is used for an image editing program (Lander et al., 2001).

Since the introduction of Sanger sequencing, it has taken about 25 years (1977-2004) to complete the human genome reference sequence. During this period, significant technological advances have been made that have enabled large-scale projects to be completed, and sequencing has become widely used. However, sequencing and annotating genomes with Sanger has still been a significant and costly endeavor requiring specialized staff and infrastructure, but this scenario changed in 2005 (Margulies et al., 2005). The method requires DNA polymerase, single-stranded DNA, four dNTPs, and a ddNTP. Complementary DNA synthesis is executing by DNA polymerase with dNTPs and the enzyme reaction is terminated when ddNTPs are present. Sequence length optimizations of the fragments obtained after the reaction are provided by testing different ddNTP/dNTP ratios. According to the dNTP data, the sequencing products are separated on the gel and the full DNA sequence is analyzed for the result (Vietina et al., 2013). The discovery

of new mechanisms and concepts in molecular biology has opened new doors in almost every field of life sciences and created its own needs for sequencing technologies. As a result, a new method for DNA sequencing, the next-generation sequencing method, has emerged.

NEXT-GENERATION TECHNOLOGY (NGS)

NGS technology has been further developed in recent years and has become an important topic for gaining information about topics such as genome diversity, genetic and epigenetic regulators, and nuclear structure. Unlike microarray techniques, array-based applications can determine the nucleic acid sequence of a complementer DNA (cDNA) molecule or a specific DNA. For example, the Human Genome Project was one of the first significant attempts at DNA sequencing. This technology was used in the Human Genome Project, which lasted about cost 3 billion dollars and 13 years, and was completed in 2003. It is also used as a chain termination method. NGS short read, a massively parallel sequencing technique, differs fundamentally from Sanger sequencing, which uses capillary electrophoresis. In particular, it has led to NGS NGS, which has introduced significant innovations in sequencing capabilities, allowing much more data to be provided at a significantly lower cost. "High-throughput sequencing" is widely used in various contemporary sequencing technologies. Sanger sequencing of DNA and RNA has been used especially in molecular biology and genomics researchThis technology has become widely used because it can achieve much faster results compared to Sanger technology (Rodriguez and Krishnan, 2023).

Since the emergence and development of NGS, technology has led to significant steps in the biological sciences. It has provided a strong step for scientific interdisciplinary studies from gene to genome-wide research. Thanks to this step, researchers have been able to ask almost any question under headings such as the genome, epigenome, and transcriptome of a living being. Today, NGS is used in many different fields such as drug discovery, biotechnology, forensic science, evolutionary biology, and environmental biology, and it is predicted that its use will explode in the future.

Whole Genome Sequencing (WGS)

In the first phase, NGS enabled millions of DNA fragments to be sequenced in parallel on solid phases or in small vesicles. The throughput of NGS technology increased as it was realized that it was feasible to sequence much smaller fragments of a selected genome, and studies have been carried out regularly since 2005. This eventually became possible in 2010 and led to WGS, which was approved by the FDA in 2018 (Margulies et al., 2005; Metzker, 2010). Genomic DNA and whole genome sequencing workflow is given in Figure 2. Because of user characteristics, it is necessary to distinguish between short and long read sequencing. Each sequencing platform has its advantages. From the user's perspective, it is most logical to distinguish between short and long-read sequencing. While short reads can read <300 base pairs (bp). But long-read sequencing can produce reads from 10 kbp to several megabases, depending on the technology. Also, long-read sequencing is used to better detect complex variants by resolving larger haplotypes. When comparing the two methods, short-read sequencing is preferred for detecting much smaller variations. This is due to the high accuracy and speed of sequencing for smaller or larger variants (Kwong et al., 2015; Choo et al., 2023).

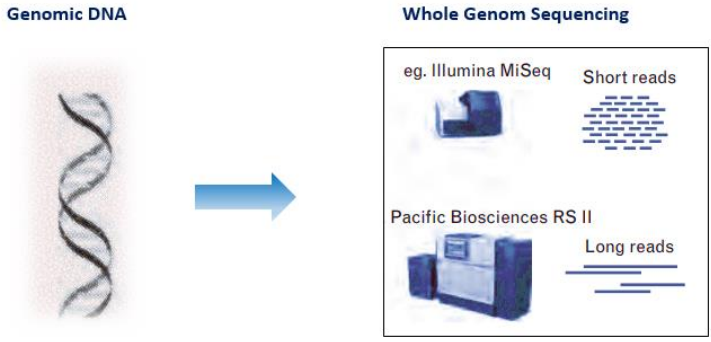

Figure 2. Whole genome sequencing (Kwong et al., 2015).

