ENVIRONMENTAL HEALTH, FOOD SAFETY, AND AQUATIC SYSTEMS

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PREFACE

Environmental health, food safety, and aquatic systems are closely interconnected areas of contemporary scientific research. Changes in environmental conditions may influence aquatic health, food quality, and ecological balance, while advances in analytical and biological sciences provide new opportunities to assess and manage related risks. Addressing these issues requires multidisciplinary perspectives that combine environmental science, microbiology, nutrition, and toxicological research.

The chapters in this volume examine selected topics within this broad field. The first chapter explores the relationship between environmental stressors and disease emergence in aquaculture, emphasizing the importance of water conditions and ecosystem health. The second chapter presents a microbial assessment of complementary food blends prepared from Nigerian yellow maize, soybean, and crayfish, contributing to discussions on food quality and nutritional safety. The third chapter investigates the integration of advanced analytical chemistry and ecotoxicological approaches for predictive environmental risk assessment.

By bringing together studies on aquatic health, food microbiology, and environmental risk, this volume highlights the importance of scientific approaches that support healthier ecosystems and safer food systems. The chapters demonstrate how environmental monitoring, microbial analysis, and analytical techniques can contribute to the identification and prevention of biological and ecological risks.

It is hoped that this book will serve as a useful academic resource for researchers, students, professionals, and scholars interested in environmental health, food safety, aquaculture, microbiology, analytical chemistry, and ecotoxicology. We extend our sincere appreciation to all contributing authors for their valuable scholarly efforts.

Editorial Team
July, 2026
Türkiye

CHAPTER 1
WHEN WATER TURNS HOSTILE: ENVIRONMENTAL STRESSORS AND DISEASE EMERGENCE IN AQUACULTURE

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INTRODUCTION

Aquaculture is one of the fastest-growing food production sectors and plays a crucial role in global food security by supplying high-quality animal protein, essential fatty acids, vitamins, and minerals to the human population (FAO, 2024). With the continued decline of capture fisheries and the increasing demand for fish and fishery products, aquaculture has emerged as a major contributor to global fish production as well as rural livelihoods (FAO, 2024). However, the rapid intensification of aquaculture systems, characterized by high stocking densities and intensive feeding practices, has led to increased physiological stress in cultured organisms, predisposing them to diseases and environmental disturbances (Austin, 2012). In aquaculture systems, water quality plays a central role in maintaining fish health, as it serves as the medium for respiration, feeding, metabolism, and waste removal. Any deterioration in water quality directly affects fish growth, survival, and resistance to diseases (Boyd, 2015). Disease outbreaks remain one of the most significant constraints to sustainable aquaculture development worldwide. Fish are susceptible to a wide range of pathogens, including bacteria, viruses, fungi, and parasites, all of which can cause severe losses in aquaculture systems (Woo and Buchmann, 2012). Among bacterial pathogens, species such as Aeromonas hydrophila and Vibrio spp. are frequently associated with disease outbreaks, leading to conditions such as ulcerative lesions, hemorrhages, and high mortality rates (Austin, 2012). The intensification of aquaculture practices, particularly under conditions of poor water management, increases stress levels in fish and weakens their immune system, making them more susceptible to opportunistic pathogens (Sindermann, 1990). Environmental conditions are therefore critical determinants of fish health and disease susceptibility. Key water quality parameters such as temperature, dissolved oxygen, pH, ammonia, and salinity directly influence physiological processes, metabolism, and immune responses (Boyd, 2015). Deviations from optimal ranges induce physiological stress, which suppresses immune function and increases vulnerability to infectious diseases (Noga, 2010). In addition, climate variability further complicates disease management in aquaculture systems by altering water temperature, rainfall patterns, and aquatic ecosystem dynamics.

These changes can enhance pathogen virulence, disrupt host–pathogen interactions, and increase the frequency and severity of disease outbreaks (Martins et al., 2012). Therefore, understanding the relationship between environmental stressors and disease emergence is essential for developing effective management strategies and ensuring the sustainability of aquaculture production systems.

1.CONCEPTUAL FRAMEWORK: WHEN ENVIRONMENT DRIVES DISEASE

1.1.The Host–Pathogen–Environment Triad

Understanding disease emergence in aquaculture requires reference to the foundational epidemiological framework proposed by Snieszko (1974), known as the Host–Pathogen–Environment (H–P–E) triad. This model explains that disease does not arise from the presence of a pathogen alone, but from the interaction between a susceptible host, a virulent or abundant pathogen, and environmental conditions that favor infection. In intensive aquaculture systems, multiple stressors often shift all three components of this triad toward conditions that promote disease, making the model highly relevant for both conceptual understanding and practical application. Disease outbreaks in aquaculture are therefore not random events but result from structured interactions among the host, pathogen, and environment. A pathogen may exist in the system without causing harm under stable conditions; however, when environmental quality deteriorates, host defenses weaken and the same pathogen can become highly pathogenic. For instance, reduced dissolved oxygen, temperature fluctuations, or the accumulation of pollutants can induce stress responses in fish, impair immune function, and enhance pathogen proliferation. Under such conditions, even otherwise healthy fish become increasingly vulnerable to infection. The H–P–E triad thus provides a clear framework for understanding the multifactorial nature of disease in aquaculture systems. It emphasizes that effective disease management cannot rely solely on pathogen control, but must also include maintaining host health and ensuring environmental stability. By addressing all three components simultaneously, the likelihood of disease outbreaks can be significantly reduced.

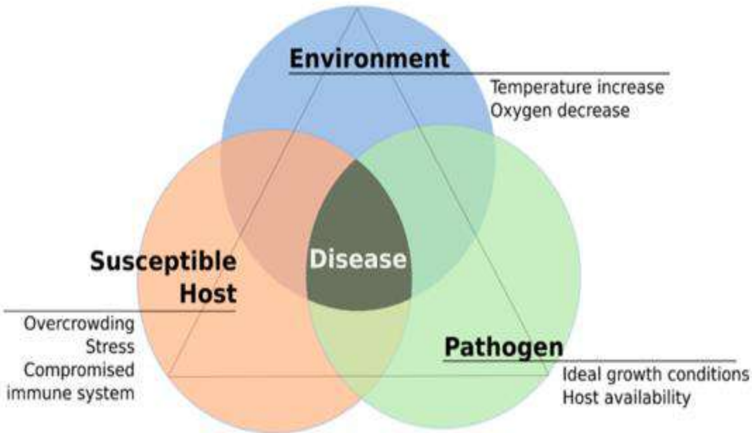

Figure 1. Conceptual illustration of the Host–Pathogen–Environment (H–P–E) triad in aquaculture

This figure illustrates the dynamic interaction between the host (fish), infectious agents (pathogens), and environmental conditions. Disease occurs when a susceptible host is exposed to a virulent pathogen under unfavorable environmental conditions. Stressors such as poor water quality, temperature fluctuations, and low dissolved oxygen weaken host immunity while enhancing pathogen activity, ultimately leading to disease outbreaks.

1.2. Stress Physiology and Immunosuppression

The primary neuroendocrine mediator of the stress response in fish is cortisol, released through activation of the hypothalamic–pituitary–interrenal (HPI) axis, the teleost equivalent of the mammalian hypothalamic–pituitary–adrenal (HPA) axis. Upon exposure to a stressor, corticotropin-releasing factor (CRF) is secreted from the hypothalamus, stimulating the release of adrenocorticotropic hormone (ACTH), which in turn drives cortisol synthesis in the interrenal cells of the head kidney. Short-term elevation of cortisol plays an adaptive role by mobilizing energy reserves and modulating inflammatory responses. However, prolonged or chronic elevation leads to immunosuppression, characterized by reduced lymphocyte proliferation, decreased mucosal IgM production, impaired phagocytic activity, and disruption of cytokine regulation (Tort, 2011).

Stressors in aquaculture systems can be broadly categorized into physical, chemical, nutritional, and biological factors. Physical stressors include handling, netting, grading, and crowding, all of which induce acute cortisol responses that transiently impair immune function. Chemical and environmental stressors encompass hypoxia, elevated ammonia and nitrite levels, carbon dioxide accumulation, temperature extremes, and exposure to pollutants. Hypoxia is particularly critical; recent studies have shown that low dissolved oxygen can activate molecular pathways such as the HIF-1α–Yki–Cactus axis, leading to suppression of cellular immunity while simultaneously enhancing the virulence of pathogens such as Vibrio spp. Nutritional stress, arising from deficiencies in essential nutrients such as vitamin C, omega-3 fatty acids, and amino acids, further compromises the synthesis of immune molecules including antibodies, complement proteins, and antimicrobial peptides. Biological and social stressors, including dominance hierarchies, co-infections, and chronic crowding, result in sustained cortisol elevation and prolonged immune dysfunction. The distinction between acute and chronic stress is critical in aquaculture health management. Acute stress responses are generally short-lived and reversible, allowing fish to recover once the stressor is removed. In contrast, chronic stress, often resulting from multiple interacting stressors, leads to persistent endocrine imbalance, long-term immunosuppression, and increased susceptibility to disease. At the cellular level, glucocorticoid-mediated immunosuppression is one of the most well-characterized mechanisms. Cortisol binds to glucocorticoid receptors on immune cells such as lymphocytes and macrophages, inducing apoptosis of lymphocytes, suppressing pro-inflammatory cytokines including IL-1β, TNF-α, and IL-6, and reducing the oxidative burst capacity of phagocytes. On mucosal surfaces, cortisol downregulates the production of secretory IgM and antimicrobial peptides, thereby facilitating bacterial colonization. Hypoxia-driven immunosuppression operates through stabilization of hypoxia-inducible factor 1-alpha (HIF-1α), which shifts cellular metabolism toward anaerobic glycolysis. This metabolic adjustment limits the energy available for immune processes such as phagocytosis and lymphocyte proliferation. In addition, disruption of the normal microbiome contributes significantly to immune dysfunction.

The commensal microbial communities present on the skin, gills, and gastrointestinal tract play an essential role in maintaining host defense through competitive exclusion of pathogens, production of antimicrobial compounds, and continuous immune stimulation. Stress, antibiotic use, and environmental degradation can disrupt these microbial communities, allowing opportunistic pathogens to colonize epithelial surfaces more effectively (Mougin and Joyce, 2023; Samsing and Barnes, 2024). Temperature also plays a crucial role in modulating immune responses. Exposure to temperatures outside the optimal range for a given species can reduce lymphocyte activity, decrease antibody production, and simultaneously enhance pathogen growth rates. This dual effect creates favorable conditions for disease development, as observed in infections caused by pathogens such as Flavobacterium psychrophilum in cold-stressed fish (Chong et al., 2023). A summary of major opportunistic pathogens associated with such conditions is presented in Table 1.

Table 1. Major opportunistic and environmental bacterial pathogens in aquaculture with key clinical signs

Pathogen	Host Species	Disease / Syndrome	Key Clinical Symptoms
Aeromonas hydrophila	Freshwater fish; catfish, carp, tilapia, salmon	Motile *Aeromonas* Septicemia (MAS); Hemorrhagic Septicemia	Hemorrhagic ulceration of skin; exophthalmia (pop-eye); abdominal dropsy with fluid accumulation
Vibrio anguillarum	Marine and estuarine fish; salmon, sea bass, turbot	Vibriosis; Hemorrhagic Septicemia	Hemorrhagic reddening at fin bases; ulcerative skin lesions; anemia with pale gills
Pseudomonas fluorescens / aeruginosa	Freshwater and marine fish; amphibians, shrimp	Pseudomonas Septicemia; Fin & tail rot	Fin and tail necrosis; diffuse hemorrhaging in musculature; reduced swimming and lethargy
Flavobacterium columnare	Freshwater fish; catfish, salmon, tilapia, koi	Columnaris Disease; Saddleback Disease	Yellow-grey saddle-shaped lesions on dorsum; frayed fins with necrotic edges; gill necrosis and respiratory distress

Pathogen	Host Species	Disease / Syndrome	Key Clinical Symptoms
Flavobacterium psychrophilum	Salmonids; rainbow trout, Atlantic salmon, ayu	Rainbow Trout Fry Syndrome (RTFS); Bacterial Cold-Water Disease (BCWD)	Peduncle erosion ('peduncle disease'); spinal deformity and abnormal swimming; hemorrhagic ulcers on skin
Tenacibaculum maritimum	Marine fish; Atlantic salmon, sea bream, turbot, halibut	Tenacibaculosis ('Yellow Mouth'); Marine Flexibacteriosis	Yellow–white necrotic mouth lesions; eroded fins and body surface ulcers; microbiome dysbiosis in gill tissue
Vibrio harveyi	Shrimp, sea bass, sea bream, marine larvae	Luminescent Vibriosis; Shrimp Acute Hepatopancreatic Necrosis (AHPND-associated)	Bioluminescence in infected tissue; body surface ulceration; mass mortality of larvae with opaque bioluminescent gut
Edwardsiella tarda	Eel, flounder, catfish, tilapia, olive flounder	Edwardsiellosis; Emphysematous Putrefactive Disease of Catfish	Gas-filled skin ulcers with putrid odour; spleen enlargement with abscess formation; exophthalmia
Streptococcus agalactiae	Tilapia, barramundi, trout, hybrid striped bass	Streptococcosis; Meningoencephalitis	Unilateral or bilateral exophthalmia; erratic corkscrew swimming (neurological signs); hemorrhagic corneal opacity

(Sources: Samsing and Barnes, 2024; Thompson et al. 2024; Mougin and Joyce, 2023; Batista et al. 2025; Chong et al. 2023)

2. AQUATIC ENVIRONMENT AS A DISEASE LANDSCAPE

2.1 Aquatic Ecosystems and Disease Ecology

The aquatic environment plays a critical role in the occurrence, transmission, and regulation of diseases in fish and shellfish. It is often described as a “disease landscape” because multiple environmental and biological factors interact to influence pathogen dynamics and host susceptibility. Disease emergence in aquaculture is therefore not solely dependent on the presence of pathogens, but is shaped by the ecological balance within the aquatic system. Pond ecosystems, although small in size, are important components of the hydrological and ecological network.

They support complex food webs, facilitate nutrient cycling, and serve as natural reservoirs that contribute to groundwater recharge and local biodiversity (Kumar et al., 2015). In aquaculture, ponds also function as controlled production systems; however, their relatively small volume makes them highly sensitive to environmental fluctuations and external disturbances. As a result, even minor changes in water quality can have pronounced effects on fish health and disease occurrence. Aquatic microbial communities include a diverse range of microorganisms inhabiting freshwater, marine, and brackish environments, encompassing members of Archaea, Bacteria, and Eukarya. Although viruses and other non-cellular entities are not classified within these domains, they play a significant role in shaping microbial population dynamics and are therefore integral to disease ecology. The composition of microbial communities is influenced by both local environmental conditions and regional ecological processes, leading to variations in community structure across different aquatic systems (Östman et al., 2012). These microbial assemblages are crucial in maintaining ecosystem stability, but shifts in their composition can favor the proliferation of opportunistic and pathogenic organisms. Fish health in aquatic systems is influenced by both natural and artificial environmental conditions. In natural ecosystems, fish allocate energy to cope with environmental variability such as changes in nutrient availability, predator pressure, competition, and physico-chemical parameters (Ikeogu et al., 2010). In contrast, aquaculture systems often impose additional constraints, including high stocking density and limited water exchange, which can intensify stress and increase disease risk. Studies have shown a strong relationship between environmental conditions and the occurrence of parasitic, bacterial, and fungal infections in fish populations. Water quality parameters such as alkalinity, temperature, and other physico-chemical factors play a significant role in disease susceptibility. Low alkalinity reduces the buffering capacity of water, leading to unstable pH conditions that can stress fish and increase their vulnerability to infections. Similarly, low environmental temperatures reduce metabolic activity and immune efficiency, making fish more susceptible to parasitic infestations, particularly during colder periods (Hossain et al., 2011). These observations highlight that disease patterns in aquatic systems are closely linked to environmental variability and ecosystem dynamics.

