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From prescription to pollution: The ecological consequences of NSAIDs in aquatic ecosystems

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ABSTRACT

Nonsteroidal anti-inflammatory drugs are widely utilised to alleviate pain and reduce inflammation in both human and veterinary medicine. Despite their therapeutic benefits, NSAIDs pose environmental concerns due to their possible ecotoxicity. NSAIDs can enter aquatic ecosystems through wastewater discharge as it is very difficult to extract NSAIDs by conventional wastewater treatment, thus affecting aquatic life. These drugs induce cytotoxic and genotoxic effects, affect the functioning of endocrine systems, influence behavioural changes, and impair reproduction in fish and other aquatic organisms. This review article also discusses the use of bio-indicators such as fish, bivalves, and crustaceans, to estimate NSAIDs exposure in aquatic ecosystems. It emphasises the importance of monitoring these organisms to evaluate potential risks linked with NSAIDs in aquatic environments. Addressing the environmental consequences of NSAIDs requires an inclusive strategy, including regulatory measures, public awareness, and the development of environmentally friendly alternatives to mitigate the risks caused by these widely used pharmaceuticals in aquatic environments.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) which are commonly recommended for reducing pain and managing inflammatory ailments, have been found to be more prevalent in aquatic environments than previously acknowledged [35]. NSAIDs are widely consumed due to their accessibility and affordability, making them a popular choice for managing pain. While these medications can be obtained without a prescription, medical professionals often prescribe them in conjunction with other treatments to address specific pain management needs during recovery from various ailments [41]. However, frequent and prolonged utilization of NSAIDs has sparked environmental concerns, particularly their effects on aquatic ecosystems [13,59]. Due to their widespread use, NSAIDs are frequently detected contaminants in aquatic environments. This high consumption rate makes them one of the most commonly used medications, contributing to their significant presence in water bodies [60]. Their persistence in these environments is facilitated by their structural and physicochemical properties. NSAIDs enter water systems mainly through wastewater treatment plants (WWTPs), which are often unable to fully eliminate pharmaceutical pollutants [36]. As a result, NSAID residues remain in treated water, which is released into rivers, lakes, and oceans. These drugs are chemically stable and resistant to biodegradation, allowing them to persist in the environment [48]. Additionally, agricultural runoff contributes to NSAID pollution when manure containing residues from animals treated with these drugs is used as fertilizer, which then leaches into water bodies during rainfall [55]. Other sources include leakage from landfills with improperly discarded medications and direct disposal of unused drugs into household drains, which ultimately flow to WWTPs [58]. The majority of these drugs enter aquatic environments through sewage effluents, where they may persist and accumulate, posing long-term risks to ecosystem health [69].

In aquatic environments, NSAIDs may undergo natural breakdown processes like photolysis, hydrolysis, and microbial degradation [49]. However, many NSAIDs resist these processes and pile up over time, raising concerns about their potential toxicity to non-target organisms [66]. The extensive use of medications prompts questions about how they may affect aquatic life. Because of their widespread distribution, these biologically active substances may be hazardous to the health of ecosystems. Various studies emphasise the importance of assessing the effects of pharmaceuticals on different aquatic species, especially their influence on microbial communities, algal growth, and fish physiology [68]. Chronic exposure to NSAIDs has also been associated with endocrine disruption, which interferes with hormonal systems, affecting

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reproduction and growth. This may result in reduced fertility, abnormal offspring development, and slower growth rates, ultimately affecting population structures and dynamics [5]. Over time, these effects can lead to a loss of biodiversity as sensitive species decline, altering ecosystem functions such as nutrient cycling and food web interactions [4]. According to the study conducted by Adeleye *et al.*, Australia, Africa, North America, and Europe contributed more to global NSAID wastewater pollution than South America and Asia. The variation in NSAID wastewater pollution across different regions may be influenced by factors such as the effectiveness of wastewater treatment systems, population size and composition, proximity to healthcare facilities and pharmaceutical manufacturing plants, prevalence of diseases, access to medical care, and medication usage habits [1].

Common NSAIDs, such as ibuprofen, diclofenac, ketoprofen and naproxen, play a key role in modern healthcare, being regularly prescribed for conditions ranging from minor issues to more serious inflammatory diseases like arthritis and post-operative recovery. While these medications offer significant benefits, they also pose environmental risks. Ibuprofen can enter the environment in its original form through improper disposal of unused or expired pills, and in modified forms (transformation products) via excretion in urine or feces. Chlorination processes in wastewater treatment plants can further transform ibuprofen into more toxic compounds [30]. Diclofenac has had a particularly detrimental impact on the environment. In a well-documented incident in Pakistan and India, vulture populations were decimated after ingesting cattle carcasses contaminated with diclofenac residues. Consequently, diclofenac was added to the watch list under the European Union Water Framework Directive [20,56,70]. Naproxen, due to its slower rate of decomposition and the limitations of current sewage treatment processes, can adversely affect kidney, liver, and jaw structures in fish, but its environmental hazards and risks are lower than diclofenac [51]. Ketoprofen poses a significant threat to aquatic ecosystems due to its slow rate of decomposition and the limitations of current sewage treatment processes. Ketoprofen tends to accumulate in wastewater, particularly hospital wastewater, as it is excreted by humans and animals in the form of metabolites. Only a small percentage of ketoprofen is excreted unchanged, further contributing to its persistence in the environment [37]. Thus, Understanding these effects is vital for developing strategies to minimise pharmaceutical pollution and protect the health of aquatic ecosystems. Addressing the environmental impact of NSAIDs might necessitate advancements in wastewater treatment technologies to efficiently eliminate these compounds. Moreover, actions such as appropriate medication disposal and regulatory controls on their use in agriculture could aid in reducing their presence in aquatic ecosystems [29,65]. The focus of the present study is to examine the impact of NSAIDs on aquatic ecosystems.