Considering the studies conducted significant efforts have been made to sequence the entire genomes of many fish species in the aquatic

ecosystem. With the emergence and development of NGS technologies, it has made significant contributions to the detection of genetic traits, single nucleotide polymorphisms, or microsatellites at the transcriptome or genome level in various fish species (Santos et al., 2015). WGS provides significant opportunities to identify genes with many important characteristics. Single nucleotide polymorphisms (SNPs), which are also one of the important reasons why individuals of the same species have different characteristics from each other, are also important for genetic diversity. SNPs also allow researchers to address challenges that may arise regarding the sustainability of aquaculture research (Montes et al., 2013; Kumar and Kocour, 2017).

Whole Exome Sequencing (WES)

Using the exome sequencing method, protein coding regions in the human genome were sequenced by whole sequence analysis. Exomes are estimated to carry a large portion of mutations, approximately 85%, that have important effects, especially in determining disease-related traits (Majevski et al., 2011). Also, exonic mutations have been shown in studies to cause the vast majority of single-gene diseases (Botstein and Risch, 2003; Kuhlenbäumer et al., 2011). These values make it difficult to identify disease-causing mutations in non-coding regions. But they confirm the advantages and growing success of exome sequencing for many single gene diseases. Exome sequencing has contributed major to the identification of Mendelian disease genes. Since its introduction, it has grown in importance (Bamshad et al., 2011). Large scale genome and exome sequencing projects have not only provided information about the variants but have also shown that approximately 20 genes in the human genome are completely inactivated (Genes, 2004). In the last few years NGS has been increasingly preferred to address human studies as well as ecotoxicological research questions (Ordas et al., 2011; Petersen et al., 2017).

Targeted Sequencing

The first genome was sequenced using the Roche/454 NGS platform. This sequencing was completed in two months by James D. Watson, with contributions from Wheeler and other scientists. WGS examines the including coding, non-coding and entire genome, DNA, and also aims to discover new and unknown genomic variants for some targeted diseases (Wheeler et al., 2008; Pei et al., 2023). WGS is widely used in cancer genome sequencing and provides data for both diagnosis and treatment of today's cancer diseases. When comparing the two methods, it can be seen that targeted sequencing is separated into specific genes and coding regions with sequencing depth in genome. It has been reported that these target genes and regions are closely related to the clinical and pathogenesis of diseases. TS sequencing was developed specially to detect and follow cancer-related gene mutations and somatic changes and schematic representation of the targeted sequence is given in Figure 3 (Pei et al., 2023).

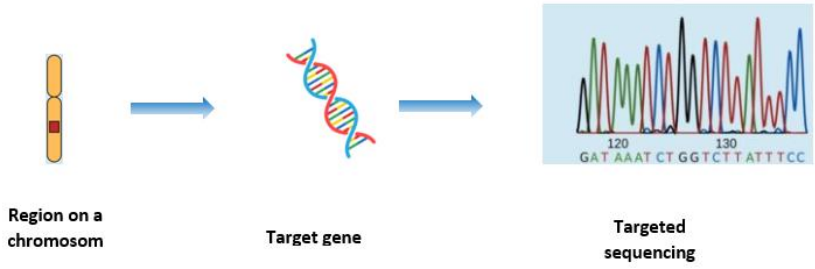

Figure 3. Schematic representation of targeted sequencing (Pei et al., 2023).

Targeted sequencing is divided into two groups: amplicon-based and capture-based. Primers designed to amplify only the regions of interest before library construction are used in amplicon-based sequencing. Capture-based DNA is first fragmented. Targeted regions are then amplified with hybridization oligonucleotide arrays coupled to biotinylated probes, isolating the remaining genetic material (Samorodnitsky et al., 2015). The cheaper of the two technologies is amplicon-based enrichment. This method allows for a higher number of target reads, but the area of these regions is more uniform than with

hybrid sequencing (Hung et al., 2018). Some amplicon platforms attempt to retain potential domain questions by using specific primers so that matching fragments can be amplified in a single PCR reaction. Hybrid capture is known to produce much fewer PCR copies than amplicon enrichment (<40%~80%) (Samorodnitsky et al., 2015; Schenk et al., 2017). These duplications are much easier to remove from a computational perspective because the random shearing of DNA in hybrid capture may reduce the probability that these two important fragments will crossover and align to the same genomic coordinates, compared to identical amplicons produced by amplicon platforms. However, the long bait sequences favored in hybrid capture allow for higher specificity when selecting regions. For small-scale experiments where sample size or cost are important factors, amplicon-based platforms are preferred (Bewicke-Copley et al., 2019).