3. ENVIRONMENTAL STRESSORS THAT TURN WATER HOSTILE

Environmental stressors are external factors that adversely affect aquatic organisms, particularly fish, which are widely regarded as sensitive indicators of ecosystem health. These stressors may be natural or human-induced and include variations in temperature, pH, salinity, dissolved oxygen, pollution, and water availability (Schulte, 2014). Such factors influence physiological processes and can disrupt homeostasis, thereby reducing the ability of fish to resist infections. Environmental fluctuations, including changes in temperature, oxygen concentration, and water chemistry, impose physiological stress that weakens immune responses and increases susceptibility to pathogens (Pinna et al., 2023). In intensive aquaculture systems, additional pressures such as overcrowding, poor water exchange, and accumulation of metabolic waste further exacerbate these effects. Exposure to pollutants, including heavy metals and chemical contaminants, can impair organ function and interfere with normal immune regulation. The severity of these impacts varies among species and depends on factors such as thermal tolerance, habitat type, and life stage. Differences between cold-water and warm-water species, as well as freshwater and marine organisms, highlight the complexity of interactions between environmental conditions and host responses (Tort and Balasch, 2022). Among environmental variables, temperature is one of the most influential factors affecting fish physiology and behavior (Tripathi and Bharathi, 2025). Both gradual and abrupt temperature changes can act as stressors, altering metabolic rates, oxygen demand, and feeding behavior (Raman et al., 2013). Elevated temperatures increase metabolic activity and energy requirements, often leading to higher oxygen consumption and altered feeding patterns. Conversely, exposure to temperatures outside the optimal range induces thermal stress, which disrupts physiological balance and compromises immune function. As a result, fish become more vulnerable to infections, and disease outbreaks often exhibit seasonal patterns linked to temperature variation (Table 2). Temperature also affects pathogen survival and virulence. Lower temperatures can prolong the persistence of certain viruses and influence host immune responses, while higher temperatures can accelerate bacterial growth and transmission.

For example, infectious hematopoietic necrosis virus (IHNV) has been reported to cause mortality within a temperature range of approximately 3–18°C, with prolonged persistence at lower temperatures (Raman et al., 2013). These observations demonstrate that both the magnitude and variability of temperature play a critical role in disease dynamics.

Table 2. Table showing different diseases and their specific causative agents in their particular seasons

Season	Disease	Causative Pathogen
Winter	Epizootic ulcerative syndrome (EUS)	*Aphanomyces invadans*
Winter	Saprolegniasis	*Saprolegnia* spp.
Summer	Lactococcosis	*Lactococcus garvieae*
Summer	Columnaris disease	*Flavobacterium columnare*
Summer	Motile Aeromonas septicemia	*Aeromonas hydrophila*
Spring/Autumn	Koi herpes virus disease	Cyprinid herpesvirus-3
Monsoon	EUS	*Aphanomyces invadans*
Fluctuating temperature	Enteromyxosis	*Enteromyxum leei*

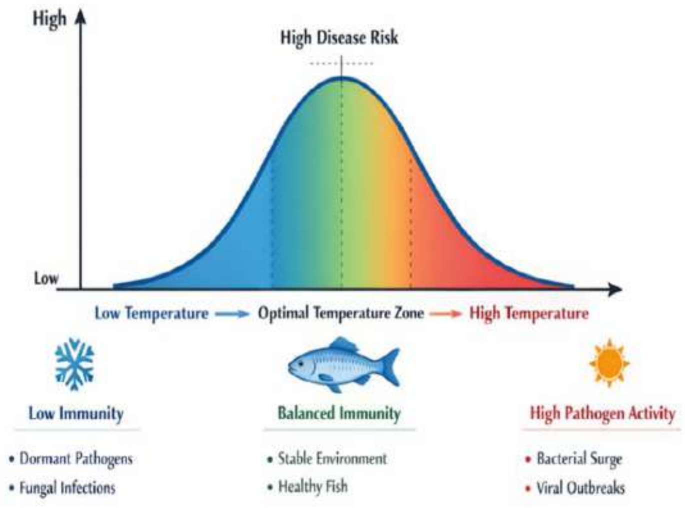

Figure 2. A diagram showing relationship between temperature and fish dynamics

Variations in pH represent another important environmental stressor influencing fish physiology and health. Most biochemical processes in fish, including enzyme activity, are highly dependent on a stable pH range. Deviations from optimal pH levels can disrupt digestive efficiency by altering enzyme function, thereby impairing nutrient absorption and overall growth (Wannas and Mohammed, 2025). In addition to digestive effects, pH fluctuations influence respiratory efficiency by affecting the oxygen-binding capacity of hemoglobin. Increased acidity reduces oxygen affinity, limiting oxygen transport in the blood, while extreme alkaline conditions can also interfere with normal respiratory processes (Walker et al., 2020). Such imbalances contribute to physiological stress and increase susceptibility to disease. Dissolved oxygen (DO) is another critical parameter governing aquatic life and is essential for respiration, metabolism, and overall physiological function in fish. Oxygen in aquaculture systems is primarily supplied through phytoplankton photosynthesis and mechanical aeration, while it is consumed through fish respiration and microbial decomposition of organic matter. When oxygen consumption exceeds production, particularly during nighttime, DO levels decline, creating stressful or hypoxic conditions (Boyd, 2017). Environmental factors such as temperature, salinity, and biological oxygen demand further influence oxygen availability in the water column (Wannas and Mohammed, 2025). When DO concentrations fall below approximately 5–6 mg/L in freshwater systems, fish experience hypoxia, leading to reduced metabolic efficiency and increased physiological stress (Wannas and Mohammed, 2025). Low oxygen conditions not only affect host physiology but also influence disease dynamics. Hypoxia weakens immune responses and creates favorable conditions for certain pathogens and parasites. The prevalence of parasitic infections may increase under such conditions, as some parasites are well adapted to low-oxygen environments and can establish more readily within host tissues. Examples include intestinal and tissue-associated parasites such as Trichodina mutabilis, Pomphorhynchus laevis, and Rhabdochona congolensis, which are capable of persisting under reduced oxygen conditions (Ito and Egwunyenga, 2024).

3.1 Pollution and Organic Load in Aquaculture

Pollution represents a major environmental stressor in aquaculture systems, arising from the accumulation of contaminants such as heavy metals, pesticides, untreated effluents, and microplastics. These pollutants introduce toxic compounds into aquatic environments, disrupting physiological homeostasis and impairing immune function in cultured organisms (Sharma et al., 2025). Chronic exposure to such contaminants can lead to sublethal effects, including oxidative stress, endocrine disruption, and reduced disease resistance, thereby increasing susceptibility to opportunistic infections. Harmful algal blooms (HABs) constitute another significant consequence of nutrient enrichment in aquatic systems, particularly in eutrophic lakes and coastal environments. These blooms are characterized by rapid proliferation of toxin-producing phytoplankton, which can severely affect aquatic organisms through both oxygen depletion and the release of bioactive toxins. The persistence of HABs, which may last from days to several months, can result in prolonged hypoxic conditions and widespread mortality of fish and invertebrates (Zhou et al., 2020). In addition, algal toxins can bioaccumulate within aquatic food webs, further amplifying their ecological and health impacts. Organic loading from aquaculture operations represents a complex and multifactorial environmental challenge. Intensive farming practices lead to the accumulation of nitrogen (N) and phosphorus (P) compounds, particulate organic matter (POM), and dissolved organic carbon (DOC), all of which alter the physicochemical properties of receiving waters. These inputs promote eutrophication, stimulate microbial proliferation, and create conditions conducive to pathogen growth (Zhou et al., 2020). Formulated feeds are the primary source of organic inputs in aquaculture systems. Although designed to optimize growth, a considerable fraction of feed remains unconsumed and enters the environment as waste. Feed conversion ratios (FCRs) in intensive finfish culture typically range from 1.2 to 2.5 kg feed per kilogram of fish produced, indicating substantial nutrient loss to the surrounding water (Bhavsar et al., 2016). The deposition of particulate organic matter beneath cages or within pond sediments leads to the development of anoxic zones characterized by elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD).

Under such reducing conditions, the production of toxic metabolites such as hydrogen sulfide (H_2S) further degrades sediment quality and adversely affects benthic communities. Spatially, the impact of organic enrichment can extend significantly beyond the culture unit. Sediment alteration and benthic disruption have been reported at distances of 25–100 meters from aquaculture installations, resulting in shifts in invertebrate community structure and alterations in nutrient cycling processes (Bhavsar et al., 2016). Additionally, the mineralization of organic waste releases dissolved inorganic nutrients, including ammonium (NH_4^+), nitrite (NO_2^-), and phosphate (PO_4^{3-}), which can stimulate phytoplankton growth and contribute to the development of eutrophic conditions and secondary algal blooms. Collectively, these processes demonstrate that pollution and organic loading not only degrade water quality but also create ecological conditions that favor pathogen proliferation and disease emergence in aquaculture systems.

3.2 Pathogen Proliferation in Aquaculture Environments

Intensive aquaculture systems, characterized by high stocking densities and elevated organic loading, create conditions that favor pathogen proliferation. Increased host density enhances transmission efficiency, while uneaten feed and metabolic waste enrich the environment with organic substrates that support microbial growth (Bhavsar et al., 2016). Such nutrient-rich conditions promote shifts in microbial communities toward opportunistic and pathogenic taxa. Bacterial genera such as Aeromonas, Vibrio, and Flavobacterium readily proliferate under these conditions, particularly in association with biofilms and organic particulates. Concurrently, environmental stressors including hypoxia, elevated temperature, and poor water quality compromise host immune function, further increasing susceptibility to infection. Aquaculture systems can also serve as reservoirs for pathogen dissemination. The release of untreated effluents and movement of infected stock facilitate the spread of pathogens to surrounding ecosystems, posing risks to wild populations and, in some cases, public health. A summary of major pathogens, host species, and favorable environmental conditions is presented in Table 3.

Table 3. Representative aquaculture pathogens categorized by type, along with associated hosts, environmental drivers of proliferation, and their transmission risk to wild populations

Pathogen type	Species / Agent	Host	Favored condition	Risk to wild population
Bacteria	*Aeromonas hydrophila*	Freshwater fish	High organic load, low DO	Moderate (via effluent discharge)
Bacteria	*Vibrio* spp.	Marine fish and shrimp	Warm, nutrient-rich water	Moderate (human health risk)
Bacteria	*Flavobacterium columnare*	Salmonids	High temperature + organic matter	Moderate
Ectoparasite	*Lepeophtheirus salmonis* (sea lice)	Salmon	High density open-net farms	High (infests wild salmon)
Virus	White Spot Syndrome Virus (WSSV)	Shrimp	Stressed, immunocompromised hosts	High (coastal shrimp zones)
Virus	Infectious Salmon Anemia (ISA)	Atlantic salmon	Poor water quality, crowding	High (documented wild outbreaks)

4. ENVIRONMENTAL STRESS AND DISEASE EMERGENCE

Disease emergence in aquaculture is closely linked to environmental stress, which disrupts physiological balance and weakens host defenses. Fluctuations in key parameters such as temperature, dissolved oxygen, and water quality impose stress that compromise's immune function, allowing opportunistic pathogens to establish infection (Snieszko, 1974; Raman et al., 2013). Under such conditions, pathogens that are otherwise present at low levels can proliferate and cause disease outbreaks. Thus, disease occurrence in aquaculture systems is not solely dependent on pathogen presence but is strongly influenced by environmental conditions that regulate host susceptibility and pathogen activity.

5. SEASONAL AND CLIMATE-DRIVEN DISEASE PATTERNS

Fish are among the organisms most sensitive to climate variability, as their ectothermic nature makes metabolic processes and physiological functions highly dependent on environmental temperature. One of the primary biological responses to climate warming in many species is a shift in distribution toward higher latitudes or elevations (Rigos et al., 2026). However, in freshwater ecosystems, where physical boundaries often restrict large-scale movement, such redistribution may be limited. In these systems, fish may instead utilize localized thermal gradients, such as depth-related temperature variation, to access more favorable conditions (Rigos et al., 2026). Thermal stress arises when fish are exposed to temperatures beyond their optimal tolerance range, which is species-specific and critical for normal growth and physiological functioning. Deviations from this range result in physiological imbalance and stress responses.

Effects of thermal stress include:

- Suppression of immune function, increasing susceptibility to infections
- Elevated levels of stress hormones such as cortisol
- Disruption of metabolic processes and feeding behavior
- Reduced growth performance and feed efficiency
- Gill damage and impaired oxygen uptake

Rapid or abrupt temperature fluctuations are particularly detrimental, as fish lack the capacity for immediate physiological adjustment. Such conditions weaken host defense mechanisms and facilitate the invasion and proliferation of opportunistic pathogens. Disease patterns in aquaculture frequently exhibit strong seasonal associations driven by temperature variation. Lower temperature ranges (approximately 5–10°C) are known to support prolonged viral persistence and can influence host immune responses, including antibody production (Snieszko, 1974). Infectious hematopoietic necrosis virus (IHNV), for example, has been reported to cause mortality within a temperature range of 3–18°C. During winter, reduced water temperatures slow metabolic activity and suppress immune function, increasing susceptibility to infections such as epizootic ulcerative syndrome (EUS), saprolegniasis, and winter kill, particularly in carp-based aquaculture systems in India.

In contrast, elevated temperatures during summer enhance metabolic rates while often reducing dissolved oxygen availability, creating conditions favorable for pathogen proliferation. Diseases such as lactococcosis, columnaris disease, and motile Aeromonas septicemia are more prevalent under these conditions, with higher temperatures accelerating bacterial growth and increasing the severity of outbreaks. These observations indicate that temperature variability, rather than absolute temperature alone, plays a critical role in disease initiation and progression. Maintaining stable thermal conditions is therefore essential for minimizing stress-induced disease outbreaks in aquaculture systems.

6. POLLUTION–DISEASE NEXUS IN AQUACULTURE SYSTEMS

The accumulation of particulate organic matter beneath net pens and within pond sediments leads to the formation of anoxic zones characterized by elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Under such reducing conditions, the production of hydrogen sulfide (H_2S) further degrades sediment quality and negatively impacts benthic biodiversity. Bhavsar et al. (2016) reported that organic enrichment beneath sea cages can extend up to 25–100 meters from the culture unit, resulting in significant disruption of benthic invertebrate communities and alterations in biogeochemical cycling. In addition to sediment impacts, the decomposition of organic waste releases dissolved inorganic nutrients that influence water column processes. Mineralization of uneaten feed and fecal matter generates ammonium (NH_4^+), nitrite (NO_2^-), and phosphate (PO_4^{3-}), which contribute to localized eutrophication and stimulate phytoplankton growth. This nutrient enrichment can promote hypoxic conditions and, in some cases, trigger harmful algal blooms (HABs), further degrading water quality and affecting aquatic life. Quantitative assessments indicate that uneaten feed may constitute approximately 5–30% of total feed inputs, depending on feeding practices, species-specific behavior, and system management (Lim et al., 2015).

Once introduced into the aquatic environment, uneaten pellets undergo rapid microbial degradation, accelerating nutrient cycling and intensifying organic loading. A summary of key parameters associated with organic load and their environmental implications is presented in Table 4.

