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# DETERMINATION OF THE CONCENTRATION OF AMMONIA THAT COULD HAVE LETHAL EFFECT ON FISH POND

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**ABSTRACT**

A local fish farmer experienced high mortality rate of fishes from his fish pond located in a city of Port Harcourt, Nigeria. In order to ascertain the cause of the death of these fishes, some selected physicochemical and microbiological parameters were used to determine the concentration which resulted in lethal effect on the fishes. Test results indicated that among the parameters determined, the concentration of ammonia in both the storage tank and aquarium exceeded the maximum limit of 0.2mg/l for aquatic life. The ammonia concentrations from test results ranged from 0.25mg/l to 0.50mg/l. The sample with 0.50mg/l also indicated the highest level of total coliform of 3.52 x $10^3$(cfu/100ml) as against the sample with 0.25mg/l with Total Coliform of 0.0 (cfu/100ml). Moreover, higher values of pH (7.45), TDS (104mg/l), Electrical conductivity (208µScm$^{-1}$), Chloride (75.76mg/l), and Nitrate (2.00mg/l), Calcium (0.94mg/l) respectively were seen in the sample with the highest level of ammonia (0.5mg/l). The death of the fishes was more evident with the sample having 0.5mg/l, followed by the sample with 0.35mg/l of ammonia. Test results, therefore, confirm that ammonia concentration of above 0.20mg/l in fish ponds has a tendency to harm the fishes.

**Keywords**: lethal effect, fish pond, mortality rate, aquatic life, aquarium, storage tank.

## INTRODUCTION

Ammonia is the major end product in the breakdown of feed in fish pond. Fish digest the protein in their feed and excrete ammonia through their gills and in their feces. The amount of ammonia excreted by fish varies with the amount of feed put into the pond or culture system, increasing as feeding rates increase. Ammonia also enters the pond from bacterial decomposition of organic matter such as uneaten feed or dead algae and aquatic plants (Robert et al., 1997).

When ammonia is dissolved in water, it is partially ionized depending upon the pH and temperature. The ionized ammonia is called Ammonium and is not toxic to the fish. As the pH drops and the temperature decreases, the ionization and ammonium increases which decreases the toxicity (Norm Meck, 1996).

Ammonia tends to block oxygen transfer from the gills to the blood and can cause both immediate and long term gill damage. The mucous producing membranes can be destroyed, reducing both the external slime coat and damaging the internal intestinal surfaces. Fish suffering from Ammonia poisoning usually appear sluggish, often at the surface as if gasping for air (Robert et al., 1997).

Total ammonia nitrogen (TAN) is composed of toxic (un-ionized) ammonia ($NH_3$) and nontoxic (ionized) ammonia. Only a fraction of the TAN exists as toxic (un-ionized) ammonia, and a balance exists between it and the nontoxic ionized ammonia. Uptake (assimilation) of ammonia by plankton algae is important in reducing the amount of ammonia coming in contact with fish. Lower water temperatures slow down aerobic bacterial activity, thus slowing the nitrification process whereby ammonia is converted to harmless nitrate (Robert M. D., *et al* 1997).

Dangerous short-term levels of toxic un-ionized ammonia which are capable of killing fish over a few days start at about 0.6 mg/l (ppm). Chronic exposure to toxic un-ionized ammonia levels as low as 0.06 mg/l (ppm) can cause gill and kidney damage, reduction in growth, possible brain malfunctioning, and reduction in the oxygen- carrying capacity of the fish. The use of lower feeding rates and good feeding practices play a big role in keeping TAN levels low. Problems with high TAN concentrations can be expected when feeding rates exceed 100 pounds per acre per day, or when excessive feed waste is occurring. Fish should not be overfed, and the feeder should be sure that fish are consuming feed offered. This is both of practical and economic importance, since feed costs are a major portion of production costs (Robert et al., 1997).

### Effect of ammonia breakdown

Nitrate is the result of the bacterial breakdown of ammonia, nitrite, and nitrate which is the final stage of the natural biological metabolic waste conversion.

