Environmental Pollution Biomarker: Fish Antioxidant Défense System Alterations

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ABSTRACT

Heavy metals (Fe, Zn, Cu, Mn and Cd) pollution in River Nile's Rosetta Branch has been studied in this work to determine the influence on the antioxidant defence system and lipid peroxidation indicator MDA levels in O. niloticus tissues (liver and white muscles). High levels of agricultural drainage water, sewage, and industrial waste water flow into the Rosetta Branch of the Nile, which has a negative impact on the living creatures, particularly fish. Water and fish samples from stations II and III showed an increase in the content of heavy metals (Fe; Zn; Cu; Mn; Cd) over the winter months. The accumulation patterns of heavy metals in the muscles of O. niloticus were as follows: Fe > Zn > Mn > Cu and Cd. During the winter months, the bioaccumulation factor (BAF) was greater than during the summer months. Oxidative enzymes (SOD, CAT, GPx, GST, and GR) as well as MDA levels in the liver and white muscles of O. niloticus (stations II and III, particularly in winter) exhibited a considerable rise. White muscles had lower activity of antioxidant enzymes and greater amounts of MDA

Keywords: Heavy metals, pollution, antioxidant defense system

1. Introduction

In order to keep up with increased worldwide demand for fish, aquaculture has grown and now accounts for 52% of the amount eaten. For more than three decades, the food industry has grown at the fastest yearly pace; 10% in the 1990s and 5.8% from 2000 to 2016. Antimicrobial resistance has spread throughout the globe as a result of the widespread use of antimicrobial agents in contemporary food animal production. Antibiotic-resistant bacteria have appeared in aquatic environments as a result of aquaculture, antibiotic resistance in fish pathogens has increased, resistance determinants have been transferred to bacteria from land animals and human pathogens, and the bacterial flora in sediments and the water column has changed. Phytotherapy is based on the use of plants to prevent or cure human or animal illnesses.. Phytotherapy and modern medicine, particularly antibiotic compounds, were competitors in the early twentieth century. More recently, the aquaculture industry has seen an increase in the usage of essential oils (EOs) and polyphenol-enriched extracts (PEEs) as a method of improving industrial and environmental sustainability. These bioactive chemicals have been shown in previous research to have favourable impacts on fish crop development, immune, antibacterial and antiparasitic activity. Streptococcus fluorescens and Pseudomonas fluorescens, both of which are common in farmed fish, were shown to be effectively treated by Ocimum basilicum.

The antioxidant and immunological defence mechanisms of fish are modulated by bioactive chemicals found in both EOs and PEEs, resulting in increased fish development. When oregano Origanum heracleoticum essential oil was given to channel catfish Ictalurus punctatus for eight weeks as a dietary supplement with a substitution of 0.05 percent, body indices (hepatosomal, visceral and condition factor indices) increased as did antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) linked to EO. For eight weeks, common carp Cyprinus carpio were given an essential oil-based diet by Abdel-Latif et al. and Zhang et al. and identical results were found. IL-1 and IL-10 transcription levels were enhanced, whereas TNF- and TGF-down transcription levels were decreased by the essential oil, which improved the fish's immunological characteristics and tolerance to Aeromonas hydrophila. As for the rainbow trout Oncorhynchus mykiss, Origanum onites EO had an equally good effect on the fish's immunological and antioxidant systems

2. Literature Review

López-Pedrouso, María & Lorenzo (2022) As a major source of protein in our diet, aquaculture production is expected to continue for many years to come. However, there is a good chance that marine pollution will have a negative impact on the safety of mollusks as food. It is well accepted that seafood is beneficial to our health because of its high nutritional content in our diets. Heavy metals, persistent organic pollutants and other new contaminants pose an increasing danger to marine ecosystems and seafood safety, thus it is important to keep an eye out for them. Improved procedures for the aquaculture sector might be achieved by new methods to the search for biomarkers and a more global perspective on pollution. Protein biomarkers might be used to quickly and accurately identify food safety issues in bivalves. The finding of protein biomarkers might be a first step in improving seafood safety standards using proteomic technology. The bivalve proteome has been shown to be altered by marine contaminants, influencing a wide range of biological processes and cellular activities. Bivalves' primary protection against oxidative stress in a polluted marine environment is the antioxidant defence system. There is a strong correlation between changes in oxidative stress owing to marine pollution and robust and confident indicators for seafood safety, according to the research.