Table 4. Estimated organic load parameters from aquaculture operations and their environmental implications (Source: Bhavsar et al., 2016)

Parameter	Typical Range	Unit	Significance
Feed Conversion Ratio (FCR)	1.2 – 2.5	kg feed / kg fish	Higher FCR indicates more waste per unit production
Uneaten feed fraction	5 – 30	% of total feed	Direct organic input to water column
Sediment impact radius	25 – 100	meters from cage	Zone of benthic disruption and anoxia
Copper sediment enrichment	Up to 10× background	mg/kg dry weight	Toxic to benthic invertebrates
BOD increase in effluent	Significant elevation	mg O_2/L	Oxygen depletion in receiving waters

7. MANAGEMENT STRATEGIES: PREVENTING HOSTILE WATER CONDITIONS

Commercial feeding represents a primary source of nitrogenous waste, particularly ammonia, in aquaculture systems. Therefore, regulating feeding practices is essential for maintaining water quality and environmental stability. Overfeeding leads to the accumulation of uneaten feed and metabolic waste, increasing ammonia concentrations and deteriorating overall water conditions. Feeding rates should be adjusted according to species-specific requirements and fish size to minimize waste generation and optimize feed utilization. Standardized feeding schedules, such as those developed for Indian Major Carps (IMC), should be followed to ensure efficient nutrient input and reduce environmental load (Lieke et al., 2020). Stocking density is another critical factor influencing water quality. Overstocking beyond the carrying capacity of the system disrupts the balance of key parameters such as dissolved oxygen, carbon dioxide, and ammonia, leading to stressful conditions for cultured organisms.

Regular monitoring of physicochemical parameters is therefore necessary to maintain optimal environmental conditions and support fish health (Lieke et al., 2020). Implementation of biosecurity measures plays a significant role in disease prevention by limiting pathogen introduction and spread within culture systems. In addition, monitoring water quality parameters can provide early indications of environmental imbalance, allowing timely management interventions that support growth and reduce disease risk. The use of fermentation-based inputs in aquaculture systems requires careful regulation. Uncontrolled application can promote excessive nutrient enrichment and lead to algal blooms, which may create unfavorable conditions for aquatic organisms. Proper management of such inputs is therefore important to maintain ecological balance and prevent the development of adverse environmental conditions.

Table 5. Size-specific feeding regimes for cultured fish, indicating daily feed allocation as a percentage of body weight and corresponding pellet size to improve feed efficiency and minimize nutrient loading.

Fish Weight	Daily Feeding Rate (% of BW)	Feed Size
5g – 10g	7.50%	1.0 mm
10g – 20g	7.00%	1.5 mm
21g – 50g	5.5% – 6.0%	1.5 mm
51g – 100g	4.00%	2.0 mm
101g – 300g	3.0% – 3.5%	3.0 mm
301g – 500g	2.0% – 2.8%	3.0 mm
> 500g	1.0% – 2.25%	3-4 mm

8. INTEGRATED PERSPECTIVE: FROM STRESS TO DISEASE CASCADE

In aquaculture systems, disease development can be viewed as a cascading process that originates from environmental imbalance and progresses toward clinical outbreaks. Deterioration in water quality, including fluctuations in temperature, dissolved oxygen, pH, and organic load, induces physiological stress in fish. This stress triggers endocrine responses, particularly elevated cortisol levels, which suppress immune function, disrupt mucosal barriers, and alter microbial communities.

As host defenses decline, opportunistic pathogens present in the environment proliferate and invade host tissues, resulting in disease manifestation. Effective management strategies focus on interrupting this cascade at early stages. Maintaining optimal water quality through regular monitoring, controlled feeding, and adequate aeration reduces environmental stress and stabilizes system dynamics. Proper stocking density prevents crowding-related stress, while biosecurity measures limit pathogen introduction and transmission. Collectively, these interventions restore system balance, reduce disease risk, and support sustainable aquaculture production.

CONCLUSION

Aquaculture systems are fundamentally dependent on water quality, which directly influences fish health, productivity, and disease dynamics. This chapter highlights that disease emergence is closely associated with environmental conditions, where stressors such as temperature fluctuations, dissolved oxygen imbalance, pH variation, pollution, and excessive organic loading disrupt physiological stability and impair immune function. Under such conditions, pathogens that are otherwise present at low or subclinical levels can proliferate and cause disease outbreaks. The interaction between host, pathogen, and environment demonstrates that disease cannot be managed in isolation but must be addressed within the broader context of ecosystem stability. Effective aquaculture management therefore requires an integrated approach that emphasizes regular monitoring of water quality, optimized feeding practices, appropriate stocking densities, and strict biosecurity measures. With increasing intensification and climate variability influencing aquaculture systems, proactive environmental management becomes essential for disease prevention. Maintaining stable and optimal rearing conditions not only improves fish health and productivity but also supports the long-term sustainability of aquaculture practices.

REFERENCES

Austin, B. (Ed.). (2012). Infectious disease in aquaculture: Prevention and control. Elsevier.

Batista, D. V. V., Owatari, M. S., & Martins, M. L. (2025). Fish health: Diagnosis and health management in farming systems.

Bhavsar, D. O., Pandya, H. A., & Jasrai, Y. T. (2016). Aquaculture and environmental pollution: A review work. International Journal of Scientific Research in Science, Engineering and Technology, 2(1), 40–45.

Boyd, C. E. (2017). General relationship between water quality and aquaculture performance in ponds. In Fish diseases (pp. 147–166). Academic Press.

Chong, C. M., Lee, P. T., Rakus, K., & Wangkahart, E. (2023). The epidemiologic triads in aquaculture: Host, pathogen and environment. Frontiers in Immunology, 14, 1305784.

FAO. (2024). The state of world fisheries and aquaculture 2024: Blue transformation in action. FAO. https://doi.org/10.4060/cd0683en

Hossain, M. K., Islam, K. T., Hossain, M. D., & Rahman, M. H. (2011). Environmental impact assessment of fish diseases on fish production. Journal of Science Foundation, 9(1–2), 125–131.

Ikeogu, F. C., Nsofor, C. I., & Ikpeze, O. O. (2010, November). A review of risk factors for fish diseases in aquatic environments. In Proceedings of the 6th National Conference of the Society for Occupational Safety and Environmental Health (SOSEH) (pp. 199–204).

Ito, E. E., & Egwunyenga, A. O. (2024). Impact of water quality and morphometric indices on the spatio-temporal prevalence of fish endo-parasites and diversity in the Ase River, Niger-Delta, Nigeria. Journal of Parasitic Diseases, 48(3), 439–449.

Kumar, G., Menanteau-Ledouble, S., Saleh, M., & El-Matbouli, M. (2015). Yersinia ruckeri, the causative agent of enteric redmouth disease in fish. Veterinary Research, 46(1), 103.

Lieke, T., Meinelt, T., Hoseinifar, S. H., Pan, B., Straus, D. L., & Steinberg, C. E. (2020). Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. Reviews in Aquaculture, 12(2), 943–965.

Lim, C., Webster, C. D., & Lee, C. S. (2015). Feeding practices and fish health. In Dietary nutrients, additives, and fish health (pp. 333–346).

Martins, C. I., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M. T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J. C., Carter, T., & Planellas, S. R. (2012). Behavioural indicators of welfare in farmed fish. Fish Physiology and Biochemistry, 38(1), 17–41.

Mougin, J., & Joyce, A. (2023). Fish disease prevention via microbial dysbiosis-associated biomarkers in aquaculture. Reviews in Aquaculture, 15(2), 579–594.

Noga, E. J. (2010). Fish disease: Diagnosis and treatment. John Wiley & Sons.

Östman, Ö., Drakare, S., Kritzberg, E. S., Langenheder, S., Logue, J. B., & Lindström, E. S. (2012). Importance of space and the local environment for linking local and regional abundances of microbes. Aquatic Microbial Ecology, 67(1), 35–45.

Pinna, M., Zangaro, F., Saccomanno, B., Scalone, C., Bozzeda, F., Fanini, L., & Specchia, V. (2023). An overview of ecological indicators of fish to evaluate the anthropogenic pressures in aquatic ecosystems: From traditional to innovative DNA-based approaches. Water, 15(5), 949.

Raman, R. P., Prakash, C., Makesh, M., & Pawar, N. A. (2013). Environmental stress mediated diseases of fish: An overview. Advances in Fish Research, 5, 141–158.

Rigos, G., Padrós, F., Constenla, M., Jerončić, A., Kogiannou, D., Consuegra, S., Adamek, M., & Mladineo, I. (2026). Global warming affects the pathogenesis of important fish diseases in European aquaculture. Reviews in Aquaculture, 18(1), e70112.

Samsing, F., & Barnes, A. C. (2024). The rise of the opportunists: What are the drivers of the increase in infectious diseases caused by environmental and commensal bacteria? Reviews in Aquaculture, 16(4), 1787–1797.

Schulte, P. M. (2014). What is environmental stress? Insights from fish living in a variable environment. Journal of Experimental Biology, 217(1), 23–34.

Sharma, M., Kant, R., Sharma, A. K., & Sharma, A. K. (2025). Exploring the impact of heavy metals toxicity in the aquatic ecosystem. International Journal of Energy and Water Resources, 9(1), 267–280.

Sindermann, C. J. (1990). Principal diseases of marine and shellfish (Vol. 1). Gulf Professional Publishing.

Snieszko, S. F. (1974). The effects of environmental stress on outbreaks of infectious diseases of fishes. Journal of Fish Biology, 6(2), 197–208.

Thompson, C. C., Wasielesky Jr., W., Landuci, F., Lima, M. S., Bacha, L., Perazzolo, L. M., Lourenço-Marques, C., Soares, F., Pousão-Ferreira, P., Hanson, L., & Gomez-Gil, B. (2024). Understanding the role of microbes in health and disease of farmed aquatic organisms. Marine Life Science & Technology, 6(4), 579–609.

Tort, L., & Balasch, J. C. (2022). Stress and immunity in fish. In Principles of fish immunology: From cells and molecules to host protection (pp. 609–655). Springer International Publishing.

Tripathi, G., & Bharathi, R. R. (2025). Diseases in cage aquaculture. In Aquatic animal health management (pp. 85–108). Springer Nature Singapore.

Walker, R. H., Smith, G. D., Hudson, S. B., French, S. S., & Walters, A. W. (2020). Warmer temperatures interact with salinity to weaken physiological facilitation to stress in freshwater fishes. Conservation Physiology, 8(1), coaa107.

Wannas, A. K., & Mohammed, S. H. (2025). The role of environmental stress in fish health: A review. GSC Biological and Pharmaceutical Sciences, 32, 17–27.

Woo, P. T. K., & Buchmann, K. (Eds.). (2012). Fish parasites: Pathobiology and protection. CABI.

Zhou, J., Lao, Y. M., Song, J. T., Jin, H., Zhu, J. M., & Cai, Z. H. (2020). Temporal heterogeneity of microbial communities and metabolic activities during a natural algal bloom. Water Research, 183, 116020.

CHAPTER 2
MICROBIAL ASSESSMENT OF COMPLEMENTARY FOOD BLENDS FORMULATED FROM NIGERIAN YELLOW MAIZE, SOYBEAN, AND CRAYFISH USING SPRAGUE DAWLEY RATS

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INTRODUCTION

In sub-Saharan Africa, particularly Nigeria, the high prevalence of infant morbidity is often linked to the microbial contamination of weaning foods. While nutritional density is vital, the safety of complementary foods is frequently compromised during processing, storage, and handling (Omemu & Oyewole, 2016). Formulations involving yellow maize, soybean, and crayfish are highly nutritious but also provide an ideal substrate for the growth of pathogenic and spoilage microorganisms. Using Sprague Dawley rats as a biological model allows researchers to assess not just the presence of microbes in the food, but their actual impact on the host's gut health and systemic safety.

Food quality is an indefinite term but can be harmoniously described as the requirements necessary for a food product to satisfy the needs and expectations of the consumers (Prache et al., 2022). The quality of food is determined by various attributes, such as nutritional content, nutrient bioavailability, functional characteristics, organoleptic properties, and preparation convenience (Prache et al., 2022). Another critical requirement for consumer satisfaction is the food safety aspect (Salvatore, 2021), which is related to health hazards but not commonly measured or take into cognizant in the household or community setting. The World Health Organization (WHO, 2024) defines food safety for complementary foods as ensuring they pose no risk to infants and young children when prepared and fed according to guidelines. Still, in the definition of Stanton (2024), food safety is simply those recommended conditions and practices starting from the farm along several chains of production till the consumption that are necessary to protect foods from pathogenic organisms, exogenous and endogenous contaminants, and even from toxic substances that are newly formed in the course of transmission along the chains of production. Food-borne diseases especially diarrhea, cancer, and other diseases of concern are caused by bacteria, viruses, and parasites or chemical substances (WHO, 2024). Food safety is always a concern when intended to human consumption. Recent years have seen a surge in food-borne diseases, with developing countries experiencing a heavier health and economic toll (Compaore et al., 2022). According to the WHO (2024), approximately 1 in 10 people globally fall ill from consuming contaminated food, resulting in 420,000 annual deaths, including 125,000 children under five.

Detecting pathogenic organisms in food products and clinical specimens is a fundamental step in ensuring food safety and regulatory compliance, enabling the identification and control of pathogens before they cause widespread outbreaks (Zadernowska & Chajecka, 2012; Verocai et al., 2020; Rapidmicrobilogy, 2023; Kabiraz, 2023). Salmonella spp is a widely distributed food-borne pathogen that serves as a marker of food products safety (Ehuwa et al., 2021), and its presence in food products or feacal specimens can be detected in the laboratory using the conventional culture-based technique, serological and biochemical identification of suspected colonies for confirmation (Paniel and Noguer, 2019). According to Rapidmicrobiology (2023), Salmonella enteritidis is highly common and usually causes gastroenteritis/salmonellosis among under-five children, though persons of all ages are affected. Salmonella infections can cause gastroenteritis, characterized by symptoms such as diarrhea, abdominal pain, nausea, and vomiting that can persist for up to 7 days, with as few as 10-100 cells capable of triggering illness. Salmonella is widespread in animals and typically linked to contaminated food consumption, although transmission can also occur through direct animal contact, water, or person-to-person contact. Intestinal parasites including protozoans and helminths are another significant cause of diarrhea, particularly in Nigeria, where they contribute to childhood morbidity and mortality (Zaman et al., 2018; National Population Commission [Nigeria], NPC and International Classification of Functioning Disability and Health Nigeria Demographic and Health Survey, ICF, 2018, 2019). Verocai et al. (2020) reported that wet mount preparation of feacal samples was mostly used for the diagnosis of intestinal parasitic infections through microscopic observation of parasitic ova in the smeared sample. Food contamination could be due to preparation under unhygienic conditions, handling with contaminated hands, cooking utensils (Gallo et al., 2020). With good treatment of materials and preservation method, pathogens threatening food safety can be effectively mitigated through growth retardation (Tumuluru, 2023).

Yellow maize is susceptible to fungal contamination, particularly by Aspergillus, Penicillium, and Fusarium species. The primary concern in the Nigerian context is the presence of aflatoxins, secondary metabolites produced by Aspergillus flavus.

