



## **The Effect of Some Toxic Anthropogenic Pollutants on Fish**

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### **Conclusion**

In conclusion, the presence of ammonium nitrate in the aquatic environment can cause genetic and carcinogenic effects on fish, Therefore, it is necessary to take strict preventive measures and hold accountable and punish anyone who dares to harm the general environment, especially the marine environment, and try to educate all segments of humanity about the need to avoid dumping pollutants into the aquatic environment. The difference here is The difference between it and the terrestrial environment is that waste on land can be easily collected, cleaned, or disposed of, while in the marine environment it is extremely difficult to do so, whether through the human, material, or time-consuming efforts it requires the decline of forest areas, soil depletion, insufficient water resources, and the deterioration of fisheries are factors that threaten the quality of life and health of developing countries and make them more vulnerable to disasters, not to mention the organic pollution that occurs in fresh river water and all of it is inorganic and is not in the interest of the people living around these water sources, from which they live off their products for themselves and others, from various crops, whether plant or animal, therefore, i continue to repeat and reiterate that it is necessary to during scientific

forums, we encourage the relevant decision-makers to put in place controls that would stop any reckless person who deliberately pollutes the environment, disregarding the interests of others in his community for the sake of his own interests and benefits personality, as well as individuals who dump their waste and filth into flowing rivers that carry everything deposited in them and distribute it to distant areas unaware of the toxins and dangers they contain for humans, the animal and plant worlds, and all living things.

### Keywords:

Waste – Pollutants – Pesticides – Poisoning – Marine Environment – Freshwater

### Introduction

As is well known, marine waters occupy approximately **71% of the total surface area of the Earth**, with a total water volume estimated at about **1.4 billion cubic meters**, of which only **2.5% is freshwater**. The importance of water lies primarily in providing **oxygen**, followed by **salts**, among which **table salt** is the most important (Dr. Abdulhamid Mohammed).

The aquatic environment also includes approximately **150,000 species of aquatic organisms**, as well as about **70 billion tons of seaweeds, grasses, aquatic plants, algae, and large quantities of organic materials**.

Scientists predict that by the year **2010**, the world's population would reach approximately **7.3 billion people**, with more than **90% living in developing countries**, where about **20% of the population suffers from chronic malnutrition**, particularly children. Recent estimates also indicate that by the year **2050**, the world population will rise to about **12 billion people**, nearly **60% of whom will reside within a coastal zone extending 60 km inland**. Agricultural and industrial activities required to serve this population are expected to significantly increase the already enormous pressures on fertile coastal regions (Ahmed Saad El-Din).

One of the most pressing global concerns today is the increasing **pollution of the marine environment**, which has begun to cause the emergence of numerous pathological symptoms among aquatic organisms, particularly fish. A notable example is what occurred in **Japan**, where mercury levels rose sharply, leading to what became known as **Minamata disease**. The amount of mercury discharged into the environment from agricultural activities reached **four times or more** than the quantity produced naturally.

In addition, many diseases are currently widespread among both **marine and freshwater organisms**, including **gill diseases, skin diseases, and liver diseases**.

As for the sources of pollution, they are numerous and difficult to enumerate individually. However, the most important major sources that primarily contribute to the pollution of **seas, oceans, and rivers** can be summarized as follows:

1. **Industrial pollutants** from factories constructed near coastlines and rivers.
2. The increasing construction of **thermal power stations and nuclear reactors** used for electricity generation, which discharge large quantities of **hot water** into lakes, seas, and oceans. This has led to increased pollution known as **thermal pollution**, causing a sharp rise in fluctuations of the **physicochemical factors** of aquatic environments and introducing new elements whose harmful effects negatively impact the living conditions of aquatic organisms. This results in what is known as a **shock to aquatic fauna and flora** in rivers, artificial lakes, seas, and oceans worldwide.
3. **Radioactive waste**, which is well known for its extreme danger.
4. **Waste disposal sites (landfills)**.
5. **Sewage effluents**, containing chemical substances such as disinfectants, detergents, and mineral oils from car-washing stations and fuel stations.
6. **Agricultural pesticides**.
7. **Mining activities** along coastlines and underwater.
8. **Hospital waste**.
9. **Coastal pollution caused by rainwater runoff** carrying various pollutants from alleys and public streets.