Although less toxic than ammonia/ammonium and nitrite, nitrate as a nitrogen compound also causes stress at all levels making a fish's organs work harder to adjust to its new environment. The increasing stress results in the loss of ability to fight diseases, the ability to heal itself, and the ability to reproduce.

The process of breaking down ammonia, nitrites, and nitrates is known as the nitrification process. It takes place in an aerobic environment. Nitrifying bacteria settle on gravel and build colonies. They need nutrients (ammonia and nitrite) and oxygen in order to perform their tasks. The result is nitrate. The removal of nitrate, if not utilized by plants, takes place in an anaerobic environment and is called denitrification (Norm Meck 1996).

Nitrates are potentially dangerous due to the effects on the water chemistry and on a healthy

environment for fish while nitrates are accumulating. The higher the nitrate levels the higher and severe the consequences due to the stress on fish and the favorable conditions for a serious algae outbreak.

Nature provides an almost nitrate free environment with levels around 5ppm or less. The higher the nitrate concentration the more stress for the fish. Extremely severe stress is reached at levels exceeding 60ppm. Most of the plants fail before reaching this level. This is due to an accumulation of live forms feeding from the waste and the consequently higher biomass (animals living in the aquarium) leads to an increasing demand of oxygen (Norm Meck, 1996).

Therefore nitrate levels ($NO_3$) should be kept below 10ppm. Nitrate is also a key nutrient source for algae. Most of the pesky and unwanted algae thrive on poor water quality (Damon, T. A., 1999), high nutrient levels and excessive nitrate. Many initially cycling tanks experience an algae bloom due to this effect.

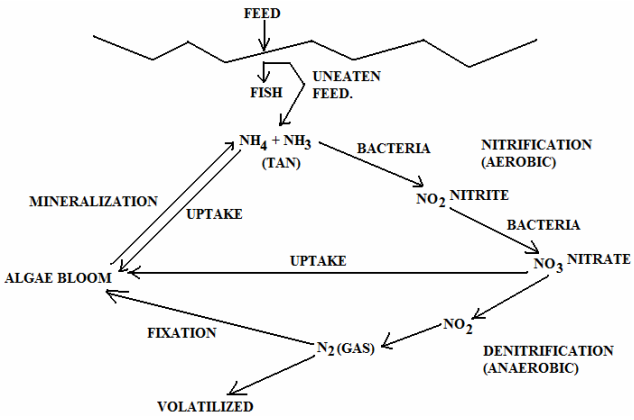


**Figure-1**. Breakdown of ammonia (Robert, M. D., *et al* 1997).

## Fish feed

Feeding is vital to the fishes well being. Feeding correctly ensures fish health, growth and ability to breed. When feeding, any uneaten food should be removed after 5minutes. This ensures that excess food will not pollute the water as it breaks down. Feeding your fish is a supplement to the natural diet they will find in the pond. This supplement is vital in ensuring provision of correct balanced diet. Over feeding will cause poor water conditions and ill health to fish (www.worldofwater.co.uk, 2009).

Once the temperature lowers to 70$^o$F, the fishes' staple food should be mixed with a wheat germ base that is lower in protein. When the temperature drops to 60$^o$F, the stable food should be switched to wheat germ based foods exclusively. When pond water temperatures decrease, the nutritional requirement of hardy pond fish change. Fish metabolism and the ability to properly digest and extract nutrients decrease as water temperatures plummet. Wheat germ diets are much easier to digest than other foods, making them the ideal food choice during periods of cool water temperatures (www.drsfostersmith.com.).