Research indicates that poor post-harvest drying of maize contributes to high fungal loads, which can lead to acute or chronic toxicity in infants (Adegoke et al., 2023). Soybean is rich in protein and moisture-absorbing carbohydrates. If not properly processed, it can harbor Bacillus cereus and Staphylococcus aureus (Obadina et al., 2013). However, traditional processing methods like fermentation are often employed to reduce these pathogens by lowering the pH through the production of organic acids by Lactic Acid Bacteria. As an animal-source food, crayfish is the most microbially sensitive component. It is often contaminated during sun-drying or open-air marketing with Salmonella spp., Escherichia coli, and Vibrio species (Amadi et al., 2021). The inclusion of crayfish in weaning blends necessitates rigorous heat treatment to ensure the final product is free from enteric pathogens. While in vitro (laboratory) testing identifies the specific microbial load present in a food sample, it cannot simulate the complex biological responses of a living host. Consequently, feeding trials with Sprague Dawley rats provide critical data on the "host-microbe" interaction (Olatoye & Arueya, 2018). These models are essential for observing how the gut environment reacts to pathogens or beneficial microbes introduced through the maize-soy-crayfish blend.

The present write-up aimed at reporting the microbial level of CFs formulated from blends of Nigerian yellow maize, soybean, and crayfish, hence the health-associated hazard. Thus, contributing to the array of knowledge on food safety and control measures in the academic research.

1. MATERIALS and METHODS

1.1. Materials

Yellow maize (Z. mays), soybean (G. max), dried crayfish (P. clarkii) and a commercial CF (Fortified Milk and Wheat Flour) were purchased from the main market, Abuja, Nigeria, and transported safely to the Nutrition Science Laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. Twenty-eight (28) male Sprague Dawley and standardized laboratory chow (SLC) were purchased from Sapphire Enterprise, Malaysia, were purchased from Sapphire enterprise, Malaysia. Approval for animal study was obtained through the Universiti Putra Malaysia Institutional Animal Care and Use Committee (UPMIACUC) as displayed in Appendix 1.

1.2. Methods

1.2.1. Foodstuff Treatment

Raw foodstuffs were treated into flours following the description of Adegbusi et al. (2022): We cleaned and sorted raw soybean seeds, removing stones, rot, and defects. The good ones were soaked for 3 hours, de-hulled, boiled for 15 minutes, oven-dried at 80°C for 24 hours, and roasted until golden brown. Maize grains and crayfish were also cleaned, washed, and oven-dried at 80°C for 24 hours. We then milled the dried ingredients, sieved them through a 710 µm aperture sieve, and obtained smooth flours, free of large particles. These were packed in air-tight bags and refrigerated at 4°C, ready for formulation and analysis. Protein contents of flours of maize (MF), soybean (SBF), and crayfish (CRF) were determined following the description of Tamangwa et al. (2023) and the data of crude protein contents were used to make the CFs blends that met the recommended infant CF protein content of 16 g/100 g Adegbusi et al. (2022) to form the formulation novel CFs, namely; blend of maize and soybean flours, MSF, in the ratio of 72:28% w/w; blend of maize, soybean, and crayfish flours, MSCF, in the ratio of 80:10:10% w/w; while maize-only flour, MF, was made of 100:0%.

1.2.2. Rat Feeding Experiment

1.2.2.1. Formulation of Experimental Diet

Experimental diets for the rat studies were made following the description of Adegbusi et al. (2022): A protein-free diet was made basal. The amount of formulated CF that provided 8% protein was incorporated into the basal diet at the expense of corn starch to make a total of 100 g diet. The requirement for protein quality determination in rat tests is commonly by 10 g protein/100 g diet level. However, other researchers have used 8 g protein/100 g diet level. To make isonitrogenous diets in this study, the formulation was made to contain 8 g protein/100 g level, because MF had a very low protein content. Corn oil was added to a total of 10% lipid which made allowances for the natural oil present in the protein source of a diet for isocaloric formulation. Standardized laboratory chow was offered to rats as supplied by the supplier. Formulated and control diets contained equal amounts of sugar, vitamin mix, mineral mix, choline bitartrate, cellulose as the basal diet.

The flours of formulated and control diets components were manually mixed in 2 g/mL water and kneaded into thick dough to avoid wastage. The dough was compressed, cut into a rectangular shape of 1 by 1 cm, and oven-dried at 60oC overnight. Dried pieces of dough were kept in well-labeled, sealed plastic containers and stored at 4oC pending use. The weighing combinations for diets fed to rats are shown in Table 1.

Table 1. Weighing combination of rat-fed diets fed (g/100.00 g DM)

Component (g)	**Diet (100.00 g)**					
	MD	**SBD**	**MSD**	**MSCD**	**FWMD**	**PFD**
Maize Flour	100.00	–	–	–	–	–
Soybean Flour	–	20.70	–	–	–	–
Maize Soybean Flour	–	–	46.30	–	–	–
MSCF	–	–	–	45.50	–	–
FWMF	–	–	–	–	55.80	–
Corn Starch	–	52.60	27.00	27.80	17.50	73.30
Corn Oil	–	10.00	10.00	10.00	10.00	10.00
Cellulose	–	5.00	5.00	5.00	5.00	5.00
Sugar	–	7.00	7.00	7.00	7.00	7.00
Choline Bitartrate	–	0.20	0.20	0.20	0.20	0.20
Mineral Mix	–	3.50	3.50	3.50	3.50	3.50
Vitamin Mix	–	1.00	1.00	1.00	1.00	1.00

MD = Maize diet; SBD = Soybean diet; MSD = Maize + soybean diet; MSCD = Maize + soybean + crayfish diet; FWMD = Fortified wheat milk diet (Nestle Cerelac diet); PFD = Protein-free diet; MSCF = Maize + soybean + crayfish flour; FWMF = Fortified wheat milk flour (Nestle Cerelac powder); DM = Dry matter. Mineral mix (AIN-76 Salt mix, Dyets # 200000, USA), Vitamin mix (AIN-76A Vitamin mixture, Dyets #300050, USA), Corn starch (Cap Bintang, Malaysia); Corn oil (Vecorn, Malaysia); Cellulose (Dyets # 401850, USA); Sugar (fine granulated, Malaysia), Choline bitartrate (Solarbio # C6850, China). The standardized laboratory chow (SLC) was a "GOLD COIN" BRAND ANIMAL FEED with the following quality specification: Crude Protein, 21-23%; Crude Fiber, 5.0%; Crude Fat, 3.0%; Moisture, 13.0%; Ash, 8.0%; Calcium, 0.8%-1.2%; Phosphorus, 0.6%-1.0%; Nitrogen Free Extract, 49.0%; Additives include; Vitamins A, D3, E, C, K, B12, Thiamine, Riboflavin, Pantothenic Acid, Niacin, Pyridoxine, Choline, Antioxidant and Microminerals.

1.2.2.2. Rat Feeding Experiment

An 11-day feeding experiment with 28 male Sprague Dawley rats was conducted, following a protocol similar to Adegbusi et al. (2022).

The rats were first acclimatized for 5 days, during which they were fed standardized laboratory chow and water, and then divided into 7 diet groups. Each rat was housed individually and fed one of 7 different diets for 5 days, with their health and behaviour monitored. Faecal samples were collected on the 11th day to test for pathogens like Salmonella and parasites. After 14 days, the rats were humanely euthanized, and their carcasses were disposed of according to established protocols. Throughout the study, adherence to the Universiti Putra Malaysia's guidelines for animal care and use, incorporating the 3Rs principle was ensured.

1.2.3. Assessment of Microbiological Quality of Experimental Diets

1.2.3.1. Media Preparation

- Selenite F Broth (SFB): about 4 g of part B powder was weighed into 1 L glass bottle of distilled water. A 19 g weight of part A was added and mixed to dissolve. The solution was boiled for 10 minutes for sterilization. Storage was at 4 °C pending used. Part A Powder Composition (g /L): Tryptone (5 g); Lactose (4 g); Sodium phosphate (10 g). Part B Powder Composition (g /L): Sodium hydrogen selenite (4 g).
- MacConkey Agar (MacA): about 49.53 g powder was weighed, dissolved in 1 L glass bottle of distilled water, autoclaved at 121 °C, poured into Petri dishes, and store at 4 °C pending use.
- Salmonella-Shigella Agar (SSA): about 57.02 g powder was weighed, dissolved in hot boiling distilled water and cooled to about 50 °C, poured into sterile Petri dishes and stored at 4 °C pending used.
- Normal saline: 8.5 g of normal saline was dissolved in 1 L distilled water.

1.2.3.2. Microbial Detection

- Microscopic observation: wet mount preparation of the feacal sample was achieved following the procedure of Demeke et al. (2021). Fresh and urine-free feacal samples were collected on the 6th day of balance period from individual rats in each of the rat-fed group, MD, SBD, MSD, MSCD, FWMD, PFD and SLC, into individual labelled sterile urine bottles and transported to the laboratory for the detection of parasite ova.

The examination was done within one hour of feacal collection. An aliquot of a urine-free feacal sample was diluted with 0.85 % normal saline on a microscopic glass slide, a cover slip was gently placed over it to spread out the emulsion into a thin transparent layer. The slide was examined by methodically scanning each field of the entire coverslip using a 10x objective and then 40x objective.

- Macroscopic observation: wet and cultured feacal samples from experimental rats were observed with unaided eyes for the presence of mucus, blood, and microbial colonies. Following the culture method of detecting Salmonella spp. from biological or clinical samples as described in Awang et al. (2021) with modifications, about 0.5 g of wet feaces from rat-fed groups, MD, SBD, MSFD, MSCD, FWMD, PFD, and SLC, was on the 6th day of balance period placed into a labelled sterile urine bottle and transported to the laboratory. Afterward, the same quantity of feaces was transferred into a correspondingly labelled sterile 10 mL test tube containing 4.5 mL of sterile SFB within 1 hr of collection, for growth enrichment of Salmonella. Culturing tubes were turned upside-down, slightly, to allow a proper mixture of contents, covered with cotton wool, and incubated aerobically for 24 hrs at 37 °C. After 24 hrs of incubation, a cultured feacal sample from each diet group was inoculated and streaked with an inoculation loop into a pair of solidified MacA and SSA media. The MacA medium served to selectively differentiate the growth of all gram-negative bacteria into lactose, pink or red colonies, and non-lactose, colorless colonies, fermenters (Jung and Hoilat, 2023), while the SSA medium served to selectively differentiate lactose, pink or red colonies, and non-lactose, colorless colonies, fermenters, and black-centered colonies of gram-negative bacteria (Neyaz et al., 2024). Following the streaking, paired agar plates were incubated for 24 hrs at 37 °C, after which they were observed for the presence of Salmonella spp, and hence the infected diet group.

1.2.3.3. Total Bacterial Count (TBC)

A feacal sample from a culturing tube that corresponded to an agar plate where Salmonella was detected was subjected to TBC using a plate counting method. According to the procedure described in Lee (2021) with slight modification. Four five-fold serial dilutions were made from the original tube (0.5 g of feacal sample/5 ml of SFB) with phosphate buffer saline (PBS) in the ratio of 5-1, 5-2, 5-3, 5-4 (Reynolds, 2021). Eight petri dishes containing solidified MacA were labelled. From each dilution, 200 µL of suspension was aseptically transferred into duplicate plates of labeled solidified MacA using pour plating (Reynolds, 2021). Plates were inverted and incubated at 37 °C for 24 hr, and after the incubation period plates were selected for counting manually.

1.3. Statistical Data Processing

Data were analyzed using a binary logistic regression model to determine the presence or absence of Salmonella and parasites, as adapted from Rodríguez-Saavedra et al. (2021) and Leong et al. (2021). Significant differences between the test diets were identified using Tukey's honestly significant difference (HSD) test at a significance level of $P < 0.05$, with the help of SPSS software version 25.

2. RESULT and DISCUSSION

2.1. Results

Macroscopic and microscopic observations were used to determine the microbial quality of rats-fed diets, hence their safety. For microscopic observation, all the representatives of the feacal samples were observed to be parasite-free.

For macroscopic observation with unaided eyes, all rats showed no sign of diarrhoea, the representative's feaces were mucus- and blood-free. Rashes and lesions on rats’ skin were not observed, and all rats were active and healthy throughout the feeding trial.

For the detection of microbial colonies, as describe in Table 2, pink or red and colorless colonies were observed in all MacA and SSA plates in all samples. However, black-centered colonies additionally appeared in SSA member plates from a rat fed each of MSD and FWMD.

Table 2. Characteristic colors observed in representative plate from rat fed on diet

Diet Sample	MacConkey Agar Plate	Salmonella Shigella Agar Plate
MD	Pink or red and colorless colonies	Pink or red and colorless colonies
SBD	Pink or red and colorless colonies	Pink or red and colorless colonies
MSD	Pink or red and colorless colonies	Pink or red, colorless, and black-centered colonies
MSCD	Pink or red and colorless colonies	Pink or red and colorless colonies
FWMD	Pink or red and colorless colonies	Pink or red, colorless, and black-centered colonies
SLC	Pink or red and colorless colonies	Pink or red and colorless colonies
PFD	Pink or red and colorless colonies	Pink or red and colorless colonies

The results of TBC conducted on feacal samples where black-centered colonies showed the absence of microbial growth, as show in Figure 1.

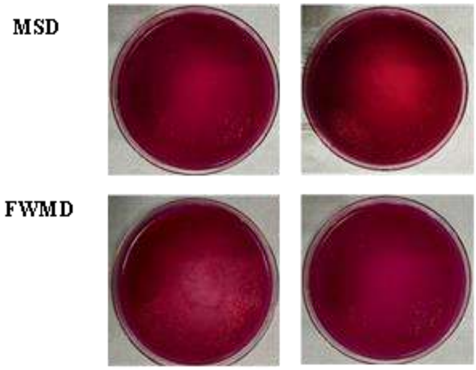

Figure 1. Representative plates from MSD- and FWMD-fed rats showing the absence of bacterial growth with buffer saline.

As in **Table 3,** a comparison of the presence of *Salmonella* spp and parasite in the feacal samples of all the rat-fed diets showed that there was no significant difference ($P < 0.05$) among MD, SBD, MSD, MSCD, FWMD, SLC, and PFD.

Table 3: Number of rats that showed *Salmonella* growth or parasite ova in a feacal sample

Diet	*Salmonella* spp	Parasite
MD	0000[a]	0000[a]
SBD	0000[a]	0000[a]
MSD	0001[a]	0000[a]
MSCD	0000[a]	0000[a]
FWMD	0001[a]	0000[a]
SLC	0000[a]	0000[a]
PFD	0000[a]	0000[a]

Samples were coded as 0 (absence) or 1 (presence) for Salmonella or parasites. Values sharing the same letter in a column indicate no significant difference ($P < 0.05$) according to Tukey's HSD test.

2.2.Discussion

The absence of parasites in all the feacal samples observed may be suggested that all the rat-fed diets have good microbial quality, hence, were safe for consumption. The appearance of pink/red and colorless colonies in MacA and SSA plates, and absence of black-centered spot in SSA plate, Table 2, is a suggestion of the absence of non-lactose fermenters gram-negative Salmonella spp in the feaces of rats-fed on MD, SBD, MSCD, PFD, and SLC, thus, those diets may be safer for consumption. Conversely, the presence of black-centered colonies in SSA plates, Table 2, is a suggestion of the presence of non-lactose fermenters gram-negative Salmonella spp in the feaces of rats-fed on MSD- and FWMD, and thus, those diets may not be safe for consumption. The presence of non-lactose fermenters gram-negative Salmonella spp in the feaces of a rat fed on each of MSD and FWMD was confirmed by the presence of black-centered colonies in their representative SSA member plates.