2. Types of NSAIDs

The categorization of NSAIDs is done based on the chemical structure, mode of action and duration of action to provide an outline for understanding the intricate details about the wide ranging NSAIDs.

2.1. Types of NSAIDs based on chemical structure

The general structure of NSAIDs consist of an aromatic ring or rings with carboxylic acid or enol functional group. Different side chains attached to the aromatic ring give diverse varieties of NSAIDs contributing to the potency of the drug [7]. According to the chemical structure, the NSAIDs is classified into the following categories (Table 1):

1. Salicylic acid derivatives
2. Aryl and heteroaryl acetic acid derivatives
3. Indole/indene acetic acid derivatives
4. Anthranilic acid derivatives
5. Enolic acid derivatives

Table 1
Different types of NSAIDs based on chemical structure.

TYPES OF NSAIDs	NAME OF THE DRUG (EXAMPLES)	CHEMICAL FORMULA OF THE DRUG
Salicylic acid derivatives	Sodium salicylate	$C_7H_5NaO_3$
	Aspirin	$C_9H_8O_4$
	Diflunisal	$C_{13}H_8F_2O_3$
Aryl and heteroaryl acetic acid derivatives	Fenoprofen	$C_{15}H_{14}O_3$
	Naproxen	$C_{14}H_{14}O_3$
	Ketoprofen	$C_{16}H_{14}O_3$
	Ibuprofen	$C_{13}H_{18}O_2$
	Oxaprozin	$C_{18}H_{15}NO_3$
Indole/indene acetic acid derivatives	Indomethacin	$C_{19}H_{16}ClNO_4$
	Etodolac	$C_{17}H_{21}NO_3$
	Sulindac	$C_{20}H_{17}FO_3S$
Anthranilic acid derivatives	Mefenamic acid	$C_{15}H_{15}NO_2$
	Diclofenac	$C_{14}H_{11}Cl_2NO_2$
	Meclofenamic acid	$C_{14}H_{11}Cl_2NO_2$
Enolic acid derivatives	Piroxicam	$C_{15}H_{13}N_3O_4S$
	Meloxicam	$C_{14}H_{13}N_3O_4S_2$
	Tenoxicam	$C_{13}H_{11}N_3O_4S_2$

2.2. Types of NSAIDs based on mode of action

NSAIDs are classified into Non-Selective NSAIDs and Selective COX-2 NSAIDs based on their inhibitory action against the enzyme.

2.2.1. Non-selective NSAIDs

Non-selective NSAIDs target both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes, with drugs like naproxen, aspirin, and ibuprofen falling under this group. Aspirin, a commonly used NSAID, provides pain relieving, antiphlogistic and antiplatelet effects. It achieves this by irreversibly inhibiting COX enzymes, leading to a reduction in the production of thromboxane and prostaglandins [18]. While aspirin is indicated for pain relief, fever reduction, and cardiovascular disease prevention, potential adverse effects include gastrointestinal ulcers, bleeding disorders and Reye's syndrome [27,42]. Ibuprofen favours freely reversible inhibition of COX enzymes, providing pain relief by reducing prostaglandin synthesis, and also aids in fever reduction [75]. Naproxen, also a nonselective NSAID, is recognized for its prolonged analgesic effects, effectively alleviating pain, inflammation, and stiffness associated with conditions like arthritis. Despite its efficacy, naproxen shares similar risks of gastrointestinal issues and heightened cardiovascular risk with other nonselective NSAIDs [62].

2.2.2. Selective COX-2 NSAIDs

Selective NSAIDs specifically inhibit COX-2, while leaving the COX-1 enzyme unaffected. This selective action preserves the protective role of prostaglandins produced by COX-1 in the gastrointestinal tract and platelets, significantly reducing the likelihood of gastrointestinal side effects. The mode of action of COX-2 inhibitors is through inhibition of the NF-κB pathway [31]. However, it is essential to consider the potential cardiovascular risks associated with these medications. Celecoxib, a widely utilised selective COX-2 inhibitor, is prescribed for medical ailments such as ankylosing spondylitis, osteoarthritis, rheumatoid arthritis, and acute pain management. While providing comparable analgesic and anti-inflammatory efficacy to nonselective NSAIDs, celecoxib offers a reduced risk of gastrointestinal complications [17]. Nevertheless, prolonged usage of celecoxib may elevate the likelihood of myocardial infarction as it has been proved to have reduced the mitochondrial complex IV activity in rat heart mitochondria [3]. Meloxicam, another selective COX-2 inhibitor, is mainly prescribed for managing chronic arthritis and juvenile idiopathic arthritis. It offers an extended duration of action as an NSAID and is associated with a lower incidence of gastrointestinal side effects compared to non-selective NSAIDs. However, it may still cause gastrointestinal issues such as ulcers and bleeding, along with fluid retention and an elevated risk of

cardiovascular events [34].

