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A critical review on the effect of nitrate pollution in aquatic invertebrates and fish

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Abstract Apart from anthropogenic pollution, nitrate contamination is prevalent in practically all developing countries as a result of increased natural activities. The accumulation of nitrates in water bodies causes cumulative effects on living species, environmental receptors, and human vitality by accumulation along the food chain. Nitrates have recently piqued the interest of academics due to their widespread pollution of surface and groundwater systems. The presence of nitrate in high amounts in surface and groundwater causes a variety of health issues, including methemoglobinemia, diabetes, the emergence of infectious diseases, and a negative impact on aquatic organisms. Sensing nitrate is an alternative method for measuring the distribution of nitrate in various bodies of water. The nitrate-laden wastes from agricultural run-off, industrial discharges, and livestock and poultry farms contaminate resources. Nitrate toxicity shows swimming alteration, growth retardation, and eventually death among aquatic organisms. In fishes, it causes histopathological alteration of gills, esophagus, and brain. Therefore, this present review discusses the toxic concentration of nitrates, its adverse effects on the aquatic animals and the assessment of the safe limit of nitrate that is crucial to prevent chronic toxicity among invertebrates and fish.

Keywords Nitrate toxicity · agricultural run-off · methemoglobinemia · fertilizers · histopathological alteration · anthropogenic pollution · food chain

Highlights
- Environmental pollution from nitrate is getting worse and is harming health of aquatic ecosystem.
- Nitrate causes growth reduction, histopathological changes, neurotoxicity, endocrine disruption, and ultimately death to aquatic organisms.
- Nitrate disrupts thyroid function, causes cancer, congenital cardiac defect, and methemoglobinemia in infants, fishes.

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1 Introduction

Nitrogen is one of the primary constituents for biomolecules including DNA, RNA, proteins, chlorophyll, etc. Although gaseous nitrogen (N_2) is very abundant in the atmosphere, it is largely inaccessible to most organisms in this form. Inorganic and bioavailable nitrogen is present in various forms like NO_3^-, NH_4^+ and NO_2^-. These compounds are interrelated via the nitrification cycle. Ammonia is a metabolic bi-product directly excreted into the water (Camargo et al., 2005; Romano and Zeng, 2013). Ammonia is converted into nitrites and subsequently nitrates through nitrification by naturally occurring bacteria *Nitrosomonas* and *Nitrobacter*, as follows:

$$NH_4^+ + CO_2 + 1.5O_2 + \text{Nitrosomonas} \rightarrow NO_2^- + H_2O + H^+$$

$$NO_2^- + 0.5O_2 + \text{Nitrobacter} \rightarrow NO_3^-$$

In an aquatic and terrestrial ecosystem, the concentration of bioavailable nitrogenous compounds is maintained at a low level. The plants and microbes assimilate those nitrogenous compounds. The limiting resource of bioavailable nitrogen maintains a balance between primary productivity and global carbon storage. In the United States, the maximum concentration level for nitrate as nitrate-nitrogen (NO_3-N) in public drinking water is 10 mg/l, which is nearly equivalent to the WHO recommendation of 11.3 NO_3-N or 50 mg/l as NO_3 (Singh et al., 2022). In India, the permitted limit for nitrate ions in drinking water is 45 mg/l (Bureau of Indian Standards) (Agarwal et al., 2019). However, due to different natural and anthropogenic sources, the level of these nitrogenous wastes including ammonia (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) are increasing in the ecosystem and becoming a global threat for the animal life due to the toxic impact (Valencia-Castañeda et al., 2019; Chong, 2022).

Increased exposure to ammonia ($NH4^+$) changes metabolic status in aquatic vertebrates, impair muscle contraction due to competition with potassium ions present in the muscle membrane (Sinha et al., 2012). It also causes neurotoxicity by depolarizing the neurons and depleting ATP, which can lead to cell death (Rodrigues et al., 2014). Elevated nitrite concentrations cause acute toxicity in aquatic animals. Nitrite exposure affects blood parameters, leading to methemoglobin or ferrihemoglobin hypoxia, and haemolytic anemia. It causes tissue impairment and metabolism injure (Xiang et al., 2010; dos Santos Silva et al., 2018). Nitrate can enter the body of fish and crustaceans through diffusion in the branchial epithelium (Jensen, 1996; Stormer et al., 1996). Elevated nitrate concentration in the body affects the food intake, growth rate (Schram et al., 2014; Stelzer & Joachim, 2010), swimming performance, reproductive capacity (Alonso & Camargo, 2013; Egea-Serrano & Tejedo, 2014), developmental alteration (Mallasen et al., 2003) and survival rate (Camargo & Alonso, 2006).

Nitrate toxicity to aquatic animals increases with the increase of concentration of nitrate and its exposure time (Camargo et al., 2005). The low pH of water also enhances the effect of nitrate toxicity on aquatic animals. At low pH, electrolytes such as Na^+ and Cl^- are lost from the body, and to balance the electrolytes metabolic energy is needed. Therefore, simultaneous exposure to both low pH and elevated nitrate concentration increases maintenance costs and reduce maximum oxygen uptake, which negatively impacts on growth and activity of animals (Wood & Rogano, 1986, 1993). In contrast, water salinity and increasing body size also cause a general decrease in nitrate toxicity (Camargo et al., 2005).