This is because Salmonella spp colonies were capable of utilizing the sodium thiosulfate, a sulfur source in SSA, to produce hydrogen sulfide which in the presence of ferric ammonium citrate, an indicator, produced the black color precipitate on SSA plates (Neyaz et al., 2024). Nevertheless, the absence of microbial growth from TBC test conducted showed that the observed colonies of Salmonella spp were not viable, and hence both MSD and FWMD may be safe for consumption. Consequently, it may be suggested that the presence of Salmonella spp in those SSA plates were due to exogenous contamination, because the components of MSD, MD and SBD, were also free of parasite and Salmonella spp. The reasons for the zero viable count and hence safety of these diets, as observed in the faecal samples of experimental rats, may be due to proper treatment, processing, handling, and storage of the foodstuffs, formulated foods and diets. This is in conformity with the suggestion of Erkmen (2022).

CONCLUSION

In this research, all rat-fed diets were considered safe for consumption due to the absence of parasite, non-significant presence of Salmonella spp in the feacal samples, and the zero viable number of bacterial determined in the representative feacal samples where the colonies of Salmonella spp were detected and confirmed. All the rats carried no parasitic infection because the parasite ovum was not detected in the feacal samples. All rats were active and healthy during the trial period. All the complementary experimented diets exhibited good microbial safety, hence quality. Therefore, MD, MSD, MSCD, and FWMD can be recommended for consumption as complementary foods. Proper treatment, processing, handling, and storage foods are good techniques for ensuring food safety.

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REFERENCES

Adegbusi, H. S., Ismail A., Mohd Esa N, Mat Daud ZA (2023) Effect of formulated Nigerian yellow maise, soybean, and crayfish blend on some growth performance and physiological status. Food Prod Process and Nutr 5:14. https://doi.org/10.1186/s43014-022-00128-7.

Adegbusi HS, Ismail A, Mohd Esa N, Mat Daud ZA (2022) Evaluation of nutritional quality of complementary foods formulated from blends of Nigerian yellow maize (Zea mays), soybean (Glycine max) and crayfish (Procambarus clarkii). J. Sci. Food Agric. vol. 15:6961–6973. doi: 10.1002/jsfa.12057.

Adegoke, G. O., Allamu, O. E., & Akanni, A. O. (2023). Quality and safety of maize-based complementary foods: A review of microbial and chemical hazards. Nigerian Food Journal, 41(1), 45-58.

Amadi, O. C., Onuoha, S. C., & Okereke, H. C. (2021). Microbiological quality and safety of dried crayfish (Procambarus clarkii) sold in local markets in Southeast Nigeria. Journal of Applied Sciences and Environmental Management, 25(4), 567-573.

Awang MS, Bustami Y, Hamzah HH, Zambry NS (2021) Advancement in Salmonella Detection Methods : From Conventional to Electrochemical-Based Sensing Detection. sensor 11:1–42. doi: https://doi.org/10.3390/bios11090346.

Compaore MKA, Kpoda SD, Bazie RBS., Ouedraogo, M., Valian, M., Gampene, M-L., Yakoro, A., Nikiema, F., Belemlougri, A., Meda, N-S-B.R., Meda, N-I.S.D, Sanon, S., Bande, M., Hien, H., Barro, N., Kabre, E. (2022). Microbiological quality assessment of five common foods sold at different points of sale in Burkina-Faso. PLoS 17:1–17. doi: 10.1371/journal.pone.0258435.

Demeke G, Fenta A, Dilnessa T (2021) Evaluation of Wet Mount and Concentration Techniques of Stool Examination for Intestinal Parasites Identification at Debre Markos Comprehensive Specialized Hospital, Ethiopia. Infect. Drug Resist. 14:1357–1362. doi: 10.2147/IDR.S307683.

Ehuwa O, Jaiswal AK, Jaiswal S (2021) Salmonella, food safety and food handling practices. Foods 10:1–16. doi: 10.3390/foods10050907.

Erkmen O (2022) Practice 2 - Plate count techniques. In: Microbiological Analysis of Foods and Food Processing Environments, Elsevier Inc, . pp 13–18. https://doi.org/10.1016/B978-0-323-91651-6.00040-9

Gallo M, Ferrara L, Calogero A, Montesano D, Naviglio D (2020) Relationships between food and diseases: What to know to ensure food safety. Food Research International 137:2020. doi: 10.1016/j.foodres.2020.109414.

Jung B, Hoilat GJ (2023) MacConkey Medium Definition. In: Bookshelf, Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557394/. Accessed 24 July 2023

Kabiraz MP, Majumdar PR, Mahmud MMC, Bhowmik S, Ali A (2023) Conventional and advanced detection techniques of foodborne pathogens: A comprehensive review. Heliyon, 9: e15482.doi: 10.1016/j.heliyon.2023.e15482.

Lee A (2021) Introduction to Enumeration of Bacteria. In: MB352 General Microbiology. LibreTexts, North Carolina State University, pp 1–164. https://bio.libretexts.org/Courses/North_Carolina_State_University/MB352_General_Microbiology_Laboratory_2021_(Lee)/05%3A_Enumeration_of_Bacteria/5.01%3A_Introduction_to_Enumeration_of_Bacteria. Accessed 03 February 2025

Leong SS, Lihan S, Ling TY, Chia HC (2021) Logistic Regression Model for Predicting microbial growth and antibiotic Resistance Occurrence in Swiftlet (Aerodramus Fuciphagus) FaeceS. J. Sustain. Sci. Manag. 16:113–123. doi: 10.46754/jssm.2021.06.010.

National Population Commission [Nigeria], NPC and (ICF) International Classification of Functioning Disability and Health Nigeria Demographic and Health Survey 2018. NPC and ICF, Abuja, Nigeria, and Rockville, Maryland, USA, 2019. [Online]. https://dhsprogram.com/publications/publication-fr359-dhs-final-reports.cfm.

Neyaz LA, Alghamdi HS, Alghashmari RM et al. (2024) A comprehensive review on the current status of culture media for routine standardized isolation of Salmonella and Shigella spp. from contaminated food. J. Umm Al-Qura Univ. Appl. Sci. doi: 10.1007/s43994-024-00205-2.

Obadina, A. O., Oyewole, O. B., & Odunfa, S. A. (2013). Microbial safety of alternative complementary foods in Nigeria. African Journal of Biotechnology, 12(34), 5341-5347.

Olatoye, K. K., & Arueya, E. B. (2018). Toxicological evaluation of a developed cereal-legume based complementary food. Journal of Food and Nutrition Research, 6(5), 312-318. https://doi.org/10.12691/jfnr-6-5-5

Omemu, A. M., & Oyewole, O. B. (2016). Food safety relevant to infant feeding: A case study of Nigeria. International Journal of Environmental Health Research, 26(1), 67-81

Paniel N, Noguer T (2019) Detection of Salmonella in Food Matrices, from Conventional Methods to Recent Aptamer-Sensing Technologies Nathalie. foods 371:1–36. doi: doi:10.3390/foods8090371.

Prache, S., Adamiec, C., Astruc, T., Prache, S., Adamiec, C., Astruc T, Baéza-Campone, E., Bouillot, P. E., Clinquart, A., Feidt, C., Fourat, E., Gautron, J., Girard, A., Guillier, L., Kesse-Guyot, E., Lebret, B., Lefèvre, F., Le Perchec, S., Martin, B., Mirade, P. S., Pierre, F., Raulet, M., Desmond, D., Sans, P, Souchon, I., Donnars, S., (2022). Review: Quality of animal-source foods. Animal 16:100376. doi: 10.1016/j.animal.2021.100376.

Rapidmicrobilogy, (2023): Salmonella Detection and Identification Methods for Food Processors. internet. https://www.rapidmicrobiology.com/test-method/salmonella-detection-and-identification-methods.

Reynolds, J. (2021): 11: Bacterial Numbers. In: LibreTexts, pp. 1–7, 2021, [Online]. https://hyp.is/go?url=https%3A%2F%2Fbio.libretexts.org%2FBookshelves%2FAncillary_Materials%2FLaboratory_Experiments%2FMicrobiology_Labs%2FMicrobiology_Labs_I%2F11%253A_Bacterial_Numbers&group=__world__.

Rodríguez-Saavedra, M., Pérez-Revelo, K., Valero, A., Moreno-Arribas, M.V. and González de Llano, D. (2021): A Binary Logistic Regression Model as a Tool to Predict Craft Beer Susceptibility to Microbial Spoilage. Foods, 10:1926. doi: 10.3390/foods10081926.

Salvatore, I. (2021): Food Microbial Quality and Safety. Arch. Clin. Microbiol., vol. 12:171. https://www.acmicrob.com/

Stanton, N. (2024): What is Food Disruption and Why is it Important? The Safety Expert. https://www.thesafetyexpert.co.uk/what-is-food-safety/.

Tamangwa, M.W., Djikeng, F.T., Feumba, R.D., Sylvia, V.Z.N., Loungaing, V.D., Womeni, H.M. (2023): Nutritional composition, phytochemical, and functional properties of six soybean varieties cultivated in Cameroon. Legum. Sci. 5:1–14. doi: 10.1002/leg3.210.

Tumuluru, J.S. (2023): Food Processing, Preservation, and Packaging – A Brief Overview. In: Food Processing and Packaging Technologies- Recent Advances, IntechOpen, 2023, pp. 1–12.

Verocai, G.G., Chaudhry, U.N. and Lejeune, M. (2020): Diagnostic Methods for Detecting Internal Parasites of Livestock Vet. Clin. North Am. - Food Anim. Pract. 36:125–143. doi: 10.1016/j.cvfa.2019.12.003.

World Health Organization, WHO (2024): Food safety. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/food-safety.

Zadernowska, A. and Chajecka, W. (2012): Detection of Salmonella spp. Presence in Food. In: B. Mahmoud (ed.). Salmonella – a dangerous foodborne pathogen. InTech, 2014:393–412. https://doi.org/10.5772/1308.

Zaman, R.F., Khanum, H., Nargis, S. and Das, P.K. (2018): Comparison of saline, iodine and koh wet mount preparations for occurrence of parasites in stool samples from patients attending ICDDR,B, Bangladesh J. Zool, 45:159–170, 2018, doi: 10.3329/bjz.v45i2.35711.

CHAPTER 3
SYNERGISTIC SPECTROCHEMISTRY: INTEGRATING ADVANCED ANALYTICAL CHEMISTRY AND ECOTOXICOLOGICAL INSIGHTS FOR PREDICTIVE ENVIRONMENTAL RISK ASSESSMENT

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INTRODUCTION

The high rate of chemical contamination of the environment has necessitated the emergence of more advanced methods of monitoring, interpretation, and prediction of risks. The classical methods of analysis, which are mostly based on concentration-related measurements, have given invaluable information on the existence and distribution of pollutants. Nevertheless, these methods are usually not sufficient to understand how chemical structure, transformation pathways, and environmental interactions are converted into biological effects (Escher et al., 2020; Schwarzenbach et al., 2010). To overcome this shortcoming, synergistic spectrochemistry is a novel interdisciplinary paradigm that is becoming a potent framework to combine the best spectroscopic analysis with ecotoxicological interpretation to provide predictive environmental risk assessment.

Simply put, synergistic spectrochemistry is a methodology that combines the methodological advantages of analytical chemistry with the mechanistic advantages of ecotoxicology. Spectroscopic methods like UV-Vis, FTIR, Raman spectroscopy, and mass spectrometry are used to give detailed information at the molecular level, including chemical speciation, functional group composition, and structural changes (Skoog et al., 2018; Hollas, 2004). However, these spectral signatures are not linked to their ecological implications, unless they are interpreted contextually in biological terms. Synergistic spectrochemistry is a method by which the results of an analytical process can be converted into ecotoxicological endpoints (e.g., bioaccumulation, oxidative stress responses, and trophic transfer) by relating spectroscopic data with ecotoxicological responses (Ankley et al., 2010; Newman, 2014).

This is especially important in the case of emerging contaminants, a heterogeneous group of substances that encompasses pharmaceuticals, personal care products, endocrine-disrupting compounds, per- and polyfluoroalkyl substances (PFAS), and microplastics (Richardson and Kimura, 2020; Gago et al., 2020). These pollutants are usually found in trace amounts, have complicated transformation characteristics, and have a dynamic interaction with the environmental matrices, including soil, water, and biota, unlike the conventional pollutants.

As an example, pharmaceutical residues such as chloroquine and other bioactive compounds are becoming more and more common in aquatic systems and can cause sub-lethal ecologically significant impacts (aus der Beek et al., 2016; Wilkinson et al., 2022). Their resistance and conversion to metabolites also make them even harder to detect and determine their toxicity, which underscores the ineffectiveness of traditional monitoring systems that only use bulk concentration measurements.

In addition, the environmental systems are heterogeneous in nature, and the behavior of the contaminants is highly dependent on physicochemical variables like pH, organic matter content, mineral composition, and redox conditions. These influence chemical speciation, adsorption-desorption equilibrium, and degradation pathways, which eventually dictate the bioavailability and toxicity of pollutants (Sparks, 2003; Alloway, 2013). The spectroscopic methods are placed in a unique position to unravel these complexities by offering in situ and molecular-level information about the interactions of contaminants (Brown et al., 2017). To translate these insights into ecological risk, however, a complementary knowledge of the effect of molecular features on biological responses is needed, traditionally the domain of ecotoxicology.

The intersection of the two fields is also motivated by the increasing need for predictive environmental risk systems. The regulatory and environmental management strategies are becoming more and more proactive rather than reactive, in which the main aim is not only to identify the contaminants but also to predict them before they occur at the ecosystem level (European Commission, 2020; OECD, 2019). Quantitative structure-activity relationships (QSAR), machine learning models, and geochemical modeling are predictive tools that are extremely dependent on high-quality, mechanistically informed data (Gramatica, 2020; Chen et al., 2021). Synergistic spectrochemistry offers this much-needed connection between spectral signatures and chemical speciation and toxicity pathways and ecological outcomes.

Notably, this integrative methodology is consistent with the concepts of green and sustainable science, which is focused on efficiency, the limited environmental impact, and the application of sophisticated but accessible analytical instruments (Anastas and Warner, 1998; Płotka-Wasylka et al., 2018).

Synergistic spectrochemistry can be used to facilitate the development of specific remediation strategies, better regulatory policies, and sustainable environmental management practices by making more accurate predictions of contaminant behavior and impact.

1. BASICS OF SPECTROCHEMISTRY

Spectrochemistry offers a unifying paradigm to the interaction of electromagnetic radiation on matter to produce measurable signals that encode chemical identity, structure, and concentration. Spectrochemical principles are essential in the detection, characterization, and quantification of pollutants in environmental systems where the contaminants are usually in trace levels and in complicated matrices. The basic mechanisms of light-matter interaction, which include the phenomena of absorption, emission, and scattering, are at the center of this field, and they form the basis of contemporary spectroscopic methods applied in environmental monitoring (Skoog et al., 2018; Hollas, 2004).

1.1 LightMatter Interaction: Theoretical Principles

The electromagnetic radiation when interacting with atoms or molecules can transfer energy provided the photon energy corresponds to the difference between quantized energy levels. This type of interaction is determined by quantum mechanics and produces specific spectral characteristics of particular chemical species (Atkins and de Paula, 2014).

Different transitions can be made depending on the energy of the incident radiation:

- Electronic transitions (UV-Vis region): The valence electrons are excited between molecular orbitals.
- Vibrational changes (infrared region): bond stretching and bond bending motions.
- Rotational transitions (microwave region): rotational transitions of the molecules.
- Atomic excitation and ionization (high-energy sources): used in elemental analysis.