RNA Sequencing (RNA-Seq)

Correlating genotypes with phenotypes is one of the fundamental elements for regulating gene expression. The synthesis and maturation of RNAs are tightly monitored and controlled, resulting in complex gene expression that drives biological processes. At the same time, they must be robust and flexible to allow rapid adaptation to genetic perturbations or environmental problems. In genetic information can be modified by environmental factors and characterize the phenotype (Kukurba and Montgomery, 2015). The transcription of genes into RNA molecules regulates biological activities within the cell and determines cell identity. RNA molecules, known as transcriptomes, are important for understanding the functional properties of the genome, development and disease. The transcriptome has a complex structure and includes multiple coding and noncoding RNA types. When RNA molecules are examined, it is known as a simple intermediate between genes and proteins, as well as being the basic dogma of molecular biology. Therefore, messenger RNAs (mRNA) have been the most frequently studied RNA type due to their ability to encode proteins via the genetic code. There is another diverse group of functional noncoding RNA (ncRNA) molecules. Most of the ncRNAs we know perform essential cellular functions, such as

transfer RNAs, small nuclear RNAs (snRNAs) involved in splicing, ribosomal RNAs involved in mRNA translation, and small nucleolar RNAs (snoRNAs) involved in the modification of rRNAs. In recent years, new RNA classes have been discovered that enrich the domains of ncRNAs. These are small noncoding RNAs, including piwi-interacting RNA (piRNA) and microRNA (miRNA). Another important class of ncRNAs are long noncoding RNAs (lncRNAs), both of which regulate gene expression at the post-transcriptional level. This group was first identified by large-scale sequencing of cDNA libraries in mice, and many molecular functions for lncRNAs have been discovered, chromatin remodeling, including transcriptional control, post-transcriptional processing, but the majority of these remain uncharacterized (Stefani and Slack, 2008; Guttman et al., 2009; Mercer et al., 2009).

Several methods have been developed to enable genome-wide measurement of transcriptomic gene expression. Early transcriptomic studies were performed using high-throughput and low-cost hybridization-based microarray technologies (Mutz et al., 2013). These methods have some limitations, such as the need to know in advance the sequences to be analyzed and the limited ability to accurately measure genes with low or very high expression. However, due to its relatively low throughput, it has not been preferred for measuring transcripts (Casneuf et al., 2007). Despite these methods limitations, serial analysis of gene expression and cap analysis of gene expression methods have been developed to measure expression levels more precisely and achieve higher throughput. Directly measuring the number of labeled sequences corresponding to the number of mRNA transcripts provides a significant advantage over measuring analogous intensities as in array-based methods. However, these assays cannot be used for new gene discovery. The need for large amounts of input RNA, coupled with the high cost of cloning sequence tags and automated Sanger sequencing, has limited their use (Velculescu et al., 1995; Shiraki et al., 2003).

Next-generation sequencing, along with RNA analysis by sequencing cDNA, has revolutionized transcriptomics (Harismendy et al., 2009). RNA-Seq has provided important data in understanding the complex and dynamic structure of the transcriptome and provides more

detailed data on gene expression, allele-specific expression and alternative splicing. Recent advances have detailed the transcriptome under different pathological and physiological conditions, from samples preparation to bioinformatics analysis and sequencing platforms (Figure 5).

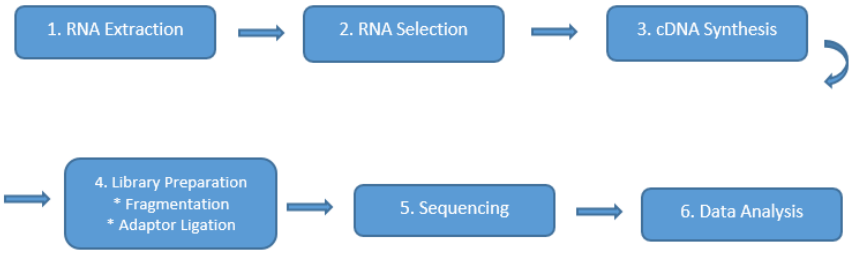

Figure 4. The general workflow of RNA-Seq.