2.3. Types of NSAIDs based on duration of action

The time it takes for the concentration of the drug in the plasma to drop by half after delivery is known as the plasma half-life, and it affects how long a medicine will take to take effect. NSAIDs are classified into short-acting and long-acting categories. Short-acting NSAIDs like aspirin, diclofenac, and ibuprofen have plasma half-lives of less than 6 hours, making them suitable for treating acute pain but requiring multiple doses. Conversely, long-acting NSAIDs, with plasma half-lives exceeding 10 hours, are better suited for managing chronic pain and necessitate less frequent dosing compared to short-acting NSAIDs. Examples of long lasting NSAIDs include naproxen and celecoxib [15].

3. Fate of NSAIDs in aquatic ecosystems

The presence of pharmaceuticals in the environment is mainly governed by the total number of pharmaceuticals manufactured and released into the water bodies, along with processes such as degradation, partial decomposition, and dissolution that reduce the concentrations of these chemical substances.The extensive utilisation of NSAIDs results in their notable identification in effluents from wastewater treatment plants and subsequent discharge into aquatic ecosystems due to their inadequate elimination in these facilities. These pharmaceutical chemicals make up around 15 % of all pharmaceuticals found in global monitoring surveys and are among the most often documented in aquatic habitats. Some anti-inflammatory drugs (ibuprofen, ketoprofen, naproxen, diclofenac and mefenamic acid) have been found in aquatic habitats at microgram-per-litre quantities; the majority of these drugs come from wastewater treatment facilities [16]. Diclofenac, naproxen, and ibuprofen have been found in various aquatic settings, including surface waters, seawaters, groundwaters, wastewater, and sludge [9, 12].

There are a number of problems related to treatment procedures and operational settings that can be blamed for the insufficient removal of pharmaceuticals from wastewater treatment plants (WWTPs). The fluctuation of the weather is one important uncontrollable aspect. A study conducted by Yu et al. examining the presence of fourteen pharmaceuticals, personal care products, and endocrine disruptors in wastewater revealed seasonal fluctuations in their concentrations. Naproxen, ibuprofen, and paracetamol were the predominant drugs detected, exhibiting higher concentrations during the winter season compared to summer. This discrepancy may stem from increased consumption of NSAIDs and paracetamol-containing medications during winter months, coinciding with heightened influenza prevalence. Another reason for the relatively higher pharmaceutical concentrations observed in winter may be their accelerated degradation during summer due to elevated wastewater temperatures. During summer, photolytic processes occur more rapidly, with effectiveness contingent upon light intensity and exposure. Photolytic degradation primarily occurs in highly transparent waters with surface layers exposed to sunlight. The pH level is an additional factor that significantly impacts the elimination of pharmaceuticals, as these compounds can undergo transformations in their ionic state under varying pH conditions. Consequently, the chemical characteristics of pharmaceuticals, including their toxic effects, mode of action and photosensitivity will fluctuate with changes in the pH of the environment [78]. Baena-Nogueras et al. [6] demonstrated the pivotal effects of pH in the photodegradation process of numerous pharmaceutical compounds. Specifically, the researchers noted that the degradation of the pain killer acetaminophen was more pronounced at pH levels of four or nine compared to a pH of seven. However, for NSAIDs such as diclofenac, ketoprofen and ibuprofen, no significant alterations were observed.

Studies on the buildup of NSAIDs in freshwater invertebrates have been carried out on a range of species belonging to several taxonomic categories. NSAID residues in invertebrates were found to be comparatively greater than in other pharmaceuticals; diclofenac and ibuprofen were the most often discovered NSAIDs. NSAIDs can go through biotransformation after it gets incorporated into the body.The oxidation of drugs in humans as well as in aquatic invertebrates depend on the cytochrome P450 mixed function oxidase (MFO) systems. In fish and invertebrates, several families of P450 genes (CYP) have been found. It has been established that the CYP2C9 subgroup of the CYP2 family is responsible for the biotransformation of NSAIDs [22]. Another biotransformation pathway for carboxylate NSAIDs, including aspirin, diclofenac, naproxen and ibuprofen, involves glucuronic acid conjugation which results in the formation of acyl glucuronides. In fish and invertebrates, several families of P450 genes (CYP) have been found. It has been established that the CYP2C9 subgroup of the CYP2 family is responsible for the biotransformation of NSAIDs. It has been shown that a variety of NSAID-derived acyl glucuronides, such as those from ibuprofen and diclofenac, form covalent connections with intra- and extracellular proteins, which can have toxicological consequences [28]. Multiple studies have investigated the impact of NSAIDs on non-target organisms. Xia et al. [76] observed a noteworthy delay in hatching among *Danio rerio* after exposure to ibuprofen and diclofenac, attributed to a suppression of overall embryo motion. Wang et al. [72] studied the exposure to four different NSAIDs - ibuprofen, ketoprofen and aspirin which resulted in growth inhibition, cellular structure damage, significant effects on photosynthesis, photorespiration and carbon assimilation in the green algae *Scenedesmus obliquus*. Ketoprofen exhibited the highest toxicity among the NSAIDs tested on *Scenedesmus obliquus*, potentially due to its high liposolubility and bioavailability. A group of researchers documented a decrease in the reproductive rates of *Moina macrocopa* and *Daphnia magna* along with reduced survival rates among juvenile *Oryzias latipes* fish following chronic exposure to naproxen. Ibuprofen induced nephrotoxicity in South American catfish, *Rhamdia quelen* [38]. Fig. 1. shows the fate of NSAIDs in aquatic ecosystem.