It is these processes that underlie spectroscopic detection, with a given chemical species having a distinct spectral signature that can be utilized to identify and quantify that chemical species.

1.2 Absorption Spectroscopy and Quantitative Analysis

One of the most popular methods of environmental analysis is absorption spectroscopy. It is founded on the attenuation of radiation when it passes through a sample, and the amount of absorption is directly proportional to the concentration of the absorbing species. The Beer-Lambert law characterizes this relationship:

$$[A=\varepsilon bc] \qquad (1)$$

A is the absorbance, ε is the molar absorptivity, b is the optical path length, and c is the concentration of the analyte. This straight-line relationship forms the basis of quantitative determination of environmental matrix contaminants in water and soil extracts (Skoog et al., 2018).

1.2.1 UV-Visible Spectroscopy (UV-Vis)

UV–Visible spectroscopy takes advantage of electronic transitions in molecules, especially $\pi\rightarrow\pi^{+}$ transitions and $n^{0}\rightarrow\pi^{+}$ transitions of chromophoric groups. Organic contaminants with conjugated systems, i.e., dyes, aromatic hydrocarbons, and some pharmaceuticals, have characteristic absorption bands in the UV-Vis region (Silverstein et al., 2014).

UV-Vis spectroscopy is commonly used in environmental applications to:

- Observation of nutrient species, e.g., nitrate and nitrite.
- Detection of colored industrial effluents and dyes.
- Measurement of pharmaceutical residues in water.

Analytically, UV-Vis has the benefits of being simple, quick, and economical to use. The parameters that are important to guarantee the reliability of data are calibration curves, detection limits, and instrumental sensitivity.

Regarding organic chemistry, the interpretation of the absorption spectrum is based on the knowledge of chromophores, auxochromes, and the degree of conjugation, which determine the wavelength and the intensity of absorption (Pavia et al., 2015).

1.3 Vibrational Spectroscopy: Molecular Fingerprinting

Vibrational spectroscopy is a technique that gives detailed information of the structure of the molecules by investigating bond vibrations. The methods are especially useful in determining functional groups and describing complex organic mixtures in environmental samples.

1.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is the absorption of infrared radiation of vibrational transitions in molecules. The functional groups absorb at characteristic frequencies, and this creates a fingerprint region, which is very specific to the molecular structure (Smith, 2011).

FTIR is applied in environmental analysis:

- Determination of organic pollutants and natural organic matter.
- Adsorption processes on the surface of soil and sediment characterized.
- Identification and sorting of microplastics using polymer signatures.

Instrumental parameters that analytical chemists focus on to identify accurately are spectral resolution, baseline correction, and library matching. Organic chemists, by contrast, are interested in the interpretation of functional group vibrations, including OH, C=O, and C-O, to infer the composition of a molecule and its chemical interactions.

1.4 Spectroscopy and Structural Inferences

Scattering methods give complementary information to absorption methods, especially in systems with weak direct absorption or masked by matrix effects.

1.4.1 Raman Spectroscopy

Raman spectroscopy is founded on inelastic scattering of light, in which incident photons are interacting with the vibrations of the molecules, causing a change in energy. Raman spectroscopy is also not as sensitive to water as FTIR, and thus it is particularly applicable to aqueous environmental samples (Ferraro et al., 2003).

Environmental science applications in environmental science include

- Detection of microplastics in the sea and freshwater.
- Inorganic and organic pollutants characterization.
- On-site chemical transformation monitoring.

Analytically, surface-enhanced Raman spectroscopy (SERS) is an important sensitivity enhancement method essential in the detection of low-concentration analytes. From an organic chemistry perspective, Raman spectra can be used to give information on the molecular symmetry, the bonding environment, and structural changes.

1.5 Elemental Analysis by Atomic Spectroscopy

Whereas molecular spectroscopy deals with compounds, atomic spectroscopy is necessary to identify and determine the elements' contaminants, especially the heavy metals.

Atomic Absorption Spectroscopy (AAS) is used to determine the atomic element composition of a sample.

1.5.1 Atomic Absorption Spectroscopy (AAS)

AAS is a technique used to determine the absorption of element-specific radiation by free atoms in the gaseous state, usually produced by flame or atomizing the graphite furnace. The method is very selective and is commonly employed in the determination of trace metals in environmental samples (Welz and Sperling, 1999).

Applications in the environment include:

- Follow-up of toxic metals like Pb, Cd, Zn, and Cu.
- Evaluation of the level of soil and water pollution.

Analytical chemists are interested in calibration methods, detection limits, and the correction of matrix interference.

Organic chemists help by elucidating the metal-ligand interactions and speciation effects that affect the behavior of metals.

Inductively Coupled Plasma Techniques (ICP-OES and ICP-MS) are used to analyze the samples.

Inductively coupled plasma methods make use of a high-temperature source of plasma to energize atoms and ions. ICP-OES measures emitted radiation, and ICP-MS quantifies ions by their mass-to-charge ratio, which has extreme sensitivity and multi-element capabilities (Hou and Jones, 2000).

The following techniques play a central role in:

Multi-elemental analysis on environmental samples.

- Trace and ultra-trace metal contaminant detection.
- Separations in conjunction with speciation.

Instrument optimization, quality control, and management of matrix effects are emphasized by analytical chemists, whereas coordination chemistry and chemical forms of metals that determine their environmental fate are emphasized by organic chemists.

1.6 Sensitivity and Enhancement of Emission Processes

Emission spectroscopy is a complementary technique to absorption methods used to measure the emitted radiation of excited species. Since the intensity of the emission is directly proportional to the number of excited atoms or molecules, it is sensitive, especially at the trace level. The methods like ICP-OES take advantage of this principle to conduct multi-element analysis simultaneously and are therefore essential in environmental monitoring (Boss & Fredeen, 2004).

1.7 Spectrochemical Principles in the Detection of Environmental Contaminants.

The combination of absorption, emission, and scattering processes allows a complete analysis of the environment by:

- Qualitative identification: Spectral fingerprints of FTIR and Raman offer molecular-level identification of contaminants.

Quantitative determination: UV-Vis, AAS, and ICP methods can be used to determine the concentration accurately.

•Speciation analysis: The separation of chemical forms that affect toxicity and mobility.

Mechanistic understanding: Knowledge of interactions, including adsorption, complexation, and degradation.

These properties are especially important in the case of the emerging contaminants, which tend to have complicated behavior depending on the environmental factors, including pH, redox potential, and the presence of organic matter (Richardson and Kimura, 2020).

1.8 Integrative Perspective

One of the strengths of spectrochemistry is that it is interdisciplinary. These guarantee accuracy, sensitivity, and methodological strength and molecular-level elucidation by comprehending functional groups, chromophores, and pathways of reaction. Such synergy improves the capacity to connect spectral data to environmental processes and eventually predict the behavior of contaminants and their ecological effects more effectively. The principles of spectrochemical analysis are the basis of contemporary environmental analysis and convert the interaction of light and matter into chemical action. Using methods like UV-Visible, FTIR, Raman spectroscopy, AAS, and ICP, one can detect, quantify, and comprehend the contaminants at a level never before seen. Spectrochemistry can be used to not only facilitate environmental monitoring by combining quantitative measurement with structural interpretation but also to offer the mechanistic insight that is required to make predictions on the risks that an environment may face and to manage the environment sustainably.

2. HIGH-ORDER METHODS OF ANALYTICAL MONITORING IN THE ENVIRONMENT.

The development of modern environmental monitoring has not only seen the progression of single-parameter measurements but also very advanced, multi-dimensional analytical approaches that are able to identify trace contaminants in very complex matrices like wastewater, sediments, biota, and atmospheric particulates.

The necessity to detect emerging contaminants, such as pharmaceuticals, endocrine disruptors, per- and polyfluoroalkyl substances (PFAS), and microplastics, at ultra-trace levels is also the reason behind these advances, as well as the need to understand their transformation products and ecological effects (Richardson and Kimura, 2020; Escher et al., 2020).

Although much has been achieved, none of the analytical techniques can completely address the chemical complexity of environmental systems. The combination of complementary methods is required due to the matrix interferences, low concentrations of analytes, and the occurrence of unknown compounds. In this regard, high-order analytical methods, especially hyphenated systems; high-resolution devices; and new sensor technologies are of primary importance in enhancing detection, identification, and predictive evaluation of contaminants (Hollender et al., 2017).

2.1 Hyphenated Techniques

Hyphenated methods use a combination of separation techniques (e.g., gas or liquid chromatography) and strong detection methods (e.g., mass spectrometry) to allow the separation of complex mixtures and the identification of the molecules in a single analysis.

The gas chromatography-mass spectrometry (GCMS) was used to analyze the samples.

GC-MS is a proven method of analyzing volatile and semi-volatile organic substances. Gas chromatography separates the analytes according to their volatility and polarity, whereas mass spectrometry identifies the structure according to the fragmentation pattern.

Applications:

Detection of pesticides, hydrocarbons, and persistent organic pollutants (POPs).

- Volatile organic compounds (VOCs) analysis in air and water.

Strengths:

High sensitivity and reproducibility.

- Strong spectral databases of compound recognition.

Excellent separation efficiency.

Limitations:

- Only thermally stable and volatile compounds.
- Needs to be derivatized in case of polar or non-volatile analytes.

Matrix effects could interfere with quantification.

In the view of analytical chemistry, GC-MS is superior in method validation, reproducibility, and spectral matching. Organically, the analysis of fragmentation patterns can give a lot of information about the molecular structure and the composition of functional groups (Niessen, 2017).

2.1.2 The Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

LC–MS/MS has proven invaluable in the analysis of polar, thermally labile, and high-molecular-weight compounds that cannot be analyzed using GC–MS.

Applications:

Pharmaceuticals and personal care products.

Antibiotics and endocrine-disrupting compounds.

Metabolites and polar pesticides.

Strengths:

- Very high selectivity and sensitivity (ng/L detection limits)
- Multi-residue analysis capability.
- Little sample preparation as compared to GC-based methods.

Limitations:

Effects of matrix suppression/enhancement.

- Optimization and development of complex methods.
- Higher operational costs

The tandem MS setup (MS/MS) increases selectivity by using several steps of fragmentation, which allows precise quantification of the analyte even in complicated environmental samples (Petrović et al., 2005).

2.2 High-Resolution Techniques

Environmental chemistry has been revolutionized by high-resolution methods of analysis that allow screening of non-targets, identification of unknowns, and characterization of contaminants at the molecular level.

2.2.1 High-resolution mass spectrometry (HRMS)

HRMS tools, including time-of-flight (TOF) and Orbitrap devices, offer the correct mass determination with a high resolving power, enabling the recognition of unknown compounds and transformation products.

Applications:

- Suspect and non-target screening of emerging contaminants.
- Determination of the transformation products.
- Environmental forensics

Strengths:

- Large mass accuracy and resolution.
- Ability to identify unidentified compounds.
- Retrospective data analysis.

Limitations:

- Massive and intricate data that needs sophisticated data processing.
  High cost of instruments and technical skills.
- Poor accessibility of spectral databases of unknowns.

HRMS is especially useful in revealing the hitherto unknown pollutants in the environmental systems, which increases the environmental monitoring range beyond the specific analysis (Hollender et al., 2017).

2.2.2 Synchrotron-Based Spectroscopy

Synchrotron radiation is an ultra-bright and tunable X-ray beam that is utilized in high-technology spectroscopic methods like X-ray absorption spectroscopy (XAS) and X-ray fluorescence (XRF).

Applications:

oven metal speciation in soils and sediments.

Mechanism of contaminant binding studied.

Nanoparticles are analyzed in terms of their behavior.

Strengths:

- Element-specific and oxidation-state-sensitive analysis.
- On-site and non-destructive measurements.
- High spatial resolution

Limitations:

- Poor access to the synchrotron facilities.
- Multidimensional data interpretation.
- High operational costs

They are especially effective in the study of chemical speciation, which regulates the mobility and toxicity of the contaminants in the environmental systems (Brown et al., 2017).

2.3 Emerging Technologies

The emerging analytical technologies are responding to the demand of real-time, on-site, and cost-effective environmental monitoring to supplement the laboratory-based methods.

2.3.1 Nanosensors

Nanosensors are based on nanomaterials (e.g., carbon nanotubes, graphene, and metal nanoparticles) to detect contaminants in a highly sensitive and selective fashion.

Applications:

- Heavy metals (e.g., Pb^{2+}, Hg^{2+}) detection.
- Pollution of organic substances and pesticides.
- Biosensing of toxins

Strengths:

- Ultra-low detection limits
- Rapid response times
- Minimization potential.

Limitations:

Problems of stability and reproducibility.

Complex matrix interference

Interference by complex matrices

Scalability and standardization problems.

Nanosensors are an interface between nanotechnology and analytical chemistry, which promises to have decentralized environmental monitoring (Rodea-Palomares et al., 2012).

2.3.2 Portable Analytical Devices.

The instruments that can be deployed in the field and analyzed onsite allow in situ analysis, eliminating the necessity of transporting samples and preserving the integrity of samples.

Examples:

Portable X-ray fluorescence (pXRF) is a technique that uses a compact and handheld device to perform X-ray fluorescence measurements at the site of an archaeological find (Brown et al., 2006). Portable X-ray fluorescence (pXRF) is a method in which a small and handheld instrument measures X-ray fluorescence at the location of an archaeological discovery (Brown et al., 2006).

- Portable Raman and FTIR spectrometers.
- Electrochemical sensors that are field deployable.

Applications:

On-site assessment of soil and water quality.

- Quick identification of contaminants.

Environmental emergency response.

Strengths:

- Real-time data acquisition
- Less cost and time of analysis.
- User-friendly operation

Limitations:

- Less sensitive than laboratory instruments.
- Calibration challenges
- Limited analytical scope

The technologies are especially useful in resource-constrained environments and quick decision-making in environmental management (Płotka-Wasylka et al., 2018).

2.4 Integrative Evaluation

The effectiveness of advanced analytical techniques in environmental monitoring depends on their ability to address fundamental challenges:

Table 1. Comparison of Analytical Performance Criteria Among Hyphenated, High-Resolution, and Emerging Spectrochemical Techniques

Criterion	Hyphenated Techniques	High-Resolution Techniques	Emerging Technologies
Sensitivity	High	Very high	Moderate–high
Selectivity	High	Very high	Variable
Field applicability	Low	Low	High
Data complexity	Moderate	Very high	Low–moderate

There is no single method that is adequate, and instead, combined methods of analysis are needed to

- Solve complicated environmental matrices.
- Determine the unknown contaminants.
- Give qualitative and quantitative data.

Modern analytical methods have transformed the process of environmental monitoring by allowing the identification of contaminants and their description at a level of detail never before seen. Hyphenated methods like GC-MS and LC-MS/MS offer powerful targeted analysis, whereas non-target screening and mechanistic understanding are possible with high-resolution methods like HRMS and synchrotron spectroscopy. These capabilities are extended to real-time and field-based applications with emerging technologies, such as nanosensors and portable devices. These approaches need to be integrated to deal with the complexity of modern environmental systems and are a fundamental basis of predictive assessment of environmental risks.

3. ECOTOXICOLOGICAL INSIGHTS

Ecotoxicology is the science of the impact of chemicals on biological systems in the ecological context, relating environmental chemistry to the biological outcome.

It combines the concepts of toxicology, ecology, and environmental chemistry to comprehend the effects of the pollutants on organisms at various trophic levels, including microorganisms and top predators. The field is critical in forecasting the ecological effects of contaminant exposure as well as in guiding regulatory systems that would safeguard the health of the ecosystem (Walker et al., 2012; Sparling et al., 2017).