Camilo Cotrim, Carlos & Reis (2021) At risk from human activity are waterways, which disrupt the natural balance of aquatic life. The goal of this research was to examine how anthropogenic effects affect fish biochemical and genetic biomarkers. An impact stream (reference) and one of its non-impacted tributaries (reference) were used to expose Astyanax lacustris individuals. Using gill and liver tissue samples, we performed biochemical analyses on the antioxidant enzymes catalase and glutathione peroxidase, as well as lipid peroxidation levels. We looked for micronuclei and nuclear abnormalities in blood samples for the genotoxicity test. The antioxidant enzymes exhibited seasonal change, independent of the stream, while lipid peroxidation did not. Due to increased hazardous chemical leaching during the rainy season, there were more micronuclei and nuclear abnormalities in the affected stream during this time period. Using nuclear biomarkers in health monitoring systems is supported by these findings.

Georgieva, Elenka & Velcheva (2021) Analyzing how organic pollution in aquatic habitats affects organisms' health, as well as the most effective biomarkers for detecting stress caused by such contamination, are the primary goals of the present research review. Water Framework Directive (WFD; 2000/60/EC) and Marine Strategy Framework Directive (MSFD; 2008/56/EC) legislations have been enacted by the European Union in order to protect the environmental state of aquatic systems. The degree of health status is thus monitored by sentinel fish species. Invertebrates, on the other hand, tend to be less susceptible to toxicants, including pesticides and other persistent organic pollutants, but fish, on the other hand, are more sensitive to a variety of toxicants. This makes fish an excellent biomonitor of water pollution. Biomarkers are characterised as changes in histology, physiology, biochemistry, genetics, and behaviour as a result of exposure to any substance. The creation of a procedure for the aquatic evaluation of organic pollutants requires the use of a multi-biomarker approach. A legal foundation for the presence of organic pollutants in aquatic ecosystems and biological reactions at concentrations equal or lower than those authorised by European and Bulgarian regulations may be provided by using this methodology in risk assessment and water monitoring programmes.

Valon, Morina & Aliko (2013) Using fish (Cyprinus carpio L.) antioxidant enzyme activity, researchers in Kosovo were able to establish how pollution in the Sitnica River has affected the fish's physiology and biochemistry. Fish blood was used as a bioindicator to measure the activity of three antioxi- dant defence enzymes, including catalase, superoxide dismutase, and glutathione-S-transferase (GST). Fish (n=21) were collected from Ferizaj, Vragoli, and Plemetin in March-May and August-September 2010-12. (Sitnica River). The concentrations of plasmatic CAT, SOD, and GST in fish from contaminated Vragoli and Plementin sites were substantially greater (P 0.001) than in fish from Ferizaj. As a river source, Ferizaj is less polluted than the other two locations. Biochemical bioindicators such as these three enzymes may be used to identify chemical contamination in fish. This river is predominantly contaminated by industrial and urban liquid waste products, according to the findings of our study. Therefore, legal action is necessary to avoid environmental degradation on the site.

Martínez-Alvarez, Rosa & Morales (2005) Many metabolic activities necessary to aerobic living need oxygen in its molecular state, O2. Oxygen is essential for the survival of aerobic species, yet it is also hazardous. It is widely documented in the scientific literature that fish are vulnerable to reactive oxygen and have efficient antioxidant defence mechanisms that are extensively documented in the scientific literature. Different biotic and abiotic (age, evolutionary position, feeding habit, environmental conditions and the presence of xenobiotics) influences on fish antioxidant defences are examined in this review. The findings are discussed. Antioxidant activity in fish has opened up a number of new research avenues that will enhance fish farming and artificial production in a variety of ways.

3. Objectives Of the Study

- the role of heavy metal contamination in Rosetta Branch on the antioxidant defence system has to be clarified
- Some heavy metals in O. niloticus water and muscles were determined and the effects of these pollutants on liver antioxidant defence enzymes were evaluated

4. Matrials And Methods

Three sites on the Rosetta Branch of the River Nile were used to collect water and fish samples: (1) El-Qanater El-Khyria. Al Qata. (3) Kafr El-Zayat, in the spring of 2014 and the winter of 2015. Polyethylene bottles with two-meter depths were used to collect water samples that were acidified with nitric acid and sent to a laboratory for analysis.