Alteration in performance of aquatic animals in nitrate toxicity is because of reduced oxygen-carrying capacity of the blood. The oxygen-carrying pigments hemoglobin and hemocyanin transformed into the non-oxygen-carrying pigment methemoglobin, which results in decreased oxygen levels in the blood. So, the toxicity mechanism of nitrate is similar to that of nitrite as nitrate is ultimately transformed into nitrate *in-vivo* (Scott & Crunkilton, 2000; Cheng & Chen, 2002; Camargo et al., 2005).

Humans are exposed to environmental nitrate through public drinking water supplies. Drinking water nitrate concentration has increased in many regions mostly due to the application of animal manure and inorganic fertilizer in agricultural lands. The safe level of nitrate in public water supply has already been set to prevent infant methemoglobinemia, although other health issues were not considered. The risk of various cancers, birth defects, thyroid disease, and other health damage can occur due to nitrate intake through drinking water that increases the formation of N-nitroso compounds (Ward et al., 2018).

The major focus of this present review is to discuss the implications of elevated nitrate levels in aquatic ecosystems and the acute toxic effect of nitrate-N on aquatic invertebrates and fish. This review also focuses on the link between nitrate contamination in drinking water and adverse health outcomes like methemoglobinemia, colorectal cancer, thyroid disease, and neural tube defect in the human population.

2 Source of nitrate pollution and its environmental fate

Some major sources of nitrogen compounds in the aquatic ecosystem include atmospheric deposition, surface, and groundwater runoff from fertilized agricultural lands, municipal and industrial waste disposal, decaying plant debris, N_2 fixation by certain prokaryotes, etc. (Dauda et al., 2019; Xue et al., 2016). Inorganic nitrogen can infiltrate aquatic ecosystems from both point and nonpoint sources resulting from human activity in addition to natural sources (Howarth, 1988; Guildford & Hecky, 2000). Due of their size and difficulty in control, nonpoint sources are typically more important than point sources (Howarth, 2005). Additionally, anthropogenic inputs of organic and particulate nitrogen into the environment can lead to inorganic nitrogen pollution (Stevens et al., 2011; van den Berg et al., 2016). As a result, inorganic nitrogenous compound concentrations (NH_4^+, NO_2, and NO_3) in ground and surface waters are rising globally, having a significant impact on a variety of aquatic creatures and ultimately contributing to the decline of freshwater, estuarine, and coastal marine environments (Galloway & Cowling, 2002; Lacerda et al., 2018; Norton & Ouyang, 2019; de Carvalho et al., 2021; Zhao et al., 2021). Nitrate is added to act as a reservoir for nitrite (Lundberg et al., 2009). The oxidation of human and animal excrement causes nitrate to enter surface water and groundwater as a result of agricultural operations such the excessive use of nitrogenous fertilisers and manures, wastewater treatment, and nitrate oxidation (Mahvi et al., 2005; Sahoo et al., 2016). Nitrate levels in surface and groundwater naturally range from 5-100 mg/l but can increase up to 500-1000 mg/l due to natural and anthropogenic activities (Galloway et al., 2004; Vitousek et al., 1997).

Although nitrate is typically thought of as a benign substance, prolonged exposure to high nitrate levels has significant effects on aquatic life (Isaza et al., 2020b). The fate of nitrate is determined by the surrounding environment and elements including the presence of organic materials and rainfall. *Nitrosomonas* transforms nitrate from nitrate in oxygen-deficient water (WHO, 2016). The nitrogenous wastes both organic as well as inorganic forms in soil, decomposed primarily to give ammonia, subsequently, oxidized to nitrite and nitrate (Bernhard, 2010). The nitrate released is drawn by plants which are required for their growth, and building of complex organic nitrogenous compounds (Fatta et al., 1999).

3 Toxicity to aquatic invertebrates

A predicted unimodal relationship states that nutrient enrichment may cause a subsidy-stress effect. A small number of nutrients prompt primary productivity, thereby, delivering advantage to the community composition as well to biodiversity. Uncurbed nutrients often lead to algal blooms, oxygen depletion, and deteriorating habitat conditions creating environmental stress, and biodiversity loss (Niyogi et al., 2007). The nitrate-nitrogen along with sediment and low flows has been predicted to have cumulative stress on freshwater communities (Wagenhoff et al., 2011; Piggott et al., 2012). Nitrate toxicity to aquatic invertebrates depends on the nitrate concentration and exposure time. Increasing body size, the salinity of water, and environmental adaptation generally decreased the toxic impact of nitrate on aquatic animals.