4. Ecotoxicity of NSAIDs

Pharmaceuticals possess a unique ability to enter the cell membranes of non-target species and interact with molecular systems, leading to unforeseen consequences across various species. Additionally, these drugs are engineered to endure over time, ensuring their chemical composition remains stable for therapeutic purposes. Despite their intended benefits for target organisms, pharmaceuticals have the potential to cause undesirable reactions, even at low concentrations, for a variety of organisms that are exposed. These effects include changes in seminal fluid quality, changes in sexual behaviour, and delays in the development of frogs and fish [23,71]. NSAIDs is one of the most popular pharmaceuticals that is extensively spread in the freshwater ecosystems. Lethal Concentration, Effective Concentration, Inhibitory Concentration, predicted no-effect concentrations, Lowest Observed Effect Concentration and No Observed Effect Concentration are the parameters used by the researcher used by the researcher to evaluate and analyse the ecotoxicological impacts of NSAIDs on various aquatic species. Each parameter serves a different purpose and it helps to identify safe exposure levels and establish environmental quality standards.

In the research performed by Huang et al. [26], the ecological risk assessment for ibuprofen was assessed. The results showed that ibuprofen posed a heightened risk to aquatic organisms, leading to increased ecological concerns for surface water in China. Also the value of the tiered approach called HQ was calculated. When the value HQ of exceeds 1, it indicates that the exposed organisms are at risk, posing a high threat to the aquatic environment. It was found that the frequency of HQ greater than 1 was up to 55.6 % for ibuprofen. In another study conducted by a team of researchers, the Predicted Environmental Concentration, Measured Environmental Concentration and the Predicted No-Effect Concentration were assessed to determine the risk quotient

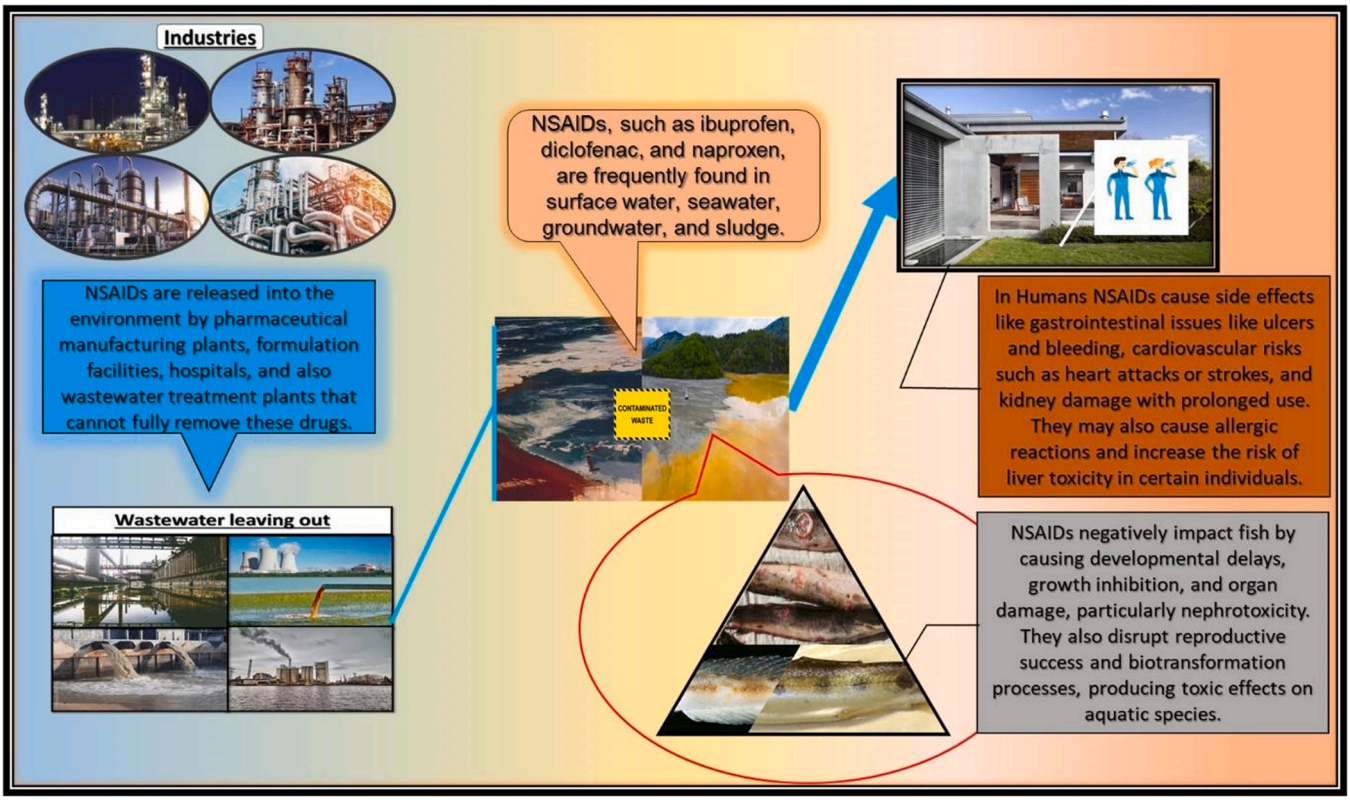

Fig. 1. FATE OF NSAIDs IN AQUATIC ECOSYSTEM.