If the food is offered when too cold then the food will remain uneaten in the pond, begin to breakdown and pollute the water, leading to water quality problems, ultimately stressing the fish (www.pond-doctor.co.uk/longgoldfishcarefeeding.htm)

## Synergistic effect of pH, temperature, oxygen and ammonia in fish pond

Ammonia, $NH_3$, measured in parts per million (ppm), is the first measurement to determine the "health" of the biologic converter. Ammonia should not be detectable in a pond with a "healthy" bio-converter. The ideal and normal measurement of Ammonia is zero. When ammonia is dissolved in water, it is partially ionized depending upon the pH and temperature. The ionized ammonia is called Ammonium and is not toxic to the fish. As the pH drops and the temperature decreases, the ionization and Ammonium increases which decreases the toxicity. As a general guideline for a water temperature of 70°F (21.11$^o$C), most fish would be expected to tolerate an Ammonia level of 1 ppm if the pH was 7.0, or even as high as 10.0 if the pH was 6.0. At a pH of 8.0, just 0.1 ppm could be dangerous (Emerson, *et al.*, 1975).

Ammonia tends to block oxygen transfer from the gills to the blood and can cause both immediate and long term gill damage. The mucous producing membranes can be destroyed, reducing both the external slime coat and damaging the internal intestinal surfaces. Ammonia is a gas primarily released from the fish gills as a metabolic waste from protein breakdown, with some lesser secondary sources such as bacterial action on solid wastes and urea.

The temperature of the pond normally follows that of its surroundings although with a delay related to the size of the pond. Direct exposure of the pond to open sky can cause larger swings in temperature. Direct sunlight during the day can cause the temperature to rise higher and heat loss on clear nights can cause the temperature to drop lower than shaded ponds. A clear night sky can absorb a large amount of heat from a small pond and actually drive the pond temperature below air temperature.

Events generally happen faster at higher temperatures and in smaller ponds. Over normal temperature ranges, biologic activity doubles for each 10° rise in temperature. The toxicity of Ammonia increases as the temperature rises and the amount of Dissolved Oxygen that the water can hold decreases. Although fish have been known to survive for limited periods at 100°F and even higher, the mortality rate of fish conditioned to 75°F water increases rapidly above 85°F. Above 80°F, supplemental air may be required (Norm Meck, 1996).

Much more important than either the actual pH and alkalinity measurements, assuming they are both in the acceptable ranges, are changes to them. A typical established pond will normally settle down into an equilibrium state with a pH of about one half unit above or below the pH of the tap water used for replenishment. Over time (months), all of the inhabitants (bacteria, plants, and the fish) become acclimated to their environmental

conditions. Stress occurs in all of them if they must adjust to any changes. Rapid changes in pH can cause extreme stress to the fish similar to shock in humans. A sudden change of a half or more pH unit in an established pond is an indication that something happened and the cause should be determined. Slow, longer term, changes provide other indications. Increasing pH and/or alkalinity trends in a pond are normally caused by lime leaching out of concrete and to a lesser degree by concentration due to evaporation and decomposing organic matter. Decreasing pH and alkalinity tendencies are primarily due to bacterial action that release acidic compounds. Concrete ponds usually stabilize at a slightly higher pH value than ponds with liners (Norm Meck, 1996).

The minimum limiting oxygen concentrations for a fish is dependent upon its genetic makeup, water temperature, level of activity, long term acclimation, and stress tolerance. Water with an oxygen concentration of less than 3 mg/l will generally not support fish. When concentrations fall to about 3-4 mg/L, fish start gasping for air at the surface or huddle around the water fall (higher concentration points). Bio-converter bacteria may start to die off dumping toxins into the water compounding the lack of oxygen to the fish. Levels between 3 and 5 mg/l can normally be tolerated for short periods. Above 5 mg/l, almost all aquatic organisms can survive indefinitely, provided other environmental parameters are within allowable limits. The fish are reasonably comfortable and healthy at 5-6 mg/L concentrations (Norm Meck, 1996).

Whenever air is in contact with the water, whether through natural or artificial means, a transfer of oxygen from the air to the water takes place until the water becomes saturated. Plants under light, convert carbon dioxide to oxygen in the water. Fish, plants at night, and aerobic bacterial action consume the oxygen.