Finally, and not least, **marine accidents involving large oil tankers** and the resulting pollution of the marine environment. Despite the strenuous and costly efforts made to clean up such pollution, they often prove to be **ineffective in practice**. Estimates indicate that **land-based sources of pollution account for approximately 44% of the pollutants that ultimately reach the sea**.



**Sewage Outfall**

Atmospheric inputs (impacts) contribute an estimated **33% of pollutants**. In contrast, **marine transportation** is responsible for approximately **12% of pollution**, which may lead to a **decrease in dissolved oxygen levels** and an **increase in carbon dioxide concentrations**, ultimately resulting in the **poisoning of fish and all aquatic organisms**.

In addition, the pollution of **inland waters (rivers and lakes)** in particular, and **seas and oceans** in general, by **industrial and consumer wastes manufactured on chemical bases**, has negatively affected the **hydrochemical system of the aquatic environment**. These adverse effects have been reflected in the living conditions of the various aquatic organisms inhabiting this environment.

This situation necessitates the activation and enforcement of **all international agreements and regulations**, as well as strict monitoring of the implementation of conditions and laws that would hold **any country in the world accountable** if it neglects proper monitoring of its waste and fails to prevent pollution of its coastlines. Otherwise, pollution—with its increasing destructive power—may become a **major environmental disaster** that is difficult to avoid in the short term and may lead to **serious problems in the long term**.

In the case of the **Exxon Valdez oil tanker**, which ran aground in **Alaska in 1989**, the effects of the oil spill that occurred more than **15 years ago** are still evident. A similar situation occurred in the **Prestige incident**, when the tanker sank off the **Spanish coast in late 2002**, resulting in **severe economic losses**, as it polluted more than **100 beaches in France and Spain** and severely impacted **local fishing activities** (Internet).

### Objective of the Study

It is of serious concern that **seafood consumed by populations in warm regions** has also been affected by **persistent organic pollutants (POPs)**. **Fat-rich fish** act as **bioaccumulative reservoirs** for these pollutants, which means that they are ultimately **transferred to consumers**. Consequently, the **processing of fish and the marketing of their meat and fats** represent important pathways for the transfer of organic pollutants to humans.

In some countries, **fish and farmed seafood**, as well as **livestock used for dairy production, poultry, and pork products**, are used as **feed ingredients for fish**, thereby creating **additional pathways** for the transfer of these substances to humans.

Regardless of the harmful substances that these products may carry—such as **fungi and pathogenic microorganisms**—it is essential to **monitor and regulate the sources of these wastes supplied to feed manufacturing plants**, to conduct thorough inspections, and to evaluate all such materials before they are used as **protein sources for other organisms**. This process can be relatively **easy to control and manage**. However, monitoring **fish carrying certain types of toxins** that have been transferred through the aforementioned sources is far more difficult, due to several factors, including:

1. The **large diversity of fish species and other mixed aquatic organisms**, which makes it difficult to examine representative samples to ensure their safety.
2. The **multiplicity of pollution sources** and the inability to fully control them or prevent human-induced environmental contamination.
3. Although only a **very limited number of countries** are committed to preserving the quality of their coastal environments, **aquatic organisms may still enter their territorial waters**, making them easily caught among other fish.
4. Despite the ability of governments to regulate and **health-monitor official seafood markets**, there remains a group of **recreational or amateur fishers** who operate **outside the scope of health surveillance**.

Accordingly, the **simplest and most effective approach** to reducing cases of **poisoning from marine organisms** is for each country to implement **awareness and guidance programs**, and to ensure that all seafood harvested from seas or rivers is **safe and fit for human consumption**. This can be achieved by establishing **multiple testing and analysis centers** to facilitate the examination of caught organisms. In addition, public awareness and warnings should be

disseminated through **local media outlets** regarding the dangers of consuming fish caught near **pollution sources**, such as **sewage outlets and drainage channels**.