3.1 Principles of Ecotoxicology

Ecotoxicology is the study of the interplay between chemical substances and biological organisms, both in terms of the exposure routes and the response of the organism. The key components include:

Exposure Assessment: The determination of the level of chemical exposure, frequency, and duration of the chemical exposure to the organisms.

Effects Assessment: It is an evaluation of the biological impact of exposure, which can be acute or chronic, lethal or sub-lethal.

Risk Characterization: This involves combining exposure and effects data in order to forecast ecological effects (Meyer et al., 2019).

Ecotoxicology, by correlating environmental concentrations with biological effects, offers a model of predictive environmental risk assessment, enabling sensitive species to be identified and important exposure limits to be determined.

Dose-response relationships: The relationship between dose and response is characterized by a linear correlation coefficient of r=0.933, indicating a positive correlation (p=0.000). <Dose-Response Relationships: The correlation between dose and response is positive with a linear coefficient of r = 0.933 (p = 0.000).

One of the foundations of ecotoxicology is the dose response relationship that explains the relationship between the extent of exposure and the biological effect observed. These relationships can be:

- Acute toxicity: Immediate effects on short-term exposure, including death.
- Chronic toxicity: Prolonged exposure that results in reproductive, developmental, or behavioral alterations.

These relationships are measured using mathematical models like LC50 (lethal concentration of 50% of organisms) or EC50 (effective concentration of 50% of a measured response), which allow making cross-species comparisons (Gibbs et al., 2018). Regulatory guidelines and environmental quality standards are based on dose-response data.

3.2 Bioaccumulation

Bioaccumulation is the slow buildup of chemicals in a living organism over a period of time, which is usually caused when the uptake is higher than the elimination. Chemical properties (e.g., lipophilicity, persistence), physiology of the organism, and the environment affect this phenomenon. The basic measures are the bioaccumulation factor (BAF), which is used to indicate the ratio of concentration of an organism to the surrounding environment (Di Toro et al., 2018).

Environmental monitoring implications:

Persistent organic pollutants (POPs), heavy metals, and hydrophobic organic compounds are likely to bioaccumulate.

Bioaccumulation gives early notices of chemical stress in an ecosystem before the impacts are felt at the population level.

3.3 Biomagnification

Biomagnification refers to the increment of chemical concentration at the successive trophic levels, usually in food chains. In contrast to bioaccumulation, where the process takes place in an organism, biomagnification is a manifestation of ecological amplification, which results in high levels in top predators.

Environmental relevance:

Mercury, PCBs, and some organochlorine pesticides have high biomagnification.

Large carnivores and fish-eating birds are especially vulnerable to predators (Ratcliffe, 2017).

- Knowledge of biomagnification aids ecological risk assessment and species at highest ecological and human health risk.

The integration at trophic levels is also involved in the fourth part.

Ecotoxicology focuses on the fact that impacts at a single trophic level are transferred through the ecosystem. Sub-lethal impacts on primary producers or invertebrates may influence the energy flow and population dynamics, which eventually have an impact on ecosystem services. Ecotoxicological research is moving towards the use of mesocosm experiments, field experiments, and modeling to predict the ecologically significant effects of chemical exposure (Walker et al., 2012).

Ecotoxicology offers the conceptual and quantitative framework required to convert the chemical exposures into ecological effects. With the knowledge of dose-response relationships, bioaccumulation, and biomagnification, scientists and regulators are able to anticipate the environmental risks and protect the environment by prioritizing chemicals and ecosystems. The combination of the principles of ecotoxicology and the sophisticated methods of analysis is the core of the predictive environmental risk assessment, which allows the proactive control of chemical pollutants in the complex ecosystems.

4. THE INTERFACE BETWEEN SPECTROCHEMISTRY AND ECOTOXICOLOGY

The ecotoxicological outcomes of chemical properties identified by spectrochemical analysis can only be understood in a holistic framework that connects the ecotoxicological fate and biological effects of contaminants. Spectrochemistry offers information on the structure of molecules, functional groups, and chemical speciation, which are important determinants of toxicity, bioavailability, and environmental persistence. With such insights combined, researchers will be able to forecast biological impacts more precisely and transition to predictive environmental risk assessment instead of descriptive monitoring (Richardson and Kimura, 2020; Hollender et al., 2017).

4.1 Structure-Toxicity Relations

The interaction of a contaminant with biological targets, such as absorption, distribution, metabolism, and excretion (ADME) in organisms, depends on the molecular structure of the contaminant.

Key structural motifs can be detected with the help of spectroscopic methods, including FTIR, Raman, and UV-Vis:

Aromatic rings and conjugated systems may intercalate DNA or disrupt the membrane integrity.

Polar functional groups (-OH, $-NH_2$, -COOH) affect bioavailability, solubility, and hydrogen bonding.

Electron-withdrawing groups (e.g., -Cl, $-NO_2$) may enhance oxidative potential and reactivity.

The correlation of spectroscopic signatures with observed toxicological endpoints can be used to derive structure-toxicity relationships (STRs) that can be used to inform hazard prediction and regulatory decision-making (Sparling et al., 2017; Di Toro et al., 2018).

4.2 Environmental Behavior and Functional Group Reactivity

The chemical reactivity of pollutants is controlled by functional groups that define the interaction of pollutants with environmental matrices and biological molecules. Spectroscopic analysis gives a direct understanding of the following reactive moieties:

FTIR determines bond vibrations of nucleophilic or electrophilic sites.

Raman spectroscopy is able to provide conjugation patterns and strain on bonds that affect degradation rates.

UV-Vis absorption is a sign of the existence of chromophores that can be involved in photochemical reactions.

Implications of ecotoxicology reactivity:

Hydroxylated and carboxylated compounds tend to be more soluble and, therefore, increase bioavailability and uptake.

Halogenated aromatics are recalcitrant, biodegradable, and bioaccumulative and biomagnifiable (Ratcliffe, 2017).

Obtaining redox-active functional groups affects oxidative stress processes in organisms.

The combination of functional group data and dose response and bioaccumulation studies increases the predictive ability of ecological risk assessment.

4.3 Chemical Speciation and Toxicity

Chemical speciation identifies the environmental form of a contaminant, which has a direct effect on its toxicity, mobility, and bioavailability. The oxidation states, metal complexes, and organometallic forms are also important to comprehend ecological impact and can be differentiated with the help of spectroscopic methods, including ICP-OES, HRMS, and synchrotron-based X-ray absorption spectroscopy (Brown et al., 2017; Hou and Jones, 2000).

- Metal speciation: Hg^{2+}, Cr^{3+}, and As^{3+} species are different in their toxicity and uptake.

Organic contaminants: Ionization states (e.g., acidic or basic functional groups) influence membrane permeability and distribution in aquatic and terrestrial organisms.

- Complexation with natural ligands: Humic acid, sulfides, or phosphates interaction alters bioavailability.

Through the correlation of spectroscopic data of chemical forms with ecotoxicological consequences, scientists can create predictive models, which predict organism-level outcomes using measurements at the molecular level.

4.4 Conceptual Framework

The simplified conceptual model of the relationship between spectrochemistry and ecotoxicology can be illustrated as follows:

Spectroscopic Detection, Structural Characterization, and Functional Group Analysis Chemical Speciation Predictive Ecotoxicological Outcomes.

Spectroscopic Detection: UV-Vis, FTIR, Raman, AAS, and ICP give data about the composition of molecules or elements.

Structural Characterization: Recognition of chemical motifs and chromophores provides reactivity and interaction potential.

Functional Group Analysis: Establishes hydrophilicity/hydrophobicity, redox activity, and potential of metabolic transformation.

Chemical Speciation: Determines the environmentally relevant form of the contaminant, which influences bioavailability.

Predictive Ecotoxicology: Combines chemical data with dose response, bioaccumulation, and biomagnification measures to predict ecological risk.

This framework highlights the interdisciplinary synergy between analytical chemistry and ecotoxicology, which can be used to monitor specifically, gain mechanistic insight, and make informed environmental management (Hollender et al., 2017; Richardson and Kimura, 2020).

The linkage of spectrochemical analysis and ecotoxicological principles can be used to transform the information on the molecular level into ecological risk forecasts. Knowledge of structure-toxicity relationships, functional group reactivity, and chemical speciation can help the researcher predict the behavior of contaminants, focus on monitoring, and inform mitigation efforts. The synergistic environmental risk assessment is based on this integrative approach, which helps to protect the ecosystem sustainably and make regulatory decisions.

5. PREDICTIVE ENVIRONMENTAL RISK ASSESSMENT

Predictive environmental risk assessment (ERA) offers a methodological framework for assessing the possible negative impact of chemical contaminants on the ecosystems prior to their substantial harm. Conventionally, ERA has used measured environmental concentrations and standardized toxicity data; nevertheless, these methods do not have the mechanistic depth to handle complex and emerging contaminants. Spectrochemical data (e.g., molecular structure, functional groups, and speciation) combined with ecotoxicological information (e.g., dose-response and bioaccumulation) can be used to develop a more robust and predictive framework, enhancing accuracy and ecological relevance (Escher et al., 2020; Richardson and Kimura, 2020).

5.1 Environmental Risk Assessment Frameworks.

Environmental risk assessment is generally done in a tiered approach that has four steps that are interrelated:

5.1.1 Hazard Identification

This is done by determining chemicals of concern and their toxic characteristics.

FTIR, Raman, and high-resolution mass spectrometry (HRMS) are spectrochemical methods that can be used to obtain detailed information about the molecules, including the identification of contaminants and their transformation products, including unknown compounds (Hollender et al., 2017).

5.1.2 Exposure Assessment

The exposure assessment identifies the concentration, frequency, and duration of the chemical in environmental compartments (water, soil, air, and biota). ICP-MS, AAS, and LC-MS/MS are the analytical methods that enable the accurate determination of contaminants at the trace level, whereas speciation analysis can give information about the bioavailable forms (Skoog et al., 2018).

5.1.3 Effects Assessment

Effects assessment is the biological response of organisms to chemical exposure and is usually based on ecotoxicological endpoints (LC50, EC50, sub-lethal biomarkers, etc.). This step can be improved by incorporating spectrochemical data to provide a connection between chemical structure and speciation and observed toxicity, thus improving mechanistic interpretation (Ankley et al., 2010).

5.1.4 Risk Characterization

Risk characterization combines exposure and effects information to approximate the probability and magnitude of negative ecological impacts. The most used methods are the calculation of risk quotients (RQ):

RQ = Predicted Environmental Concentration (PEC)/Predicted No-Effect Concentration (PNEC)... (2)

RQ values above 1 are a sign of possible ecological danger. The inclusion of spectrochemical and ecotoxicological data enhances the accuracy of PEC and PNEC estimates because they consider chemical speciation, transformation, and bioavailability (European Commission, 2020).

5.2 Spectrochemistry-Ecotoxicology Integration.

The combination of spectrochemical and ecotoxicological data improves predictive ERA in a number of ways:

- Better chemical identification: Spectroscopy of structural features and transformation products.
- Improved speciation analysis: Establishes bioavailable and toxic contaminants.

Mechanistic toxicity knowledge: This is the relationship between functional groups and molecular structure and biological effects.
Less uncertainty: Analytic precision and biological relevance.

Such synergy enables better prediction of the behavior of contaminants in complex environmental matrices, especially with regard to new pollutants, which do not have extensive toxicity information (Escher et al., 2020).

5.3 Predictive Risk Assessment Modeling Tools.

Developed modeling methods are important in converting chemical and biological data into predictive knowledge.

5.3.1 Quantitative Structure-Activity Relationships (QSAR)

QSAR models are mathematical correlations between biological activity or toxicity and descriptors of chemical structure. These descriptors could be:

- Molecular weight
- Hydrophobicity (log P)
- Functional group presence
- Electronic properties

QSAR models can be used to predict the toxicity of chemicals with limited experimental data using spectrochemical data, which is useful in screening a large number of contaminants (Gramatica, 2020).

Strengths:

- Minimizes the requirement of biological testing.
- Facilitates quick screening of new compounds.
- Assists in making regulatory decisions.

Limitations:

- Quality and diversity of training data are important to accuracy.
- Poor extrapolation to new chemical classes.
- Fails to measure complicated interactions with the environment.

5.3.2 Machine Learning Methodologies.

Machine learning (ML) methods have become potent predictors of the environment, able to work with large, complex data sets obtained through spectroscopic and ecotoxicological methods.

Common algorithms include:

Artificial neural networks (ANNs) are algorithms that mimic the behavior of neural networks in the human brain. Artificial neural networks (ANNs) are algorithms that achieve the behavior of neural networks in the human brain.

- Random forest models
- Support vector machines (SVM)

Applications:

- Fate and transport of contaminants.
- Toxicity level classification.
- Patterns of non-target screening data.

Strengths:

- High predictive accuracy
- Multi-source integration capability.
- Flexibility to complicated environmental systems.

Limitations:

- Needs huge amounts of data to train.
- Risk of overfitting
- Some models have limited interpretability.

Machine learning can improve ERA by combining various datasets, such as spectral fingerprints, chemical descriptors, and biological reactions, which increases environmental risk prediction (Chen et al., 2021).

5.4 Integrated Predictive Framework

A current predictive ERA model can be modeled as

Spectrochemical Data, Structural and Speciation Analysis, Ecotoxicological Response Modelling (QSAR/ML), and Risk Prediction.

This integrative method allows the following:

- Early detection of the high-risk contaminants.
- Mechanistic knowledge of the toxicity pathways.
- Evidence-based environmental management policies.

Predictive environmental risk assessment is a shift in the paradigm of environmental management, which is reactive to proactive. With the combination of spectrochemical knowledge and ecotoxicological information and the use of the latest modeling techniques, including QSAR and machine learning, one can considerably improve the precision and dependability of risk estimates. This interdisciplinary method is necessary to tackle the issue of the emerging contaminants and to create sustainable solutions to ensure the health of the ecosystems.

6.CASE STUDIES: SYNERGISTIC SPECTROCHEMISTRY IN THE ENVIRONMENT

Interdisciplinary case studies give real-world examples of how synergistic spectrochemistry, the combination of spectroscopic analysis and ecotoxicological interpretation, can be used to better understand the behavior of contaminants and ecological risk. These methods can be used to predict environmental effects in a variety of systems more accurately by integrating molecular-level characterization with biological response data (Escher et al., 2020; Richardson and Kimura, 2020).

6.1 Interactions of Heavy Metal-Soil.

Pb, Cd, Zn, and Cu are examples of heavy metals that are environmental pollutants that are hard to get rid of, and they are found in soils, especially mining and industrial areas. Chemical speciation, soil properties, and binding mechanisms are very strong in controlling their ecological impact.

Spectrochemical Insights:

The following techniques are employed: FTIR, X-ray diffraction (XRD), and inductively coupled plasma methods (ICP-OES/MS).

- Determine functional groups that bind metal (e.g., -COOH, -OH).

Describe mineral phases (e.g., clay minerals, iron oxides)

Measure total and bioavailable metal.

X-ray absorption spectroscopy using a synchrotron can also be used to determine the oxidation state and coordination environment, which is direct evidence of metal speciation (Brown et al., 2017).

Ecotoxicological Interpretation:

- Metal toxicity is based on bioavailable fractions as opposed to total concentrations.

Soil pH, organic matter, and cation exchange capacity (CEC) have an effect on plant and microorganism uptake.

Trophic transfer can occur in soil organisms as a result of bioaccumulation.