(RQ) of pharmaceuticals including NSAIDs like ibuprofen and diclofenac. RQ was calculated with following equations:

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

(or)

RQ = Measured Environmental Concentration (MEC) / Predicted No-Effect Concentration (PNEC)

For ibuprofen, The PEC/PNEC ratio, which equalled 600 and the MEC/PNEC ratio, which equalled 1.9, both the ratios exceeded 1, indicating a likely environmental risk. Whereas for diclofenac, the resulting RQ from the PEC/PNEC ratio, which equalled 15, exceeded 1, indicating a probable environmental risk. Conversely, the RQ assessed through the MEC/PNEC ratio was below 1. Hence, it was proved that Ibuprofen presented a significant environmental hazard as it is the only NSAID in the research for which the RQ evaluated through both the ratios PEC/PNEC and MEC/PNEC respectively, exceeded one [10].

5. Effects of NSAIDs on aquatic ecosystems

5.1. Effects of NSAIDs on oxidative stress parameters

NSAIDs have become increasingly recognized for their harmful impact on oxidative stress parameters in aquatic organisms, leading to significant ecological risks. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the organism's capacity to neutralize them, often resulting in cellular damage. NSAIDs, including commonly used drugs such as ibuprofen, diclofenac, and naproxen, have been demonstrated to induce oxidative stress in various aquatic species. These drugs increase ROS production, disrupt antioxidant defense systems, and affect key biomarkers like catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH). Research has shown that NSAID exposure leads to heightened ROS generation, causing lipid peroxidation, protein oxidation, and DNA damage in aquatic species like fish, mollusks, and crustaceans [24]. For example, increased malondialdehyde (MDA) levels—a marker of lipid peroxidation—have been observed in fish exposed to diclofenac, indicating oxidative stress-induced cellular injury [47]. This oxidative stress not only compromises essential biological functions but also weakens immune responses, disrupts reproduction, and stunts growth, all of which can impact population dynamics within aquatic ecosystems. Several studies have emphasized the role of NSAIDs in altering antioxidant enzyme activities. Notably, exposure to NSAIDs has been associated with decreased SOD and CAT activity, enzymes that play a crucial role in neutralizing free radicals in aquatic organisms. This decline signals a reduction in the organism's ability to counteract oxidative damage [40]. In particular, diclofenac has been shown to significantly reduce GSH levels in species such as *Oncorhynchus mykiss* and *Danio rerio*, leading to a weakened capacity to detoxify ROS and increasing vulnerability to oxidative stress [8]. NSAID-induced oxidative stress is also linked to mitochondrial dysfunction, which further heightens ROS production, disrupts energy metabolism, and intensifies stress responses in aquatic organisms [64]. These mitochondrial disruptions can result in impaired locomotion and altered behavior, further diminishing the ability of species to thrive in polluted environments. The long-term ecological consequences of NSAID-induced oxidative stress are particularly worrisome due to the potential for bioaccumulation and trophic transfer throughout aquatic food webs [77]. As NSAIDs accumulate in primary consumers such as zooplankton and bivalves, oxidative stress can propagate to higher trophic levels, affecting species like fish and birds, thus amplifying ecological disruption [25,26]. In zebrafish (*Danio rerio*), exposure to NSAIDs has been shown to hinder larval development and reduce hatching success due to oxidative damage in embryonic cells. Such developmental disturbances can cause population declines, threatening biodiversity and ecosystem resilience over time. Given the persistence of NSAIDs in aquatic environments and the inefficiency of wastewater treatment plants in fully removing these pollutants, it is

critical to investigate the long-term effects of NSAID-induced oxidative stress on aquatic ecosystems. Understanding the mechanisms by which NSAIDs disrupt antioxidant defenses and cellular homeostasis is essential for assessing ecological risks and developing mitigation strategies. Current research underscores the urgent need for enhanced wastewater treatment methods and improved pharmaceutical management practices to mitigate the oxidative impact of NSAIDs on aquatic life [24].