Although the world has already begun to move toward the establishment of (**Inshore Aquaculture Farms**) and to reduce the use of **floating cage systems**, due to the potential harm caused by exposure to various pollutants and the spread of diseases among fish living in natural aquatic environments—as well as the environmental and technical challenges associated with this type of aquaculture—concerns still remain regarding the **introduction of external pollutants** through the **water sources on which aquaculture farms depend**. For this reason, the discussion here has been intentionally limited to pollutants that have **actually entered aquaculture ponds**, exerting severe effects that have led to the **suspension of entire production seasons**, including **mineral oil (petroleum-based) pollutants** and **chemical substances** discharged into the sea, among others.



### Marine Water Pollution by Human Waste

Accordingly, and in light of these environmental phenomena—which have become increasingly **abnormal and alarming**—it has become **imperative for every aquaculture farmer** to adhere to certain essential guidelines to ensure that **their farms are not contaminated**, and consequently that **their fish or shellfish are not poisoned**. This can be achieved through **continuous monitoring and careful management of the water intake sources** supplying their ponds.

### Research Methods

#### First: Materials Used (Water and Fish)

To maintain **water quality** and prevent sudden problems that may lead either to **fish mortality** or to the **accumulation of toxic substances in fish tissues**, which would subsequently be transferred to human consumers, it is essential to conduct **daily tests of several key parameters** important for fish health. Some parameters require **continuous monitoring throughout the day**, such as **dissolved oxygen levels in ponds**, and appropriate precautionary measures must be taken to address any deficiency.

Accordingly, **daily monitoring of water temperature**, testing of **alkalinity and acidity (pH)**, **microscopic examination of water**, and other relevant analyses should be carried out regularly.

Periodic examination of fish is also necessary to assess their **health status**, record **growth rates**, and conduct **external examinations of sampled fish** to detect and observe any **clinical signs, pathological symptoms, or external parasitic infections** that may occur. It is well known that fish are among the **most sensitive organisms**, and therefore any negligence or failure to implement the above measures may lead to a **serious disaster that would be difficult to compensate for**.

### Main Methods for Measuring the Above Factors

#### First: Measurement of Salinity

Salinity is measured using a device known as a **Salinometer**, a **Refractometer**, or by **volumetric analysis (Mohr's method)**.

#### Second: Measurement of Dissolved Oxygen

The concentration of **dissolved oxygen in water** is measured using a device known as a **Dissolved Oxygen Meter (DO meter)**.

#### Third: Measurement of Nitrate Concentration

Nitrate concentration is determined using the **Sodium Salicylate method**, with measurements taken using a **spectrophotometer** at a wavelength of **420 nanometers**.

#### Fourth: Measurement of Fish Growth Rates

Growth rates in fish are calculated as follows:

##### a. Growth rate / weight gain

= (Final fish weight – Initial fish weight) ÷ Initial fish weight

##### b. Daily weight gain (DWG)

= (Final weight – Initial weight) ÷ (Number of experimental days)

##### c. Feed Conversion Ratio (FCR)

= Dry feed supplied (g) ÷ Weight gain of fish (g)

##### d. Protein Efficiency Ratio (PER)

= Weight gain of fish (g) ÷ Protein intake (g)

### Second: Methods Used

#### Experiment No. (1)

**Relationship between variations in water temperature, salinity, and dissolved oxygen (D.O.) in a tilapia fish tank**



1. Fish tanks were prepared for the experiment with dimensions of **30 × 40 × 40 cm**. Each tank was equipped with an **air pump** to supply atmospheric oxygen in the form of bubbles, and **automatic electric heaters** were installed to allow precise control of the required temperature. The initial water temperature was set at **20°C**. The tanks were filled with **tap water to 80% of their capacity** (approximately **40 liters per tank**) and left for **48 hours** to allow the removal of chlorine, with aeration devices operating immediately after filling.

A total of **10 healthy, active, disease-free live fish** of Nile tilapia (*Tilapia nilotica*) were placed in **Tank No. (1)**. The fish were left for **one week** to acclimatize to the new glass tank environment, initially **without feeding**.