Soucek and Dickinson (2012) tested the 96 h LC_{50} value for two freshwater unionid mussels (*Lampsilis siliquoidea* and *Megalonaias nervosa*), a fingernail clam (*Sphaerium simile*), two stonefly species (*Allocapnia vivipara* and *Amphinemura delosa*), and an amphipod (*Hyalella azteca*). They observed a wide variety of sensitivity to nitrate, with the LC_{50} value ranging from 357 to 937 mg of NO_3-N/l. The nitrate sensitivity order was as follows: *L. siliquoidea*>*S. simile*>*A. delosa*>*H. Azteca*>*A. Vivipara*>*M. nervosa*. Therefore, no clear trend in nitrate sensitivity for particular taxonomic groups was observed by Soucek and Dickinson (2016).

Wang et al. (2020) examined the acute and chronic toxicity of sodium nitrate to unionid mussel

(*Lampsilis siliquoidea*) and a midge (*Chironomus dilutus* and reported the midge as more sensitive to nitrate compared to the unionid mussel. The mussel and midge showed the median effect concentrations (EC_{50}) of 665 mg NO_3-N/l and 189 mg NO_3-N/l respectively and chronic effect concentrations of 17 mg NO_3-N/l and 9.6 mg NO_3-N/l respectively. Yildiz (2004) reported the acute toxicity range of nitrite on narrow-clawed crayfish, *Astacus leptodactylus,* between 22 and 70 mg/l after 48 hours (mean 29.43 mg/l) of exposure. Environmental chloride (100mg/l chloride) elevated the toxicity of nitrite ranging between 31-80 mg/l after 48 hours (mean 49.20 mg/l) of exposure (Yildiz, 2004). Garai et al. (2022) investigated the toxicity of nitrate on freshwater oligochaeta (Annelida) worm *Tubifex tubifex*. The 96 h LC_{50} value of nitrate to *T. Tubifex* is 664.38 mg/l. The LC_{50} values for different aquatic invertebrate species reported by different investigator are denoted in Table 1.

In order to investigate effect of ionic strength on nitrate toxicity Baker et al. (2017) conducted a chronic toxicity test for aquatic invertebrates *Hyalella azteca*, midges (*Chironomus dilutes*), daphnids (*Ceriodaphnia dubia*) with different of ion concentration. The result explained that lower nitrate toxicity associated with higher concentrations of major ions in water. *C. dubia* was found to be the most sensitive species, with IC_{25} values ranging from 13.8 to 47.5 mg/l NO_3-N, as the increase of water hardness. IC_{25} values of *C. dilutus* and *H. azteca*, were from 48.8 -178 mg/l NO_3-N and 12.2 - 181 mg/l NO_3-N respectively, as the water hardness increases. Further, Soucek and Dickinson (2016) have reported a chronic nitrate toxicity test results in various chloride concentrations of water for two crustaceans *Hyalella azteca* and *Ceriodaphnia dubia*. *H. azteca* appeared to be very sensitive to nitrate exposure and *C. dubia* was not being as sensitive. There was a clear relationship between chloride concentration in water and chronic nitrate toxicity in the case of *H. Azteca,* but this relationship was not established for *C. dubia.*

To understand the toxic impact of nitrate on marine and freshwater invertebrates and to provide a general nitrate sensitivity order, we have generated a species sensitivity distribution (SSD) curve by considering available 96h LC_{50} values. SSD is a bell-shaped distribution curve represents the species sensitivity distribution to a particular environmental stressor (Posthuma & de Zwart, 2012). Among the aquatic invertebrate species taken, the sensitivity order for nitrate is *Hydropsyche occidentalis, Cheumatopsyche pettiti*, *Lampsilis siliquoidea, Sphaerium simile, Amphinemura delosa, Potamopyrgus antipodarum, Hyalella azteca, Allocapnia vivipara, Megalonaias nervosa, Penaeus monodon* and *Crassostrea virginica* respectively (Fig. 1). This SSD curve agreeing the nitrate sensitivity data with higher ion concentration and showed that freshwater invertebrates are more sensitive towards nitrate exposure than marine invertebrates.

In addition to acute and chronic toxicity, the question of whether nitrate accumulates in body tissue arises. Cheng et al. (2002) investigated nitrate build-up in different tissues of the penaeid shrimp *Penaeus monodon*. After 24 h of exposure to 3.646, 21.234 and 36.079 mM of nitrate, the accumulation was measured in muscle (0.202, 0.854 and 0.980 μmol/g), hepatopancreas (0.330, 1.139 and 1.552 μmol/g), heart (0.527, 1.468 and 1.879 μmol/g), foregut (0.632, 2.195 and 3.341 μmol/g), gills (0.927, 2.398 and 3.325 μmol/g), hemolymph (0.946, 3.327 and 3.948 μmol/g), eyestalk (1.214, 3.461 and 4.264 μmol/g) and midgut (1.529, 3.343 and 5.239 μmol/g) respectively. The concentration of nitrate in muscle was lowest, and the midgut was highest among different tissues tested. Taken together the accumulation was increased directly with nitrate concentration in the surroundings and the exposure time, except for muscle. The investigation by Garai et al. (2022) elucidated that the combined effect of physiological stress biomarkers gradually increased with increasing exposure time and nitrate in *Tubifex tubifex*. The increased level of stress biomarker during chronic exposure of nitrate indicates higher oxidative stress response in *T. tubifex*.