As sequencing technologies advance day by day, they will need to evolve to support current applications and resolve technical errors. As sequencing of small amounts of RNA becomes possible in the laboratory, advanced statistical applications will be needed to obtain clear results. In addition, combining WGS with RNA-Seq in much larger sample types will provide more data on genetic regulators. Bioinformatics advances will make the transcriptome a very powerful tool for analyzing basic biological questions and its increasing impact on personalized medicine (Kukurba and Montgomery, 2015).

Chromatin Immunoprecipitation Sequencing (ChIP-Seq)

ChIP-Seq is one of the methods used to find DNA binding sites of a DNA binding protein of interest in the genome (Valouev et al., 2008; Furey, 2012). The ability of proteins to cross-link DNA to which they are bound in vivo forms the basis of the method. Specific protein-DNA complexes can also be purified by immunoprecipitation (de Folter et al., 2007). Antibodies specific to specific protein modifications, such as histone methylation, are preferred to explore the epigenetic mechanism of the genome. NGS technologies are being used to reveal genome-wide binding profiles of some specific proteins (Kim and Ren, 2006; Bhinge

et al., 2007). In the field of ChIP-Seq analysis, histone modifications are preferred to study epigenetic properties and biological functions. Significant advances in computational analysis and NGS technology have provided important systematic data on how the field of epigenomics contributes to development, cancer, cell identity, and various diseases (Ernst et al., 2011; Yamaguchi et al., 2018; Zhao and Shilatifard, 2019). ChIP-Seq computational analysis workflow is performed in two stages and these are separated into different phases. The first one is sample preparation and sequencing, and the other one is computational analysis.

DNA binding proteins have led to the development and updating of experimental methods over the years to better define these interactions. ChIP-Seq remains the first to be used to localize binding sites for histone modifications and individual proteins. The limitations of antibody development, modification, static snapshots of a living cell require the use of complementary methods and extensions of ChIP-Seq to ensure transcriptional regulation (Nakato and Sakata, 2021).

Metagenomic Sequencing

Due to the lack of available technology and reference genomes, analysis of any bacteria was rare until recent years. The process of sequencing DNA from an organism's genome is called metagenomics. It is an important method used to study both the structure and function of the microbiome population (Roumpeka et al., 2017). However, the rapid increase in genome sequences, along with the development of NGS, has led to rapid progress in metagenomic sequencing. The increasing sequencing throughput, low cost, and additional technological advances have also increased interest in metagenomics new NGS technologies continue to develop, the metagenome field will continue to adapt to new sequencing data (Watson, 2014). Studies on many different microbiomes have brought significant innovations, including genes that code for proteins of significant industrial value. In studies conducted, researchers have provided bioinformatics tools that allow them to analyze large metagenomic data sets and extract possible new protein/gene/enzyme. It has been revealed that it can provide new enzymes that are of significant

value for the characterization of protein and function. However, studies are needed in this area (Scholz et al., 2012; Roumpeka et al., 2017).

DNA Methylation Sequencing (Methyl-Seq)

Methylation sequencing is known as the process of DNA methylation using sodium bisulfite and then nucleotide base conversion. When analyzing methylation sequencing results, a detailed overview of DNA methylation patterns across the genome is revealed. It also helps researchers understand the epigenetic mechanisms that regulate cellular development, environmental responses, and diseases. When we look at how it works, it works by first converting unmethylated cytosines to uracil using sodium bisulfite. After PCR amplification and sequencing, uracil is read as thymine, and direct PCR sequencing or cloning sequencing is used to measure the thymine read counts. Since methylated cytosine is not converted by sodium bisulfite, methylation sequencing separates methylated cytosine residues from unmethylated cytosine/transformed thymine. After these steps, it can be determined whether DNA methylation has occurred, and the converted thymine can be quantified from the total read counts from direct PCR sequencing or cloning sequencing. Methylation sequencing allows us to map and quantify the presence of methyl groups in DNA strands; it is also a critical component of gene regulation and expression (Schumacher et al., 2006; Morrison et al., 2021).