Integrated Insight:

Through the connection between spectroscopic identification of metal binding sites and ecotoxicological endpoints (e.g., plant uptake, microbial inhibition), researchers can better predict metal mobility, bioavailability, and toxicity (Alloway, 2013; Sparks, 2003).

6.2 Pharmaceutical Contaminants: Chloroquine Case.

The presence of pharmaceutical residues in water is on the rise because of the lack of complete elimination by wastewater treatment plants. Chloroquine, which is commonly used in malaria-prone areas, can be regarded as a typical example of emerging contaminants with complicated environmental behavior.

Spectrochemical Insights:

UV-Vis spectroscopy allows the determination of the amount of a substance using typical absorption bands.

LC-MS/MS offers high sensitivity in detecting and identifying transformation products.

FTIR and Raman spectroscopy show structural characteristics and decomposition mechanisms.

These methods enable the monitoring of the chloroquine persistence and conversion in different environmental conditions (aus der Beek et al., 2016).

Ecotoxicological Interpretation:

Even when used in low concentrations, chloroquine may cause sub-lethal impacts on aquatic organisms, including oxidative stress.

Bioaccumulation potential: It is based on physicochemical characteristics like polarity and ionization state.

- Transformation products can have varying toxicity.

Integrated Insight:

Combining spectrochemical data with ecotoxicological endpoints makes it possible to:

- Determination of toxic metabolites.
- Knowledge of degradation mechanisms (e.g., photolysis, biodegradation)
- Better forecasting ecological risks in water (Wilkinson et al., 2022)

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6.3 Microplastics and Organic Pollutants

Microplastics are a challenging group of new pollutants that are not only pollutants but also carriers of other toxic elements, such as persistent organic pollutants (POPs) and heavy metals.

Spectrochemical Insights:

FTIR and Raman spectroscopy are the major instruments used in identifying polymers.

- Spectral fingerprinting can be used to classify plastic (e.g., polyethylene and polypropylene).

Surface characterization methods indicate that organic pollutants are adsorbed on microplastics.

There are sophisticated methods, including pyrolysis-GC-MS, which can give a detailed chemical composition and additive profiling (Gago et al., 2020).

Ecotoxicological Interpretation:

Aquatic organisms may ingest microplastics, which leads to physical and chemical stress.

Pollutants adsorbed can be desorbed in organisms and become toxic. Bioaccumulation and biomagnification may take place via food webs.

Integrated Insight:

Through the integrated approach of spectroscopic identification of polymer types and related contaminants and ecotoxicological data, researchers can:

- Determine the importance of microplastics as toxic vectors.
- Assess physical and chemical stressor interactions.
- Anticipate long-term ecological effects at trophic levels (Rochman et al., 2013).

6.4 Synthesis of Case Studies

Across these case studies, a consistent pattern emerges:

System Spectrochemical Contribution Ecotoxicological Outcome

Table 2. Spectrochemical Contributions to Ecotoxicological Assessment Across Environmental Systems

System	**Spectrochemical Contribution**	**Ecotoxicological Outcome**
Heavy metal–soil	Speciation, binding mechanisms	Bioavailability and plant uptake
Pharmaceuticals	Molecular structure, degradation	Sub-lethal toxicity and persistence
Microplastics	Polymer identification, adsorption	Trophic transfer and combined toxicity

These illustrations point to the fact that synergistic spectrochemistry fills the gap between chemical detection and biological effect, allowing more thorough environmental measurements.

Synergistic spectrochemistry has been shown to be useful in solving complex contamination problems in the real-world environment. With the combination of the cutting-edge analytical methods and ecotoxicological knowledge, one can go beyond mere detection and proceed to mechanistic knowledge and predictive risk evaluation.

These case studies highlight the relevance of an interdisciplinary approach in addressing emerging environmental issues and informing sustainable environmental management strategies.

7. CHALLENGES AND LIMITATIONS

Although much has been done to combine the spectrochemical methods with the ecotoxicological understanding, there are still a number of limitations that limit the full accomplishment of predictive environmental risk assessment. These issues cut across analytical limitations, data uncertainties, and modeling uncertainties, which are inherent to environmental systems.

7.1 Analytical Constraints

The modern spectrochemical methods like LCMS-MSMS, HRMS, and ICP-MS have outstanding sensitivity and selectivity, but these are usually limited by the complexity of the matrix. Environmental samples, especially soils, sediments, and wastewater, are characterized by a wide variety of organic and inorganic components that may lead to signal suppression, spectral overlap, and interferences (Hollender et al., 2017). Also, although high-resolution methods allow the identification of unknown compounds, their structural elucidation is difficult, particularly in the case of transformation products that have no reference standards. Other spectroscopic methods like FTIR and Raman can also have poor sensitivity in cases where the analyte is on ultra-trace levels.

The other limitation is also the lack of interrelationship between total concentration and bioavailable fraction, which makes the interpretation of spectrochemical data more challenging in ecotoxicological matters (Escher et al., 2020).

7.2 Data Gaps and Ecotoxicological Uncertainty

One of the greatest obstacles to integration is that there is no complete toxicity information on the emerging contaminants and their metabolites. Although thousands of compounds can be identified by non-target screening using analytical methods, ecotoxicological data is not always available on most of them (Richardson and Kimura, 2020).

Moreover, conventional toxicity tests are often based on single-species laboratory tests that are not representative of ecosystem-level interactions, including trophic transfer, synergistic interactions, and long-term ecological effects (Walker et al., 2012).

The major limitations of the data are:

- Lack of chronic toxicity information.
- Lack of knowledge on mixture toxicity.
- Absence of standardized biomarkers between molecular exposure and ecological effects.

7.3 Modeling Uncertainties

QSAR and machine learning predictive models are highly sensitive to the quality and representativeness of the input data. Nevertheless, the environmental data is usually heterogeneous, incomplete, and biassed, which creates uncertainties in the model predictions (Gramatica, 2020; Chen et al., 2021).

QSAR models can be inaccurate when used on new chemical classes that fall outside their training range, whereas machine learning models can be prone to overfitting and low interpretability. Also, the majority of models fail to include environmental factors, including pH, temperature, and microbial activity, which determine the behavior of contaminants.

7.4 Integration Challenges

The reconciliation of chemical, biological, and computational data sets is necessary to bridge the gap between spectrochemistry and ecotoxicology, and these data may vary in scale, format, and resolution. Interdisciplinary research is also complicated by the absence of standardized procedures to integrate and interpret the data.

Therefore, although synergistic spectrochemistry has a promising framework, its application remains limited to technical, methodological, and conceptual constraints.

8. FUTURE PERSPECTIVES

The further development of tIe combination of spectrochemistry and ecotoxicology will involve the creation of analytical technology, data science, and sustainable practices. New trends are more holistic, real-time, and environmentally conscious methods of monitoring and risk assessment.

8.1 Analytical Chemistry and Artificial Intelligence

Analytical chemistry is being changed by artificial intelligence (AI) and machine learning in ways that allow the following:

Automated spectral interpretation.

- Large-scale screening of non-targets.

Prediction of toxicity and environmental fate.

The models based on AI can combine spectroscopic fingerprints and ecotoxicological endpoints to enhance predictive power and decrease the use of large-scale experimental tests (Chen et al., 2021).

Possible future developments will involve:

- Spectral deconvolution using deep learning.
- Unknown contaminant identification with the help of AI.
- Multi-omics ecotoxicology prediction.

8.2 Real-Time and In Situ Monitoring

The creation of real-time analytical systems is one of the significant breakthroughs in the field of environmental monitoring. Nanosensors, portable spectroscopic devices, and remote sensing technologies can be used to continuously analyze contaminants on-site.

Advantages:

- Real-time monitoring of pollution incidences.
- Less degradation and transportation errors of the samples.
- Improved time scaling of environmental data.

The technologies are especially useful in the observation of dynamic systems, including rivers, wastewater treatment facilities, and industrial discharge points (Płotka-Wasylka et al., 2018).

8.3 Green Analytical Methods

Analytical chemistry is now starting to focus on sustainability, and green analytical methods are being developed to reduce environmental impact. Key strategies include the following:

- Cutting of solvent and reagent consumption.
- Reduction of the size of analytical systems.
- Biodegradable and non-toxic materials.
- Energy-efficient instrumentation

Green analytical chemistry is consistent with the sustainability agenda worldwide and makes sure that the environmental monitoring in itself does not pollute the environment (Anastas and Warner, 1998; Płotka-Wasylka et al., 2018).

8.4 Interdisciplinary Integration

The future research will focus more on cross-disciplinary collaboration and integrate the following:

- Molecular-level analysis by spectrochemistry.
- Biological interpretation of ecotoxicology.
- Predictive modeling data science.

This kind of integration will facilitate the creation of multi-level environmental monitoring systems that can handle multi-contaminant systems.

Integration is a revolutionary way of dealing with environmental science. With accurate chemical detection and mechanistic knowledge of molecular structure and biological response, synergistic spectrochemistry allows the creation of predictive environmental models that transcend conventional monitoring.

Through this interdisciplinary framework, it is possible to:

- Proper identification and measurement of contaminants.
- Structural and speciation-based mechanistic understanding of toxicity.
- Better environmental fate and ecological impact prediction.

Notably, these innovations have serious policy and regulatory consequences.

Evidence-based decision-making, better environmental standards, and sustainable management of the available resources can be supported by predictive risk assessment tools based on integrated data (European Commission, 2020).

Moreover, the implementation of AI-powered analytics, real-time surveillance systems, and green analytical solutions will make sure that the environmental assessment in the future will be more accurate and sustainable at the same time. With the ever-changing nature of environmental issues, the combination of chemical and biological sciences will still be critical to the well-being of the ecosystem and global sustainability objectives.

REFERENCES

Alloway, B. J. (2013). Heavy metals in soils (3rd ed.). Springer.

Anastas, P. T., & Warner, J. C. (1998). Green chemistry: Theory and practice. Oxford University Press..

Ankley, G. T., et al. (2010). Adverse outcome pathways: A conceptual framework. Environmental Toxicology and Chemistry, 29(3), 730–741.

Arnold, K. E., et al. (2014). Impacts of pharmaceuticals on wildlife. Philosophical Transactions B, 369, 20130570.

Atkins, P., & de Paula, J. (2014). Physical chemistry (10th ed.). Oxford University Press.

aus der Beek, T., et al. (2016). Pharmaceuticals in the environment. Environmental Toxicology and Chemistry, 35(4), 823–835.

Boss, C. B., & Fredeen, K. J. (2004). Concepts, instrumentation and techniques in ICP-OES. PerkinElmer.

Boxall, A. B. A., et al. (2012). Pharmaceuticals and personal care products. Philosophical Transactions B, 367, 396–409.

Brown, G. E., Foster, A. L., & Ostergren, J. D. (2017). Mineral surfaces and bioavailability of heavy metals. Proceedings of the National Academy of Sciences, 96(7), 3388–3395.

Chen, B., et al. (2021). Machine learning in environmental science. Environmental Science & Technology, 55(1), 502–515.

Chen, B., et al. (2021). Machine learning in environmental science. Environmental Science & Technology, 55(1), 502–515.

Chen, B., et al. (2021). Machine learning in environmental science. Environmental Science & Technology, 55(1), 502–515.

Di Toro, D. M., McGrath, J. A., & Hansen, D. J. (2018). Bioaccumulation in aquatic systems: Concepts and modeling approaches. Springer.

Di Toro, D. M., McGrath, J. A., & Hansen, D. J. (2018). Bioaccumulation in aquatic systems: Concepts and modeling approaches. Springer.

Escher, B. I., et al. (2020). Effect-based trigger values for water quality assessment. Environmental Science & Technology, 54(14), 8470–8481.

.European Commission. (2020). Chemicals strategy for sustainability. Brussels.

Ferraro, J. R., Nakamoto, K., & Brown, C. W. (2003). Introductory Raman spectroscopy (2nd ed.). Academic Press.

Gago, J., et al. (2020). Microplastics in seawater: Distribution and analysis. Science of the Total Environment, 709, 134455.

Gibbs, P. D., Becker, D., & Chapman, P. M. (2018). Dose–response models in ecotoxicology. Environmental Toxicology and Chemistry, 37(6), 1584–1596.

Gramatica, P. (2020). Principles of QSAR models. Chemosphere, 244, 125526.

Gramatica, P. (2020). QSAR models in environmental chemistry. Chemosphere, 244, 125526.

Hollas, J. M. (2004). Modern spectroscopy (4th ed.). Wiley.

Hollender, J., et al. (2017). High-resolution mass spectrometry-based non-target screening. Environmental Science & Technology, 51(20), 11505–11512.

Hou, X., & Jones, B. T. (2000). Inductively coupled plasma/optical emission spectrometry. Encyclopedia of Analytical Chemistry, 9468–9485.

Jones, O. A. H., et al. (2005). Pharmaceuticals in wastewater. Water Research, 39(14), 3139–3148.

Kah, M., et al. (2018). Nanopesticides in the environment. Nature Nanotechnology, 13, 677–684.

Kim, S., et al. (2017). High-resolution mass spectrometry. Analytical Chemistry, 89(1), 60–80.

Newman, M. C. (2014). Fundamentals of ecotoxicology (4th ed.). CRC Press.

Niessen, W. M. A. (2017). Liquid chromatography–mass spectrometry (3rd ed.). CRC Press.

OECD. (2019). Guidance document on aquatic toxicity testing. OECD Publishing.

Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. R. (2015). Introduction to spectroscopy (5th ed.). Cengage Learning.

Petrović, M., et al. (2005). LC–MS for analysis of pharmaceuticals in environmental samples. Journal of Chromatography A, 1067, 1–14.

Płotka-Wasylka, J., et al. (2018). Green analytical chemistry. TrAC Trends in Analytical Chemistry, 100, 1–14.

Ratcliffe, D. A. (2017). Biomagnification in food chains: Ecological perspectives. Journal of Applied Ecology, 54(3), 712–723.

Richardson, S. D., & Kimura, S. Y. (2020). Emerging environmental contaminants. Analytical Chemistry, 92(1), 473–505.

Rochman, C. M., et al. (2013). Ingested plastic transfers hazardous chemicals. Scientific Reports, 3, 3263.

Rodea-Palomares, I., et al. (2012). Ecotoxicological effects of nanoparticles. Environmental Science & Technology, 46(6), 3482–3490.

Rodea-Palomares, I., et al. (2012). Toxicity of nanoparticles. Environmental Science & Technology, 46(6), 3482–3490.

Schwarzenbach, R. P., et al. (2010). Global water pollution. Science, 313(5790), 1072–1077.

Silverstein, R. M., Webster, F. X., & Kiemle, D. J. (2014). Spectrometric identification of organic compounds (8th ed.). Wiley.

Skoog, D. A., Holler, F. J., & Crouch, S. R. (2018). Principles of instrumental analysis (7th ed.). Cengage Learning.

Smith, B. C. (2011). Fundamentals of Fourier transform infrared spectroscopy (2nd ed.). CRC Press.

Sparks, D. L. (2003). Environmental soil chemistry. Academic Press.

Sparling, D. W., Linder, G., & Bishop, C. A. (2017). Ecotoxicology of amphibians and reptiles (2nd ed.). CRC Press.

Walker, C. H., Sibly, R. M., Hopkin, S. P., & Peakall, D. B. (2012). Principles of ecotoxicology (4th ed.). CRC Press.

Welz, B., & Sperling, M. (1999). Atomic absorption spectrometry (3rd ed.). Wiley.

Wilkinson, J. L., et al. (2022). Pharmaceutical pollution of the world's rivers. Proceedings of the National Academy of Sciences, 119(8), e2113947119.

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