4 Toxicity to fish

Nitrite toxicity to fish also varies with different external and internal factors i.e. water quality, fish species, body size, and individual fish susceptibility (Kroupova et al., 2005).

Table 1. Comparative toxicity of nitrate-nitrogen (NO_3-N) to aquatic invertebrates

Species	Developmental stage	Aquatic medium	Toxicological parameter (mg NO_3-N/l)	References
Hydropsyche occidentalis	Early instar larvae	Freshwater	148.5 (72 h LC_{50})	Camargo & Ward (1992)
	Early instar larvae	Freshwater	97.3 (96 h LC_{50})	
	Early instar larvae	Freshwater	65.5 (120 h LC_{50})	
	Late instar larvae	Freshwater	183.5(72 h LC_{50})	
	Late instar larvae	Freshwater	109.0 (96 h LC_{50})	
	Late instar larvae	Freshwater	77.2(120 h LC_{50})	
Cheumatopsyche pettiti	Early instar larvae	Freshwater	191.0 (72 h LC_{50})	Camargo & Ward (1992)
	Early instar larvae		113.5 (96 h LC_{50})	
	Early instar larvae		106.5 (120 h LC_{50})	
	Late instar larvae		210.0 (72 h LC_{50})	
	Late instar larvae		165.5 (96 h LC_{50})	
	Late instar larvae		119.0 (120 h LC_{50})	
Paracentrotus lividus	Juveniles (2.7–5.9 g)	Seawater	100 (15 days safe level)	Basuyaux & Mathieu (1999)
Haliotis tuberculata	Juveniles (12–14.4 g)	Seawater	250 (15 days safe level)	Basuyaux & Mathieu (1999)
Ceriodaphnia dubia	Neonates (<24 h)	Freshwater	374 (48 h LC_{50}) 21.3(48 h NOEC) 42.6(48 h LOEC)	Scott & Crunkilton (2000)
Daphnia magna	Neonates (<48 h)	Freshwater	462(48 h LC_{50}) 358 (48 h NOEC) 717 (48 h LOEC)	Scott & Crunkilton (2000)
Potamopyrgus antipodarum	Adults (2.6–3.8 mm)	Freshwater	535(96 h LC_{50})	Alonso & Camargo (2003)
Lampsilis siliquoidea	Juveniles (5 days old)	Freshwater	357 (96 h LC_{50})	Soucek & Dickinson (2012)
Megalonaias nervosa	Juveniles (5 days old)	Freshwater	937 (96 h LC_{50})	Soucek & Dickinson (2012)
Sphaerium simile	Juveniles (14 days old)	Freshwater	371 (96 h LC_{50})	Soucek & Dickinson (2012)
Allocapnia vivipara	Late-instar nymphs.	Terrestrial	836 (96 h LC_{50})	Soucek & Dickinson (2012)
Amphinemura delosa	Late-instar nymphs.	Terrestrial	456 (96 h LC_{50})	Soucek & Dickinson (2012)
Hyalella azteca	Adults	Freshwater	667 (96 h LC_{50})	Soucek & Dickinson (2012)
Hyalella azteca	Adults (7-9 days old)	Freshwater	210 (96 h LC_{50} at chloride conc. 9.9 mg/l) 516 (96 h LC_{50} at chloride conc. 24.6 mg/l) 736 (96 h LC_{50} at chloride conc. 97.6 mg/l)	Soucek & Dickinson (2016)
Ceriodaphnia dubia	Neonates (< 24 h old)	Freshwater	665 (48 h LC_{50} at chloride conc. 10.1 mg/l) 671 (48 h LC_{50} at chloride conc. 24.9 mg/l) 502 (48 h LC_{50} at chloride conc. 48.7 mg/l) 453 (48 h LC_{50} at chloride conc. 96.6 mg/l)	Soucek & Dickinson (2016)
Hyalella azteca	6-8 days old amphipods	Freshwater	124-622 (14 days LC_{50} with the increase of water hardness)	Baker et al. (2017)
Chironomus dilutes	Third-instar larvae	Freshwater	114-342 (10 days LC_{50} as the water hardness increases)	Baker et al. (2017)
Ceriodaphnia dubia	< 24 h old daphnia	Freshwater	62-127 (7 days LC_{50} as the water hardness increases)	Baker et al. (2017)
Lampsilis siliquoidea	Juveniles (6 days)	Freshwater	665 (Acute EC_{50})	Wang et al. (2020)
Chironomus dilutus	Larvae (7 days)	Freshwater	189 (Acute EC_{50})	Wang et al. (2020)
Tubifex tubifex	Adults	Freshwater	664.381(96h LC_{50})	Garai et al. (2022)