Single-Cell Sequencing (SCS)

SCS has been important in investigating genomic, epigenomic, and transcriptomic heterogeneity in cellular populations and explaining their variability levels. In addition, they have rapidly developed to observe single cells in more detail. SCS has become a powerful tool for analyzing omic-scale properties of different and heterogeneous cell populations, including stem cells. One of the advantages of this method is that it makes a significant contribution to the study of cellular heterogeneity without any prior knowledge of the cell population. The first genome-wide single-cell DNA and RNA sequencing methods for mammalian cells brought this method to the best level (Grun et al., 2015). These early

studies led to the emergence of SCS. SCS methods have proven to be more difficult compared to RNA. The reason given is that a single cell contains only two copies of each DNA molecule, while most contain thousands of copies of RNA molecules. Surani laboratory in 2009, single-cell transcriptome sequencing was first applied. Single-cell mRNA sequencing methods have started to be used after technical improvements have been made and completed (Sasagawa et al., 2013). This developed method has been used in many different studies, such as discovering rare cell types, determining tumor heterogeneity, and distinguishing cell types in healthy tissues (Patel et al., 2014). The use of SCS has provided important data in many biological phenomena such as gene transcription, carcinogenesis and embryo development. Single-cell multi-omics sequencing has become the preferred method to establish core gene regulatory relationships differentiation stages of stem cells and within a cell during the development. New methods will likely emerge shortly that cover more layers of different omics. It will also be an ideal method to establish a causal relationship between genotype and phenotype using gene editing technologies and will also provide new insights into the biological causes of diseases (Grun et al., 2015).

USE OF SEQUENCE ANALYSIS IN AQUATIC ORGANISMS

Many stress factors such as human activities, nutritional changes, global climate change, biological and chemical pollution negatively affect aquatic ecosystems (Marushka et al., 2019). These stress factors affect organisms at almost every level, from individual and biological processes to global and local ecosystems (Mushtaq et al., 2020; Carrier-Belleau et al., 2021). As a result of long-term exposure, these stress factors are also transferred to future generations. When recent studies are examined, it has been examined how epigenetic regulation should adapt to environmental factors and stress responses (Lee et al., 2022). With the recent importance of human activities, climate change and environmental epigenetics studies have become very important in understanding the negative effects on aquatic ecosystems. More than 70% of the world's surface consists of water and can be directly affected

by any environmental stress factors. Several recent review articles have attempted to regulate aquatic environmental epigenetics by focusing on ecotoxicology, chemical pollutants, or epigenetic analytical approaches to specific ecosystems in oceans and freshwaters (Šrut, 2021; Xu et al., 2022; Pham and Lin, 2023).

In recent years, aquatic animal models have been widely used in different studies. Such animal models contribute to the determination of various disease pathologies at the molecular and cellular biology levels, and the development of some therapeutic methods and diagnostic. The genomes of these species allow the application of physiological and pathophysiological discoveries obtained by linking phenotypic changes to genetic characteristics to human disease research. Sequencing their genomes provides important data for researchers in the field of biomedical research. NGS is routinely used in environmental monitoring studies because it allows the sequencing of multiple species in many samples simultaneously with a single instrument. As a result, it provides both cost and time advantages in terms of processed samples. The most preferred NGS technique today is 454 Pyrosequencing. Compared to other techniques, it can produce longer sequences for accurate identification (Hajibabaei et al., 2011).

To conduct environmental epigenetic studies on aquatic species such as model organisms such as zebrafish and *Daphnia* or organisms such as cnidarians, scallops, and mollusks, genome types depend on their sources and reference libraries. Insufficient information about the genomes of such organisms will hinder future environmental epigenetic studies (Pham et al., 2023). For example, analytical and epigenetic approaches are very important for studying the interactions between the environment and genotype. It is of great importance to monitor organisms that have an important place in aquatic ecosystems against the possible harmful effects of environmental pollution (Eirin-Lopez and Putnam, 2019). Toxic pollutants that pollute aquatic ecosystems accumulate in the affect all trophic levels and food chain which is why they are a concern for human health and both ecosystems. In addition, it is important to use better methods and tools to measure and monitor the effects of such harmful substances on ecosystems and natural

populations. For example, transcriptome analysis has become an important tool used to biological functions that will respond to toxic substances (Kumar and Kocour, 2016).