5.2. Effects of NSAIDs on immune system functions

NSAIDs are widely recognized for their analgesic and anti-inflammatory properties, but their growing presence in aquatic ecosystems has become a source of concern due to their immunomodulatory effects on aquatic species. NSAIDs can disrupt immune system function in aquatic organisms by interfering with crucial signaling pathways, leading to abnormal immune responses. A key mechanism of NSAID action involves the inhibition of cyclooxygenase (COX) enzymes, particularly COX-2, which is essential for the production of prostaglandins—lipid molecules that regulate inflammation and immune function [66]. The reduced production of prostaglandins can weaken inflammatory responses, potentially compromising the ability of aquatic species to defend against pathogens. Long-term exposure to NSAIDs has been shown to result in immunosuppression, making organisms more susceptible to infections. This has been demonstrated in studies involving fish and mollusks, where a decrease in immune cell activity, including diminished phagocytosis and lymphocyte proliferation, was observed. Additionally, NSAIDs have been found to alter the expression of cytokines, which are critical for immune cell communication. For instance, diclofenac exposure has been linked to the suppression of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α), thereby weakening the host's defense mechanisms [33]. Chronic exposure to NSAIDs can also induce oxidative stress, which further disrupts immune function. Elevated levels of ROS associated with oxidative stress can damage immune cells, decreasing the immune system's overall efficiency [32]. In aquatic environments, where organisms are continuously exposed to low concentrations of NSAIDs, oxidative stress can exacerbate immunosuppressive effects, leading to broader, population-level impacts. Several studies have documented the immunotoxic effects of NSAIDs on various aquatic species. For example, alterations in immune organs like the spleen and thymus have been observed in fish, leading to impaired immune surveillance and an increased risk of disease [63]. NSAID exposure has also been associated with disruptions in the adaptive immune system, specifically in the production of antibodies, which decreases the ability to develop long-term immune memory and resist pathogens. The adverse effects of NSAIDs on the immune systems of aquatic species extend beyond individual organisms to entire ecosystems. Impaired immune function in key species, such as fish and invertebrates, can disrupt ecological balance, affecting predator-prey relationships and making ecosystems more vulnerable to disease outbreaks. The persistent release of NSAIDs into aquatic environments, combined with their resistance to degradation, poses a significant threat to biodiversity and the overall health of ecosystems [24]. In conclusion, the immunotoxicity of NSAIDs in aquatic species is a complex issue involving disruptions in immune signaling, oxidative stress, and adaptive immunity, which collectively increase disease susceptibility and contribute to broader ecological consequences. These findings highlight the urgent need for stronger regulations on NSAID disposal and further research into the long-term ecological impacts of pharmaceutical contaminants [54].

5.3. Effects of NSAIDs on growth, development, and reproduction

NSAIDs are increasingly found in aquatic environments, raising serious concerns about their effects on the growth, development, and reproduction of aquatic organisms. NSAIDs such as ibuprofen, diclofenac, and naproxen interfere with critical physiological processes by inhibiting COX enzymes. These enzymes are vital for producing prostaglandins, which not only regulate inflammation but are also key players in reproductive and developmental pathways [52]. Chronic exposure to NSAIDs has been linked to growth retardation and developmental abnormalities in aquatic species, especially fish and amphibians. For instance, research has shown that zebrafish larvae exposed to ibuprofen exhibit reduced body length and impaired skeletal development, as prostaglandins are involved in bone growth and overall development [76]. Similar growth disruptions have been observed in mollusks and other invertebrates, indicating that the continued presence of NSAIDs in aquatic environments could have long-term consequences for species survival and population dynamics. The reproductive health of aquatic organisms is also significantly affected by NSAIDs. These drugs can disrupt hormone signaling pathways, particularly those involving prostaglandins, which are essential for regulating reproductive processes such as ovulation, sperm motility, and fertilization [54]. For example, fish exposed to diclofenac experience a marked decline in egg production and sperm quality, resulting in reduced fertilization rates and compromised reproductive success. Embryos and developing offspring are especially vulnerable, with studies demonstrating that NSAID exposure during embryonic stages can delay hatching, cause developmental malformations, and lower survival rates [39]. This reproductive toxicity is not limited to fish; bivalves and other invertebrates also show decreased reproductive output, with long-term exposure leading to fewer viable offspring and increased embryo mortality. Additionally, NSAIDs interfere with the endocrine system, causing hormone imbalances that further impair reproductive functions. Research has shown that ibuprofen exposure in aquatic invertebrates significantly disrupts estrogen and androgen signaling, both of which are crucial regulators of reproductive health [73]. The negative effects of NSAIDs on growth and reproduction extend beyond individual organisms, potentially impacting entire ecosystems. Reduced reproductive success and developmental impairments can contribute to population declines, particularly among species that play important roles in aquatic ecosystems. The persistence and bioaccumulation of NSAIDs in water bodies pose significant long-term threats to biodiversity and ecosystem health [11]. For instance, declining reproductive success in fish and amphibian populations can alter predator-prey dynamics, ultimately affecting the stability and structure of aquatic food webs. Moreover, NSAIDs may have transgenerational effects, where developmental and reproductive impairments are passed onto offspring, suggesting that the ecological impacts of these drugs could extend far beyond the populations currently exposed. Given the significant ecological risks posed by NSAIDs in aquatic environments, there is an urgent need for stricter regulations and improved wastewater treatment practices to limit the release of pharmaceutical contaminants [46].

6. Concentrations of specific NSAIDs resulting in specific effects on the aquatic system

The impact of NSAIDs on aquatic systems is closely related to their concentration and the duration of exposure. Diclofenac, one of the most studied NSAIDs, is commonly found in concentrations ranging from 0.1 to 1.0 μg/L in rivers and streams. Even at low concentrations, such as 0.1 μg/L, it has been linked to severe damage in fish, particularly affecting organs like the gills, liver, and kidneys. For example, it causes kidney damage and disrupts osmoregulation in rainbow trout (*Oncorhynchus mykiss*) [81]. At higher concentrations, around 1 μg/L, diclofenac has been shown to induce oxidative stress, compromise immune function, and reduce survival rates in both fish and invertebrates [61]. Similarly, ibuprofen is another widely detected NSAID in aquatic ecosystems, with concentrations varying from 0.1 to 2.0 μg/L in surface waters. Exposure to ibuprofen at just 1 μg/L can impair reproductive processes in fish, leading to decreased sperm motility and egg production. At higher levels, such as 10 μg/L, it has been associated with delayed hatching, skeletal deformities, and lower survival rates in