2. The temperature of **Tank No. (1) (test tank)** was gradually increased from **20°C to 22°C, 24°C, 26°C, and finally 28°C**, using the heater installed inside the tank.
3. The **salinity level** in **Tank No. (1)** was increased as follows: **less than 5, 10, 15, 20, 30, and 35 mg/L**, by adding different measured amounts of **sodium chloride (NaCl)**. All other factors—such as **lighting, water hardness, fish age, and feed weight**—were kept constant.
4. The same conditions were applied to **Control Tank No. (2)**, where the temperature was maintained at **22°C**, the **pH** was adjusted to **7.8**, the **dissolved oxygen concentration** was kept at **5 mg/L**, and the **salinity** did not exceed **20 mg/L**. A total of **10 healthy fish** of the same species used in Tank No. (1) were then introduced.
5. The **dissolved oxygen concentration** in **Tank No. (1) (test tank)** was measured, and changes in dissolved oxygen were recorded with each increase in **temperature and salinity**. Salinity measurements were conducted using **volumetric analysis (Mohr's method)**.



**Tank No. (1)**



**Tank No. (2)**

- **Experimental Tank (Tank 1) Control Tank (Tank 2)**

**Measurement of Nitrate Concentrations ( $\text{NO}_3^-$ ) Formed from the Ionization of Ammonia in Tilapia Tanks as a Result of Overfeeding**

## Experiment No. (2)

1. The tanks were prepared in the same manner as described in **Experiment No. (1)**, and **ten Nile tilapia (*Tilapia nilotica*)** were placed in **Tank No. (1)**.
2. Water temperature was gradually increased according to the following sequence: **10 – 12 – 16 – 18 – 20 – 22 – 24 – 26 – 28 °C**.
3. The **pH level** was gradually increased as follows: **7.8 – 8.2 – 8.4 – 8.6 – 8.8 – 9.0 – 9.2 – 9.4 – 9.6**.
4. A quantity of **5 mg of feed** was added daily for a period of **30 days** to allow for **metabolic conversion**, whereby protein-containing feed is metabolized and excreted by fish in the form of **ammonia**, which is subsequently oxidized in the tanks from **unionized ammonia to nitrate ( $\text{NO}_3^-$ )**.
5. The **nitrate concentrations** resulting from oxidation in the tanks were measured with each change in **pH** and increase in **water temperature**.
6. **Ten Nile tilapia** were placed in **Control Tank No. (2)** under the same water volume, with a constant temperature of **22°C**, suitable **pH (7.8)**, **dissolved oxygen concentration of 5 mg/L**, and **constant salinity not exceeding 20 mg/L**, while maintaining all other factors constant, such as **tank lighting and water hardness**.

## Method for Measuring Nitrate Concentration

1. A solution of **sodium potassium tartrate** was prepared by dissolving **60 g of pure salt** and **400 g of sodium hydroxide** in **1000 mL of distilled water**, and stored in a plastic container.
2. **Dry sodium salicylate** was used.
3. A **standard nitrate solution of known concentration** was prepared by dissolving **0.7218 mg of potassium nitrate**, previously dried at **105°C for 24 hours**, in a small amount of distilled water. The solution was then diluted to a final volume of **1 liter** with distilled water after calculating the solution volume, followed by the addition of **2 mL of chloroform**. The solution was stored in a **colored glass bottle**. This stock solution contained **100 µg nitrate per 1 mL**.

From this stock solution, a series of standard concentrations were prepared to construct a **calibration curve** as follows: **10, 20, 30, 40, 50, 60, 70, 80, 90, and 100**.

## Measurement Procedure

1. **100 mL** of the tank water sample was transferred into a glass beaker, to which **0.5 g of sodium salicylate** was added. The sample was then evaporated at **105°C**, transferred to a desiccator until cooled, followed by the addition of **2 mL of concentrated sulfuric acid**, **10 mL of distilled water**, and **15 mL of sodium potassium tartrate solution**. The mixture was then transferred to a **100 mL volumetric flask** and diluted with distilled water to the mark. A **pale yellow color** developed within **10–20 seconds**.
2. The **color intensity** was measured using a **spectrophotometer** at a wavelength of **420 nm**.

## Continuation of Previous Procedures



The **same steps** described previously were carried out with the **control sample**, using **distilled water instead of the experimental sample** (control condition), and measurements were recorded at **420 nm**.