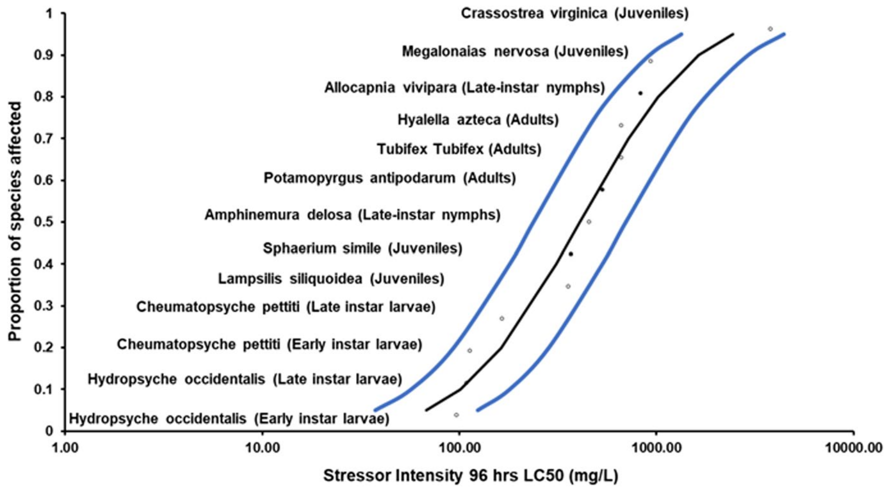

Fig. 1 Species sensitivity distribution curve for aquatic invertebrates to nitrate toxicity. The black line denotes central tendency and blue lines denotes 95% confidence intervals

Kincheloe et al. (1979) showed species-specific early fry mortality at increasing nitrate concentration. At 20 mg/l of nitrate exposure, chinook salmon egg and fry showed a significant increase in mortality rate. Rainbow trout fry showed increased mortality at 10 mg/l of nitrate exposure. Early fry of coho salmon and steelhead trout had no significant alteration in mortality rate after increasing the dose of nitrate. The early fry stage of lahontan cutthroat trout showed significantly high mortality at 30 mg/l of nitrate concentration. LC_{50} values obtained due to exposure of nitrate-nitrogen (NO_3-N) to fishes are listed in Table 2.

In order to compare nitrate-nitrogen (NO_3-N) to different freshwater and marine fishes, we have generated a species sensitivity distribution (SSD) curve by considering available 96 h LC_{50} values. Among the freshwater and marine fish species taken, the order of sensitivity for nitrate is *Poecilia reticulates*, *Monocanthus hispidus*, *Litopenaeus vannamei*, *Raja eglanteria*, *Trachinotus carolinus*, *Micropterus treculi*, *Oncorhynchus tshawytscha*, *Pimephales promelas*, *Ictalurus punctatus*, *Rachycentron canadum*, *Lepomis macrochirus*, *Centropristis striata* and *Pomacentrus leucostritus* respectively (Fig. 2). *Poecilia reticulates* is a freshwater fish is the most sensitive fish to nitrate. On another hand, *Centropristis striata* and *Pomacentrus leucostritus* are two marine fish species have very high LC_{50} value as compared to other freshwater fish. So overall, this SSD curve also explains that marine fish species are generally less sensitive towards nitrate toxicity than freshwater fish species. Yildiz et al. (2006) reported that nitrite exposure in the range of 0.50 and 1.38 mg/l caused a rise in methemoglobin levels in Nile tilapia (*Oreochromis niloticus*), however, methemoglobin percentages ranging from 16% to 42% represented a mild methemoglobinemia. Yildiz et al. (2006) also observed considerable lowering of hematocrit and haemoglobin levels in Nile tilapia after exposure to nitrite.

Freshwater fishes take up nitrite through gills, which leads to a high rate of accumulation. Seawater fishes take nitrite through both gills and intestines (Jensen, 2003). Histopathological alteration of gills, esophagus, and brain was observed in acute exposure to sub-lethal concentrations of nitrate. Gill revealed lamellar fusion and hyperplasia of epithelium and lamellar shortening induced by necrosis. The esophagus showed hyperplasia of mucus cells and epithelium. The proliferation of glial cells and satellitosis (microglial cells surrounding neurons with swollen and prenecrotic neurons) were observed in the brain (Rodrigues et al., 2011). Davidson et al. (2014) observed slow growth and decreased survival rate in juvenile rainbow trout *Oncorhynchus mykiss* at high nitrate (80-100 mg/l) exposure. Luo et al. (2016) also observed decreased growth rate, developmental retardation and increased mortality rate in rare minnow *Gobiocypris rarus* to *a* high dose of nitrate exposure.