It is not difficult to get all the air into the water that the fish need. Oxygen is continually transferred into the water at the surface of the pond and normally only a small water fall will bring the pond water to or near to saturation. Heavily populated ponds may need supplemental air and ponds with a large amount of algae may need supplemental air at night when the plants are not making oxygen but consuming it. It is very important that sufficient circulation is provided within the pond so that all areas have proper oxygenation.

Almost all of the oxygen dissolved into the water from an air bubble occurs when the bubble is being formed. Only a negligible amount occurs during the bubbles transit to the surface of the water. This is why an aeration process that makes many small bubbles is better than one that makes fewer larger ones. The breaking up of larger bubbles into smaller ones also repeats this formation and transfer process.

## METHODOLOGY

Water samples were collected from four different tanks of the fish farm. The tanks where the water samples were collected included the storage water tank, the aquarium tank from Elekahia in Port Harcourt, Nigeria, and storage water tank and the aquarium tank from Degema also in Port Harcourt, Nigeria. The samples collected were used for the analysis of both microbiological and physicochemical qualities.

## PHYSICO-CHEMICAL ANALYSIS

### pH, Temperature, conductivity, TDS and dissolved oxygen

These chemical properties were determined electrometrically with a multi- parameter data logger (Hanna model HI991300).

### Chloride and salinity as chloride

These were determined titrimetrically in accordance with APHA 2520A. About 25ml of the sample was measured in a beaker and a drop of potassium chromate indicator added. The solution was titrated with $AgNO_3$ solution until the appearance of brick red colour as the end point.

$$\text{Calculations: } Cl^- \text{ (mg/l)} = \frac{\text{Volume of } AgNO_3 \text{ titrated} \times 0.3 \times 1000}{\text{Volume of sample}}$$

$$\text{Salinity as chloride} = Cl^- \times 1.65$$

### Ammonia

About 50ml of the sample was measured into distillation flask. 0.4g of magnesium oxide was added and distilled into a beaker containing 10ml of 2% boric acid and combined indicator. This was titrated back with 0.1M HCl and the titre value was recorded.

$$\text{Calculation: } NH_3 \text{ (mg/l)} = \frac{\text{Titre value} \times 100}{\text{Sample volume used}}$$

### Nitrate

About 1ml of the sample was transferred into a 100ml volumetric flask. 0.5ml of brucine reagent was added, followed by rapid addition of 2ml concentrated sulphuric acid. This solution was stirred to mix for 30sec. This was allowed to stand for 5minutes, and stirred again to mix. 2ml distilled water was added and the mixing continued for about 30secs. The flask was allowed to stand in cold water for about 15minutes. The sample was measured at 410nm absorbance using UV Spectrophotometer.

$$\text{Calculation: } NO_3\text{-N (mg/l)} = \frac{\text{Mg/l } NO_3\text{-N from standard curve}}{\text{Sample volume used}}$$

### Nitrite

About 50ml of the sample was placed in a 50ml-Nessler tube. 2ml of buffer colour reagent was added to the sample and mixed. The solution was allowed for at least 15mins for colour development, making sure that the pH is maintained between 1.5 and 2.0. The sample was measured at 540nm absorbance using UV Spectrophotometer.

$$\text{Calculation: } NO_2\text{-N (mg/l)} = \frac{\text{Mg/l } NO_2\text{-N from standard curve} \times 50}{\text{Sample volume used}}$$

## HEAVY METALS DETERMINATION

Samples were pre-treated with 2ml conc. $HNO_3$ per litre of sample. The equipment was conditioned by auto-zeroing it with distilled water and with conc. $HNO_3$. The pre-treated sample was analysed for heavy metals using the appropriate hollow cathode element of each metal of interest at the appropriate wavelength, lamp current, band-pass, and background correction.