The **calibration curve** was then calculated directly.

## Histological and Genetic Effects of Ammonium Nitrate on Fish Kidney and Blood

### Experiment No. (3)

1. The two tanks from previous experiments were prepared: **Test Tank (1)** and **Control Tank (2)** under suitable conditions for the fish, as follows:
  - **Temperature:**  $22 \pm 2$  °C
  - **pH:** 7.8
  - **Dissolved oxygen (O<sub>2</sub>):** 5 mg/L
  - **Salinity:** not exceeding 20 mg/L
  - Other factors such as **lighting, water hardness, and feeding** were kept constant.
2. **Tank (1) (Test)** was exposed to **ammonium nitrate at a concentration of 0.2 mg/L for one month**.  
After this period, **blood samples were drawn from the gills of fish in both tanks** to observe **histological and genetic changes**, and the **kidneys of fish from both Tank (1) and Tank (2)** were collected for further analysis.

### Preparation of Tilapia Chromosomes

1. Fish were **injected intraperitoneally** with **colchicine** at a dose of **0.01 mL per gram of fish weight**.
2. After approximately **three hours**, fish were sacrificed, and the **anterior portion of the kidney** was removed using forceps. The tissue was **Crushing in a hypotonic potassium chloride solution**, then fixed in a solution of **methanol and glacial acetic acid (1:3)**.
3. Cells were **spread on slides** and allowed to dry.
4. Slides were **stained with 7% Giemsa solution** prepared in **Phosphatase Solution**.
5. Cells were examined under a **light microscope** during the **metaphase stage**.

### Blood Sampling Method

1. The **gill arch** was removed from under the operculum.
2. The gill was placed on a **clean microscope slide**, leaving a drop of blood on the slide.
3. The blood drop was **quickly spread along the slide** before clotting occurred.
4. The sample was allowed to **air dry for approximately 2 hours**.
5. The slide was **stained with 7% Giemsa solution**.
6. Slides were examined under a **light microscope** to observe **changes in red blood cell morphology**.

### Experiment No. (4): Measurement of Growth Rates

1. The tanks were prepared as previously described (**Tank 1 and Tank 2**).
2. **Optimal growth conditions** were maintained, including:
  - **Water hardness:** 200 mg/L

- **Temperature:**  $22 \pm 2$  °C
  - **pH:** 7.8
  - **Dissolved oxygen (O<sub>2</sub>):** 5 mg/L
  - **Appropriate lighting**
3. **Feeding protocol:**
- **10 g of dry feed** containing **40% protein** (equivalent to **4 g protein per liter of tank water**) was added daily for **30 days**.
  - **Monitoring parameters:** respiration rate, growth, and sudden mortality were observed daily.
  - Water quality factors described in **Experiment No. (2)** were measured daily for each tank.
  - Feed was replenished **weekly** at the same rate.
4. **Fish weighing:**
- Before starting the experiment, all fish were weighed using a **precision balance**.
  - Final weight was recorded after **30 days of feeding**.

### Calculation of Growth Rates

(%) a. Weight Gain

$$100 \times \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} = (\%) \text{ Weight Gain}$$

b. Daily Weight Gain (DWG)

$$\frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Number of experimental days}} = \text{DWG (g/day)}$$

c. Feed Conversion Ratio (FCR)

$$\frac{\text{Dry feed given (g)}}{\text{Weight gain of fish (g)}} = \text{FCR}$$

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d. Protein Efficiency Ratio (PER)

$$\frac{\text{Weight gain of fish (g)}}{\text{Protein intake (g)}} = \text{PER}$$

4. **protein).**

This approach allows assessment of **maximum growth performance** over the three-month period.

### Results and Discussion

From the results obtained in **Tables 5 and 6** and **Figures 5 and 6**, it was observed that the **concentrations of ionized ammonia** increased with rising **pH** and **temperature**. The highest percentage of ionized ammonia was recorded at **pH 9.6** and temperatures of **10, 12, 16, 18, 20**,

22, 24, 26, and 28 °C, reaching 20%, 22%, 26%, 28%, 30%, 32%, 36%, 41%, and 42%, respectively.