Table 2. Comparative toxicity of nitrate-nitrogen (NO_3-N) to fishes

Species	Developmental stage	Aquatic medium	Toxicological parameter (mg NO_3-N/l)	References
Poecilia reticulatus	Fry	Freshwater	267 (24 h LC_{50}) 219 (48 h LC_{50}) 199 (72 h LC^{50}) 191 (96 h LC_{50})	Rubin & Elmaraghy (1977)
Lithognathus mormyrus	Fingerlings	Seawater	3450 (24 h LC_{50})	Brownell (1980)
Diplodus sargus	Fingerlings	Seawater	3560 (24 h LC_{50})	Brownell (1980)
Heteromycteris capensis	Fingerlings	Seawater	5050 (24 h LC_{50})	Brownell (1980)
Micropterus treculi	Fingerlings	Freshwater	1261 (96 h LC_{50})	Tomasso & Carmichael (1986)
Pimephales promelas	Larvae	Freshwater	1341 (96 h LC_{50})	Scott & Crunkilton (2000)
Catla catla		Freshwater	1565.43 (24 h LC_{50}) in static system 1484.08 (24 h LC_{50}) in continuous flow-through system	Tilak et al. (2021)
Rachycentron canadum	Juvenile	Seawater	2407 (24 h LC_{50}) 1829 (96 h LC_{50})	Rodrigues et al. (2011)
Cyprinus carpio	Juvenile	Freshwater	1075.10 (24 h LC_{50}) in static system 967.63 (24 h LC_{50}) in continuous flow-through system	Rodrigues et al. (2011)
Litopenaeus vannamei	Juvenile	Seawater	900 (96 h LC_{50}) at a salinity concentration 3 g/l.	Valencia-Castañeda et al. (2019)

Nitrate transfer across erythrocytes membrane causes oxidation of hemoglobin to methemoglobin (metHb) which impairs the blood oxygen transport (Cameron, 1971). Smith and Williams (1974) reported methemoglobinemia and a 40% mortality rate after nitrite exposure in Rainbow Trout and Chinook Salmon. Gisbert et al. (2004) reported acute nitrite toxicity in *Siberian sturgeon* yearlings which caused severe methemoglobinemia, change in plasma electrolyte imbalance, and kidney Na^+–K^+ ATPase activities. At acidic pH, chronic exposure to nitrate (50 mg/l and 100 mg/l) in juvenile spangled perch, reduced the functional performance and compromised the blood oxygen-carrying capacity due to the

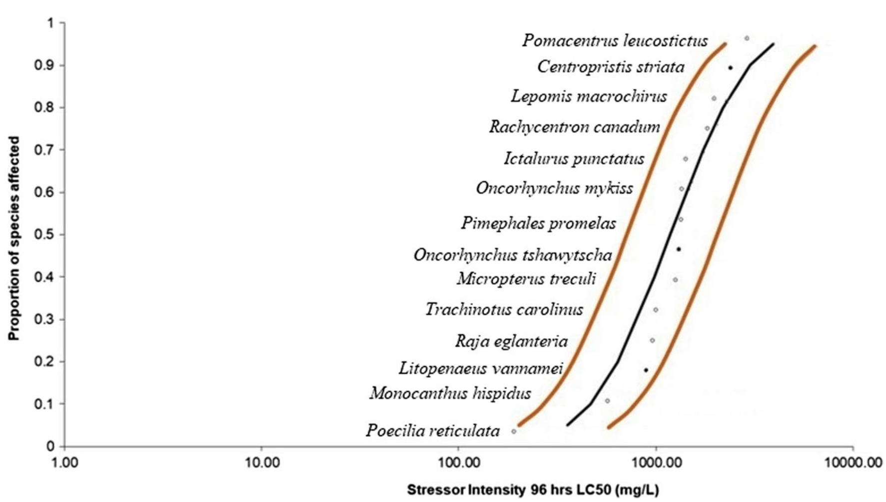

Fig. 2 Species sensitivity distribution curve for fishes to nitrate toxicity. The black line denotes central tendency and orange lines denotes 95% confidence intervals

reduction of hematocrit value, hemoglobin content, an increase of methemoglobin concentration (Isaza et al., 2020a). Nitrate accumulation in the blood plasma lead to depletion of chloride ions and caused potassium ion loss from skeletal muscle and erythrocytes, resulted in a reduction in cell volume and extracellular hyperkalemia (Knudsen & Jensen, 1997).

Aggergaard and Jensen (2001) reported that nitrite exposure to rainbow trout *Oncorhynchus mykiss,* increased the heart rate and influenced the cardiorespiratory function. Fish exposed to increased nitrate concentration showed higher creatinine levels in serum and lower chloride levels compared to the control fish (Hrubec et al., 1997). Nitrite can mimic nitric oxide and thereby inhibit the processes regulated by local hormones (Jensen, 2003). Nitrate exposure to zebrafish embryos showed neurotoxicity and acted as an endocrine disruptor possibly by the conversion to nitric oxide to downregulate the activity of dopaminergic neurons (Jannat et al., 2014).