## MICROBIOLOGICAL QUALITY

### Total heterotrophic bacteria

About 1ml of the water samples was aseptically transferred, using a sterilized dropper, into a sterile test tube containing 9ml of the diluent. This gave $10^{-1}$dilution. Subsequently, four fold ($10^{-4}$) serial dilutions were prepared from the $10^{-1}$ dilution.

### Inoculation and enumeration of water samples

0.1ml aliquot of $10^{-4}$ dilution was aseptically removed with a sterile pipette and spread plated with flame sterilized glass spreader on well dried agar plates. This was incubated at 28 ± 2$^{o}$C for 24hrs. The colonies counted were expressed as colony forming unit per ml.

### Total coliforms

About 100ml of the water samples was filtered through membrane filter with the aid of vacuum pump. The filter membrane was placed in the m-HPC agar plate. This was then incubated using an incubator pre-set to 35 ± 5$^{o}$C for 24hrs. Observation was made for colony development on the filter membrane. The colonies were then counted as colony forming unit per 100ml.

### Fecal coliforms

About 100ml of the water samples was filtered through membrane filter with the aid of vacuum pump. The filter membrane was placed in A MacConkey agar plate. This was then incubated using an incubator pre-set to 44.5 ± 2$^{o}$C for 24hrs. Observation was made for colony development on the filter membrane. The colonies were then counted as colony forming unit per 100ml.

## RESULTS

**Table-1**. Physicochemical analysis of water collected from storage water tank at elekahia, Port Harcourt, Nigeria (sample 1).

| S. No. | Parameters | Sample 1 |
|---|---|---|
| 1. | pH | 5.59 |
| 2. | Temp ($^{o}$C) | 30.6 |
| 3. | TDS (mg/l) | 67 |
| 4. | EC (µScm$^{-1}$) | 132 |
| 5. | Ammonia (mg/l) | 0.25 |
| 6. | Chloride (mg/l) | 36.36 |
| 7. | Nitrite (mg/l) | 1.4 |
| 8. | Nitrate (mg/l) | 0.12 |
| 9. | Salinity as chloride (mg/l) | 60 |

**Table-2**. Physicochemical analysis of water collected from aquarium at elekahia, Port Harcourt, Nigeria (sample 2).

| S. No. | Parameters | Sample 2 |
|---|---|---|
| 1. | pH | 6.49 |
| 2. | Temp ($^{o}$C) | 30.5 |
| 3. | TDS (mg/l) | 48 |
| 4. | EC (µScm$^{-1}$) | 79 |
| 5. | Ammonia (mg/l) | 0.35 |
| 6. | Chloride (mg/l) | 66.67 |
| 7. | Nitrite (mg/l) | 2.0 |
| 8. | Nitrate (mg/l) | 0.16 |
| 9. | Salinity as chloride (mg/l) | 110 |

**Table-3**. Physicochemical analysis of water collected from storage water tank at degema, Port Harcourt, Nigeria (sample 3).

| S. No. | Parameters | Sample 3 |
|---|---|---|
| 1. | pH | 6.79 |
| 2. | Temp ($^{o}$C) | 27.1 |
| 3. | TDS (mg/l) | 41 |
| 4. | EC (µScm$^{-1}$) | 105 |
| 5. | Ammonia (mg/l) | 0.25 |
| 6. | Chloride (mg/l) | 60.61 |
| 7. | Nitrite (mg/l) | 1.50 |
| 8. | Nitrate (mg/l) | 0.08 |
| 9. | Salinity as chloride (mg/l) | 100 |

**Table-4**. Physicochemical analysis of water collected from aquarium at degema, Port Harcourt, Nigeria (sample 4).

| S. No. | Parameters | Sample 4 |
|---|---|---|
| 1. | pH | 7.45 |
| 2. | Temp ($^{o}$C) | 27.1 |
| 3. | TDS (mg/l) | 104 |
| 4. | EC (µScm$^{-1}$) | 208 |
| 5. | Ammonia (mg/l) | 0.5 |
| 6. | Chloride (mg/l) | 75.76 |
| 7. | Nitrite (mg/l) | 2.00 |
| 8. | Nitrate (mg/l) | 0.09 |
| 9. | Salinity as chloride (mg/l) | 125 |