Factors contributing to increased ammonia concentration in water include **higher pH** and **overfeeding**, which elevate the metabolic and catabolic conversion of food inside the fish, producing ammonia. This was clearly evident in **Table 5**, where the **highest percentage of ionized ammonia** reached **42%** at **pH 9.6** and **28°C**.

**Table 6** and **Figure 5** illustrate the **concentration of unionized ammonia** in the tanks, which reached its **maximum value (1.3 mg/L)** at **pH 9.6** and **28°C**. Similarly, **nitrate concentrations (NO<sub>3</sub><sup>-</sup>)** in tank water increased with higher pH and temperature (**0.66, 0.78, 0.84, 0.90, 0.96, 1.18, 1.30 mg/L**) at **pH 9.6** and temperatures of **10–28 °C**.

Increased nitrate levels can enter the **fish bloodstream through the gills**, combining with **hemoglobin** to form **methemoglobin**, which imparts a brownish color to the blood. This effect can be mitigated by **continuous water renewal** and providing **appropriate feed levels**. If uncontrolled, it may lead to **increased respiration rates, gill damage**, and alterations in the **spleen, liver, kidney, and blood**, as well as **genetic changes in fish chromosomes**, potentially resulting in **fish mortality** (Abdelfattah, 1994).

Studies on **Nile tilapia exposed to ammonium nitrate (0.2 mg/L)** showed **chromosomal abnormalities** in kidney cells. The **frequency of chromosomal aberrations** increased with longer exposure time, compared to control tank fish, which exhibited **normal chromosomes (2n = 44)** without any abnormalities. Observed chromosomal aberrations included **structural anomalies** such as **chromatid gaps, chromosome gaps, and breaks**.

**Cytological effects** of ammonium nitrate exposure included the appearance of **micronuclei in polychromatic erythrocytes**, confirming its impact on red blood cells.

Exposure of fish and aquatic organisms to industrial pollutants and waste—**major sources of water contamination**—induces **cellular and genetic lesions**, leading to **mutations**, which may affect **embryonic development**, cause **sterility, malformations in future generations**, or **reduced fertility** (Soldatovic et al., 1994; Rabber et al., 1992).

This study revealed that **ammonia accumulation**, arising from **fish excretion** and **food decomposition due to overfeeding**, and its interaction with nitrates in water, can cause **toxic effects** on aquatic organisms, including fish. Zhestyanikov (1982) and Yonis (1983) highlighted that **chromosomal aberrations and micronuclei** are reliable **genotoxicity indicators** to assess the effect of chemicals on somatic cells, including aquatic species.

Moreover, Abu-Qare et al. (2000) reported that substances like **braniterphenol** are rapidly absorbed in fish, can pass through the brain to embryos, and may induce **adverse health effects during pregnancy**, which aligns with the findings of this study.

## Conclusion

The presence of **ammonium nitrate** in aquatic environments can induce **genetic and carcinogenic effects** on fish. Therefore, it is essential to implement **strict preventive measures** and to **hold accountable and penalize** anyone who deliberately harms the general or marine environment. Public awareness campaigns should be conducted to educate all societal groups about the importance of **avoiding the disposal of pollutants into aquatic ecosystems**.

A key distinction between terrestrial and aquatic environments is that **terrestrial waste** can be **collected, cleaned, or safely disposed of**, whereas in **marine environments**, this is extremely difficult due to the **human, material, and temporal resources required**.

The **decline of forest areas, soil depletion, insufficient water resources, and the deterioration of fish stocks** threaten the quality of life and health of people in developing countries, making them **more vulnerable to disasters**. Additionally, the pollution of freshwater rivers, whether **organic or inorganic**, adversely affects the populations living around these water sources, who rely on them for **agricultural and livestock activities**.

It remains crucial to **urge policymakers through scientific platforms** to implement regulations that **prevent reckless pollution**, protecting the interests of communities from individuals prioritizing personal gain. Likewise, individuals who **discharge waste directly into flowing rivers** contribute to the **spread of toxins and hazards** over wide areas, negatively impacting **humans, animals, plants, and all living organisms** in these ecosystems.

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