NGS technologies, which have become quite popular in recent years, are preferred for such studies. This technology is used to investigate topics such as bacterial toxicity, taxonomic classification, Cyanobacteria, and functional and catabolic gene characterization, making microbial studies related to aquatic toxicity and the wastewater industry important (Garner et al., 2021). For example, although microarray beams are at the forefront of transcriptome analysis, these technologies have been widely used to measure transcripts in recent years. The toxic effects of copper (Cu) and cadmium (Cd) were studied in yellow perch and the conditions in zebrafish embryos after short-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were investigated. 454 sequencing technology was used to determine the effects of NGS combined with real-time PCR on miRNA expression (Jenny et al., 2012). As a result of the study, they emphasized that TCDD exposure may affect hematopoiesis and cardiovascular development by changing miRNA expression. In another study, it was concluded that oxidative stress was caused by gene expression in Burbot fish (*Lota lota*) exposed to persistent organic pollutants (POPs) (Olsvik et al., 2013In this study conducted with NGS technology, the molecular mechanism of toxicity of silver nanoparticles in zebrafish embryos was studied. As a result, differences in gene expression, especially protein synthesis and oxidative phosphorylation were observed (Van Aerle et al., 2013). Sun et al. (2016) used NGS technology to examine liver histology in *Megalobrama amblycephala* to investigate the mechanisms underlying nitrite toxicity. As a result of the study, they showed that nitrites can cause oxidative stress, liver damage, histotoxic hypoxia and cell death. When the studies conducted are examined, it has been shown that NGS provides important mechanistic information in addition to toxicity, not only to determine gene expression changes as a response to toxic substances, but also to all animal physiology. In the study, gene expression analysis showed that the temperature difference during early larval and embryonic development changed the miRNA profile in the

short/long term. It is suggested that the increasing sea temperature may affect the life history of fish species (Bizuayehu et al., 2015). NGS, which has commercially important features, is preferred in the examination of related genome regions and ecotoxicological studies. It is also used to obtain more information in genome-wide characterization, in the study of the control of biological processes, and in the extraction of messenger RNA and micro-RNA profiles. In recent times, RNA-seq analyses have been used to measure mRNA expression levels and are becoming increasingly important for better examination of gene expression in fish. RNA-seq analyses have been increasingly used in recent years to study gene expression in fish. Since transcriptomes are directly associated with genes or functional regions in the genome, they have generally become a main research topic in aquaculture species. In a study, transcriptome analysis was performed in the testes, ovaries, brain and gills of rainbow trout (*Oncorhynchus mykiss*) and the genes that were dominantly expressed in the tissue were determined (Le Cam et al., 2012).

Transcriptomes are a fundamental and important research topic in aquatic species and are closely related to genes in the genome. Sea bream (*Sparus aurata*) was preferred to identify genes required for a specific function and the determined tissue-specific expression profile. Transcriptome analyses were performed on genes identified for muscle development and myogenesis in sea bream due to its rapid skeletal muscle properties (Garcia de la Serrana et al., 2012). The arrival of next-generation sequencing analysis has led to a decrease in costs and an increase in the speed of DNA sequencing. It has provided a significant convenience, especially in life sciences. This technology has facilitated the reading of all or nearly all the genomes and transcriptomes of various fish species and other aquatic organisms. In addition to NGS analysis, appropriate experimental models must be selected and applied to obtain statistically accurate and strong results.

CONCLUSION

Although Sanger sequencing is still considered the gold standard method for DNA sequence analysis today, it has disadvantages such as

long turnaround time and high cost. NGS technologies, which are cheaper and faster than traditional Sanger methods, have become an important step in genomics due to their ability to sequence hundreds of billions of base pairs simultaneously. Performing millions of sequencing reactions in parallel and sequencing each base multiple times increases the depth and therefore accuracy of the data. Next-generation DNA sequencing technology provides an unimaginable amount of information and new approaches. However, both storing and analyzing and evaluating this much information poses great challenges. With the importance given to it in recent years, there is a need for Next Generation DNA sequencing technology and bioinformatics analysis tools. Future studies should be extended not only to model organisms but also to non-model species, and appropriate sampling should be performed accordingly. Because then it is possible to better understand the genetic and biological importance of the aquatic species under study.

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