zebrafish (*Danio rerio*) [80]. Naproxen, another commonly detected NSAID, is found at concentrations between 0.05 and 1.5 μg/L. Its effects are similarly concerning, particularly for mollusks. Concentrations exceeding 0.5 μg/L have been linked to reproductive disruptions, such as impaired gametogenesis and reduced larval development [74]. Additionally, naproxen has been found to cause endocrine disruption in fish, with concentrations around 1 μg/L altering critical hormone levels that regulate reproductive health. Ketoprofen, which is present in aquatic environments at concentrations ranging from 0.1 to 1.0 μg/L, also has detrimental effects on aquatic organisms. It interferes with prostaglandin synthesis, leading to reproductive challenges and developmental anomalies in fish. Concentrations of 1 μg/L have been reported to significantly reduce sperm motility and disrupt hormone balances essential for reproduction [73]. Moreover, the combined exposure to sub-lethal concentrations of ibuprofen and diclofenac (approximately 0.2 μg/L each) has been shown to have synergistic effects, amplifying oxidative stress and impairing detoxification mechanisms in fish [67]. The persistence of NSAIDs in aquatic environments, even after wastewater treatment, compounds their ecological risks. Studies have demonstrated that treated wastewater can still contain harmful concentrations of NSAIDs, such as diclofenac, ibuprofen, and naproxen. These compounds are resistant to conventional treatment methods, allowing their widespread detection in both freshwater and marine systems [54]. Long-term exposure to low concentrations of NSAIDs has significant implications for aquatic populations. Diclofenac, for example, bioaccumulates in fish, leading to population-level declines due to increased mortality and reproductive failure. The presence of multiple NSAIDs in aquatic ecosystems can further intensify these impacts, as their combined presence often results in additive or synergistic toxicity, leading to more severe effects than single-drug exposure. In summary, specific concentrations of NSAIDs, including diclofenac, ibuprofen, naproxen, and ketoprofen, can cause extensive harm to aquatic organisms, affecting growth, reproductive health, immune response, and survival. Even at concentrations below 1 μg/L, these drugs can cause significant physiological disruptions in fish, invertebrates, and other species. The persistence and bioaccumulation of NSAIDs in aquatic systems, combined with their potential for synergistic toxicity, highlight the critical need for improved wastewater treatment technologies and more stringent regulatory measures to reduce the ecological risks associated with pharmaceutical pollutants [50].

7. Bioindicators of NSAIDs exposure

Evaluation of exposure to NSAIDs can be accomplished by employing aquatic organisms as indicators. The species acting as biomarkers gather pollutants in their tissues from the nearby surroundings, thus serving as significant biomonitoring tools.

7.1. Bivalves as bioindicators

Bivalves such as clams and mussels are widely recognized as valuable bioindicators of NSAIDs owing to their global presence, filter-feeding behaviour, high tolerance to environmental variations and efficient accumulation of both organic and inorganic chemicals. Additionally, their ability to measure various biomarkers further enhances their suitability for studying the impacts of chemical pollutants [2].

7.2. Mussels as bioindicators

The findings of the experiments conducted by Parolini et al. [53] show that when exposed to different doses of diclofenac and ibuprofen, the circulation cells in the hemolymph of freshwater mussels *Dreissena polymorpha* showed fragmentation of DNA and an increased percentage of apoptotic cells. Also it was noticed that ibuprofen induced changes in the antioxidant enzymes and collapsed the stability of lysosomal membrane. Fontes et al. [19] used *Perna perna* which is a brown mussel in the research to identify the ill effects caused by diclofenac. The exposure of diclofenac led to alterations in the action of the immune system, imbalance between free radicals and antioxidants, injury to DNA and a significant decrease in the expression of COX and lysosomal stability of the lysosomal membrane.

7.3. Clams as bioindicators

Matozzo et al. [43] tested the consequences of ibuprofen in *Ruditapes philippinarum*. In haemocytes, ibuprofen did not cause fragmentation of DNA. Despite the ibuprofen concentrations tested in this research being higher than typical levels found in aquatic environments, the findings suggest that exposure of *Ruditapes philippinarum* to ibuprofen leads to notable changes in immune parameters and hints at possible immunosuppression in clams exposed to ibuprofen. *Corbicula fluminea* were subjected to environmentally relevant doses of diclofenac for 28 days as part of the study by Yuan et al. [79], and the possible negative effects of diclofenac on siphoning behavior, antioxidant responses, and apoptosis were examined. According to the findings, clam siphon efficiency was severely reduced while they were under diclofenac stress. By decreasing acetylcholinesterase (AChE) activity in the digestive gland and gills of *C. fluminea*, diclofenac causes neurotoxicity. The gills and digestive gland of *C. fluminea* exhibited elevated levels of MDA and oxidative stress as a result of exposure to diclofenac. Also, diclofenac might cause apoptosis by starting the mitochondrial apoptotic pathway.