5 Nitrate toxicity and disease progression

The use of high nitrate-containing drinking water is a very common risk factor for methemoglobinemia from fishes to human. Fish gills actively carry nitrates, which easily oxidise haemoglobin to produce methemoglobin. The detailed molecular mechanism of methemoglobinemia studied in human. The hemoglobin (Fe^{2+}) of the affected individuals transforms to methemoglobin (Fe^{3+}). 1.8 Å crystal structure of human hemoglobin (PDB: 3D7O) shows the nitrite anion binds at the sixth coordination position at the ferric (Fe^{3+}) moity of heme, hence become unable to transform oxygen (Fig. 3a-b). It causes 'blue-baby syndrome' to the affected infants. The principal characteristics of this syndrome are cyanosis (blue-grey skin colour) and become irritable or lethargic. If not treated properly; this condition can progress to some other severe symptoms like loss of consciousness, seizures, and ultimately death (Knobeloch et al., 2000). Infants with the age group of < 3 months are most susceptible to methemoglobinemia than adults because of less synthesis of NADH-cytochrome b5 reductase, which is the key enzyme to convert methemoglobin back to haemoglobin (Savino et al., 2006). Methemoglobinemia causes severe hypoxia in fishes that can result in abrupt death. Fish blood with high methemoglobin levels appears is brown in color, also refers to "brown blood illness" (Chong, 2022).

In addition to methemoglobinemia, nitrate exposure also causes cancer in various organs like the oesophagus, bladder, stomach, and colon (Ward et al., 2018). Nitrate possibly converts to nitrite under the condition of gastric achlorhydria and subsequently transforms into nitrosamines-substances, which is known for causing cancer in animals (Magee & Barnes, 1967). Morales-Suarez-Varela et al. (1995)

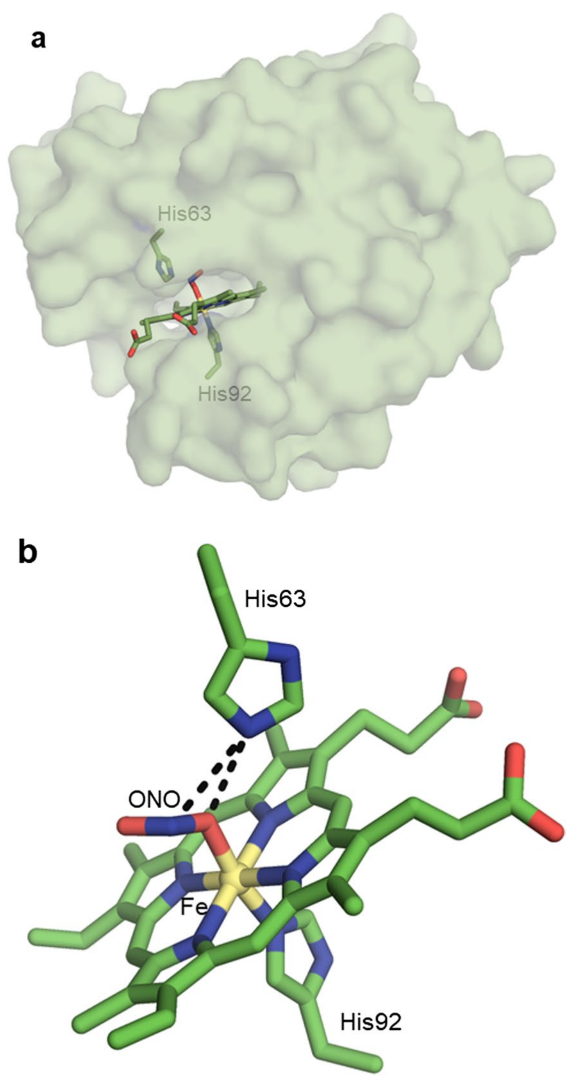

Fig. 3 Structure of methemoglobin. **a** Structure of hemoglobin protein shown in surface. Structure of heme bind with ortho-nitrito (ONO) shown in steak. **b** Zoomed in view of structure of ferric- heme- (ONO). (PDB ID: 3D7O)

reported a relationship between nitrate exposure and mortality rate in cancer of different age groups of peoples in the province of Valencia, Spain. They observed an increased mortality rate due to the relatively high risk of gastric and prostate cancer at the nitrate concentration > 50 mg/l in the drinking water. The person of age group 55-75 years, showed relative gastric cancer risk 1.91 for males and 1.81 for females. In the case of prostate cancer, the elevated relative risk was 1.86 and 1.80 for the age group of 55-75 and > 75 years respectively. Taneja et al. (2017) studied the case of gastrointestinal cancer (GI) in a population of 234 individuals from a rural area of Nagpur and Bhandara district of India. They reported 78 cases of GI cancer in 2 years period due to nitrate exposure from drinking water at the concentration of > 45 mg/l. Espejo-Herrera et al. (2015) reported a high risk of bladder cancer in Spain due to long-term exposure to nitrate-contaminated drinking water.

Ingestion of nitrate from contaminated drinking water, also affects the function of the thyroid gland, leading to the alteration of thyroid hormone concentration. Nitrate competitively inhibits the sodium-iodine symporter of thyroid follicles and thereby blocks the iodine intake by the thyroid gland. Therefore, ingestion of nitrate compromises the thyroid hormone synthesis, causes thyrotropin elevation. Ward et al. (2010) reported an increased risk of thyroid cancer in the population taking high concentrations of nitrate (>5 mg/L nitrate-N) through public water supply for a longer duration. Consumption of drinking water with high nitrate contamination (> 50 mg/l) caused the development of hypertrophy (van Maanen et al., 1994). Tajtáková et al. (2006) reported increased thyroid volume and a higher frequency of thyroid disorder in schoolchildren from high nitrate contaminated areas compared to low nitrate contaminated areas. Aschebrook-Kilfoy et al. (2012) observed that the exposure to nitrate at the concentration > 6.5 mg/l caused a high risk in subclinical hypothyroidism for women. An increased risk of type 1 diabetes mellitus was also found in people exposed to a high nitrate concentration of > 40-80 mg/l (Bahadoran et al., 2016).