**Table-5**. Heavy metal determination.

| S. No. | Parameters | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|---|
| 1. | Magnesium (mg/l) | 0.61 | 0.62 | 0.50 | 0.62 |
| 2. | Copper (mg/l) | <0.001 | <0.001 | <0.001 | <0.001 |
| 3. | Lead (mg/l) | <0.001 | <0.001 | <0.001 | <0.001 |
| 4. | Zinc (mg/l) | <0.001 | <0.001 | <0.0010 | <0.001 |
| 5. | Iron (mg/l) | <0.001 | <0.001 | <0.001 | <0.001 |
| 6. | Calcium (mg/l) | 0.14 | 0.20 | 0.26 | 0.94 |
| 7. | Potassium (mg/l) | <0.001 | <0.001 | <0.001 | <0.001 |
| 8. | Sodium (mg/l) | 0.66 | 1.06 | 1.22 | 1.14 |

**Table-6**. Micro-biological analysis.

| # | Parameter | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|---|
| 1 | Total heterotrophic bacteria (cfu/ml) | 0.3 | $4.08 \times 10^1$ | 0.4 | $4.96 \times 10^1$ |
| 2. | Total coliform (cfu/100ml) | 0 | $2.24 \times 10^3$ | $4.0 \times 10^1$ | $3.52 \times 10^3$ |
| 3. | Fecal coliform (cfu/100ml) | 0 | $1.0 \times 10^1$ | 0 | 0 |
| 4. | Mould (cfu/ml) | $2.56 \times 10^1$ | $4.0 \times 10^1$ | 3 | 2.6 |

## DISCUSSIONS

Results of physicochemical analysis (Tables 1 to 4) indicated pH value of 5.59 for sample 1, 6.49 for sample 2, 6.79 for sample 3 and 7.45 for sample 4, respectively. The ammonia concentration level was highest with sample 4 (0.50mg/l), followed by sample 2 (0.35mg/l) and least with samples 1 and 3 (0.25mg/l and 0.25mg/l), respectively. Samples 1 and 3 were from the storage tanks while sample 2 and 4 were from the fish ponds. There were also noticeable higher values of TDS (104mg/l), Calcium (0.94mg/l), Electrical conductivity (208µS/cm), chloride (75.76mg/l) and Nitrite (2.00mg/l) respectively with sample 4 that had the highest level of ammonia compared with other samples.

This agrees with previous findings that increase in ammonia concentration in fish ponds could trigger other reactions which have tendency to increase the concentration of the other physicochemical parameters.

The concentration of the heavy metals (Table-5) determined had values that were not of concern to the health of the fishes.

The microbiological analysis (Table-6) indicated highest level of Total heterotrophic bacteria ($4.96 \times 10^1$cfu/ml) and Total coliform ($3.52 \times 10^3$cfu/100ml) respectively for sample 4 that also showed the highest level of ammonia, with the least value observed with sample 1 (0.3cfu/ml and 0cfu/100ml), respectively. However, the mould was higher in sample 1 ($2.56 \times 10^1$cfu/ml) and sample 2 ($4.0 \times 10^1$cfu/ml) respectively compared with results from sample 3 (3.0cfu/ml) and sample 4 (2.6cfu/ml).

## CONCLUSIONS

Prudent pond management, effective choices of fish feed and good feeding practice of fish ponds are very vital to the fishes well being. Feeding correctly ensures fish health, growth and ability to breed. When feeding, any uneaten food should be removed as quickly as possible. This ensures that excess food will not pollute the water as it breaks down.

The concentration of ammonia in fish pond should be closely monitored to avoid both chronic acute toxic effects. Care should be taken to closely monitor the level to always be below 0.2mg/l. It is imperative to ensure that the pH and temperature values are adequately controlled to avoid negative synergistic effect with the presence of ammonia in the pond.

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