7.4. Crustaceans as bioindicators

In another research conducted by Gómez-Oliván et al. [21], *Hyalella azteca,* a crustacean, was used as the bioindicator to determine the presence of NSAIDs with the expression of suitable markers due to its high sensitivity to various drugs. It was proved that NSAIDs like diclofenac, paracetamol, naproxen, ibuprofen and aspirin triggered oxidative stress on *H. azteca.* Using *Gammarus pulex* as a model organism, Cikcikoglu Yildirim et al. [14] worked to evaluate the toxicity of the ibuprofen. Acetylcholinesterase (AChE), catalase, and superoxide dismutase activity were assessed. Ibuprofen exposure caused a reduction in SOD activity in *G. pulex*. At 24 and 96 hours, CAT activity rose at varying intensities in all groups exposed to ibuprofen.All application groups exposed to ibuprofen for 96 hours saw an increase in AChE activity. To sum up, there was a greater incidence of oxidative damage after exposure to ibuprofen.

7.5. Plants as bioindicators

Plant species are still employed today as a genetic model to assess the toxicity of chemical substances. Given that plants are among the creatures that are naturally exposed to various environmental toxins, it is crucial to employ them as eukaryotic models in ecotoxicological research in order to quantify environmental risk [57]. Mercado and Galvis, [44] used *Lens culinaris* and *Pisum sativum* to test the potential cytotoxic effects of paracetamol. At all employed concentrations, inhibition of root growth, aberrant mitotic activity, and a concerning micronucleus index were noted. These critical findings indicate that the drug has a significant level of toxicity, and these facts present a challenge for plant metabolism.

8. Threats and future challenges for aquatic ecosystems

Pharmaceutical-related environmental consequences in aquatic environment are now recognized as an urgent issue with a global distribution and negative effects ranging from population-level effects to molecular modifications on non-target species. Although it is crucial for providing a more accurate understanding of the biological impacts and environmental toxicity of these substances in natural settings, interdisciplinary methods that combine drug bioaccumulation analyses with the

detection of negative effects assessed at the genetic and biochemical levels are not commonly used. Drug bioaccumulation emphasises the need for additional research to clarify long-term ecological effects and potential mitigating measures. The ecological effect of NSAIDs received little research to date—will present issues in the future. Research is predicted to provide the motivation for knowledge-driven reduction and preventive measures that integrate "green pharmacy" ideas to produce more ecologically friendly procedures for the synthesis and degradation of NSAIDs in wastewater. Growing public awareness should coincide with scientific advancements in order to prevent medicines from accidentally entering aquatic habitats. This is because advocating for safer disposal of home medications would help prevent their unintentional penetration into the water bodies. By defining an inventive "circular knowledge" strategy, it would be possible to address environmental challenges more effectively [45]. Future multiple-stressor experiments should combine pharmaceutical pollutants with other known major stressors, such as sedimentation, climate change and pollution of nitrogen and phosphorus, in order to learn more about how NSAIDs may mediate the degree of ecosystem alteration caused by major stressors. The integration of community-level endpoints with biomarker endpoints can lead to a more precise assessment of harmful effects in subsequent ecotoxicological investigations. In addition to helping to resolve some of the ambiguities surrounding the interpretation of biomarkers and offering an acceptable explanation of cause for any changes observed at the community or ecosystem level, this approach produces compelling evidence relevant to environmental legislation and management.

9. Conclusion

NSAIDs are classified into several categories depending on their chemical structure, method of action, and duration of action. The use of NSAIDs in aquatic environments raises questions about ecotoxicity and their effects on aquatic life, even though they are essential for managing pain and have other therapeutic advantages. Research suggests that NSAIDs may negatively impact aquatic creatures by causing physiologic and behavioural disruptions. Fish may experience oxidative stress, immune system disruption, and problems with growth, development, and reproduction. These impacts have the potential to upset ecosystems and endanger the wellbeing of the aquatic inhabitants. Because bioindicators are susceptible to NSAID exposure, they are useful instruments for assessing the health of the ecosystem. The ecological effects of NSAIDs on aquatic environments must be understood in order to save vulnerable non-targeted species. In order to save aquatic ecosystems, it is imperative that efforts be made to reduce the pollution that NSAIDs produce. This will help to maintain ecological integrity and biodiversity. The number of studies that demonstrate the negative effects of NSAIDs on different trophic levels in different biological systems is quite concerning. In addition to therapeutic doses, the concentrations of NSAIDs already present in the environment have the potential to significantly damage aquatic ecosystems and have a disruptive effect on social and economic concerns pertaining to the aquatic industry and environmental responsibilities.

Abbreviations

AChE, Acetylcholinesterase; CAT, Catalase; COX, Cyclooxygenase; GSH, glutathione; MEC, Measured Environmental Concentration; MDA, Malondialdehyde; NSAIDs, Non-steroidal anti-inflammatory medicines; PEC- Predicted Environmental Concentration; PNEC, Predicted No-Effect Concentration; PPCPs, Pharmaceuticals and personal care products; ROS, Reactive oxygen species; RQ, Risk quotient; SOD, Superoxide dismutase; STPs, Sewage treatment facilities; WWTPs, Wastewater treatment plants.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: VIBHA MURALI reports was provided by Rajalakshmi Engineering College. DIVYA LAKSHMI S reports a relationship with Rajalakshmi Engineering College that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

No data was used for the research described in the article.

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