Cedergren et al. (2002)reported an increased risk for a congenital cardiac defect in a population after exposure to nitrate contaminated drinking water. They have collected data from 58,669 women, who have taken nitrate-contaminated drinking water from the public water supply. Among the infants born, 753 showed cardiac defects.

Bukowski et al. (2001) investigated a major impact of groundwater nitrate concentration on intrauterine growth retardation and prematurity on Prince Edward Island. Among 4098 controls from the database, 210 cases showed intrauterine growth retardation and 336 cases showed premature birth. Maternal exposure to nitrate through drinking water during pregnancy reduced the weight and length of offspring which are the markers of intrauterine growth (Coffman et al., 2021). Tabacova et al. (1997) investigated the pregnant women, who have taken drinking water from an area contaminated by oxidized nitrogen compounds, showed complications in pregnancy. Among them, 67% of cases showed anaemia, 33% of cases had premature labour, and 23% of cases showed preeclampsia. Tabacova et al. (1998) showed that exposure to oxidized nitrogen compounds is linked with increased risk in neonatal health and more lipid peroxidation in both maternal and cord blood. A risk in central nervous system malformation due to nitrate exposure in drinking water was reported in New Brunswick, Canada by Arbuckle et al. (1988).

6 Concluding remarks

The data presented in this present review suggest that nitrate toxicity due to natural and anthropogenic sources may seriously affect both aquatic animals and human health. Nitrate concentrations in the water resources are increasing due to the use of nitrogen fertilizer and animal agriculture. Therefore, a safe level of nitrate as recommended by WHO guidelines should be maintained in the environment to protect living organisms. National and global efforts are needed to mitigate the nitrate concentration in water resources. Some of such water quality protection agencies which prevent nitrate pollution in surface and groundwater are International Nitrogen Initiative and EU Nitrates Directive (Musacchio et al., 2020; Bowen et al., 2005). Efforts of the EU Nitrates Directive include identification of most exposed areas, the establishment of good agricultural practices, crop rotation, national observation, and reporting that decrease the nitrate concentration in groundwater in some European countries (Hansen et al., 2011). Although in the U.S., the application of nitrogen fertilizer in the crop field is not regulated and efforts to maintain the nitrate concentration in surface and groundwater are voluntary (Dinnes et al., 2002).

Long-term studies are necessary to verify and enhance the suggested safe amount of nitrate for aquatic species notwithstanding the endorsement of this level. Further research is required into the effects of water parameters such as pH, temperature, hardness, salinity, dissolved oxygen content, and other chemical components on the toxicity of nitrate for aquatic species. In addition, aquatic species engage in a variety of biotic interactions, including as competition, parasitism, and predation. In order to comprehend how nitrate contamination affects biotic interactions and natural selection for aquatic organisms, field and laboratory investigations are required. For the human population, the most adverse health effect occurs due to the intake of nitrate-contaminated drinking water and an increase of endogenous nitrosation. Some recent studies have identified subgroups of the population with the increased potentiality of endogenous nitrosation. However, a direct method is needed to assess the individuals. New tools are now available for epidemiologic studies to quantify the bacterial DNA, the relative abundance of oral microbiomes, and to characterize them (Vogtmann et al., 2017; Sinha et al., 2017). More studies are also needed to understand the nitrate-reducing capacity of oral microbiomes and to determine the factors that can modify their capacities like oral hygiene, food, and periodontal disease.

Most countries throughout the world, including the United States, South Korea, Europe, and India, are considered nitrate contaminated as the concentrations detected there exceed the allowed and acceptable limits set by environmental agencies such as the USEPA, WHO, and others. Several studies have been undertaken in India to determine the extent of nitrate contamination in ground and surface water habitats. As a result, the only way to lessen the related health risks is to remediate nitrate-contaminated soil and water. Reverse osmosis, chemical denitrification, biological denitrification, electro dialysis and adsorption are some of the processes that have proved effective and are now in use. The employment of greener and newer ways to remove nitrate, such as various nanocomposites, halloysite nanotubes, and nanorods, may improve nitrate removal efficiency. The limitations of recycling and separation from water can be overcome by immobilising these nanocomposites or nanotubes on a membrane. Sensing approaches might appear to be a more suitable and reliable tool for quantifying and estimating nitrate ions in soil and water. However, further studies are needed to develop advanced systems for removing nitrate from the environment and sensing its presence when it exceeds permissible levels.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration

Conflict of Interest The authors declare no competing interests.

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