Fished O. niloticus, which had an average weight and length of 150 gramme and 19 cm, were used to collect fish samples for the study. The accumulation of heavy metals in fish muscle tissue was assessed upon dissection. To examine antioxidant enzyme activity and MDA levels, the right lobe of liver and white muscle tissues were meticulously excised.

4.1 Heavy metals in water and fish muscle analysis:

Acidified water samples of 500ml were cooked on a hot plate with conc. nitric acid. Adding conc. Nitric acid to the heated solution was maintained until the heavy metals' digestion was completed, as described by (APHA, 2012). After drying, Ghazaly's technique was used to digest the fish samples (1988). GBC atomic absorption reader data (Model SavantAA AAS with GF 5000 Graphite Furnace) were used to estimate the concentrations of heavy metals in water and fish muscles, and were expressed in (mg/L) and in mg/kg dry weight, respectively.

4.2 Heavy Metal Bioaccumulation Factor in Fish Muscle (BAF)

The BAF is defined by the Environmental Protection Agency (EPA) as the proportion of the organism's chemical concentration to the water's surrounding concentration.

BAF = M (tissue) / M (water).

Where; M tissue is the metal concentration in fish tissue mg/kg and M water, metal concentration in water mg/L.

4.3 Biochemical analysis

On ice, the fish were meticulously dissected, and white muscles or the right lobe of the liver of O. niloticus were removed. Tissues were immediately homogenised in ice-cold 50 mM phosphate buffer, 1 percent Triton X100 (pH 7.4) to yield a 10% homogenate after fish were rinsed with isotonic saline, dried with filter paper, and weighed (n= 8). A 15-minute cooling centrifuge centrifugation at 4°C produced a 6,000 xg supernatant, which was used for an enzyme activity test in the liver and white muscles right away.

SOD activity in fish tissues was measured using Paoletti and Mocali's NADH oxidation technique (1990). The enzyme activity was measured by measuring the absorbance at 340 nm and calculating the enzyme activity using a calibration curve constructed from standard SOD to determine the unit of activity. Wet weight tissue (U/mg) units were used to represent the particular activity. The quantity of extract required to degrade one mole of H2O2 per minute was measured using the Xu et al. (1997) technique, which uses a newly generated H2O2 substrate solution and absorbance measurements at 240 nm. The activity was measured in units of mol/min/g w.w.t.

A glutathione reductase (GR) and NADPH assay was used to determine the activity of glutathione peroxidase (GPx), and the reaction was triggered by the addition of H2O2 as per the technique of Paglia and Valentine (1967). Monitoring NADPH to NADP+ oxidation at 25°C every 15 seconds for 300 seconds was done by monitoring absorbance at 340 nm. The data were presented in (mM /min/g w.wt.) units of measurement.

Using the approach of Habig et al. (1974), the generation of CDNB (S-2,4-dinitrophenyl glutathione) adducts was used to measure the activity of Glutathione-S-transferase (GST). The GST activity was measured in units of M /min/g w.wt..

The technique of Smith et al. was used to quantify the activity of Glutathione Reductase (GR) (1988), In this experiment, the oxidised glutathione (GSSG) was used to begin the reduction of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) to TNB. Over the course of five minutes, the absorbance at 340 nm was monitored, and the GR activity was measured in M /min/g w.wt. Responsive oxygen species degrade lipids, which results in a chain reaction and the creation of end products such malondialdehyde (MDA), a valuable diagnostic for oxidative stress. This process is known as lipid peroxidation. The Buege and Aust technique was used to determine the MDA concentration (1978). conjugation of MDA with TBA (thiobarbituric acid) is measured at 532 nm against the blank reagent and expressed in mg protein/ml.

4.4 Statistical Analysis

ANOVA was used to determine the significance of the differences between the experimental and control groups, and the Dunnett test was used to determine if the differences were statistically significant (GraphPad InStat Software, Inc.). One-way ANOVA (P 0.05) found a significant difference when each reading represented (Mean S.E.) 8 fish

5. Results

5.1 Concentration of heavy metals in water:

The table below shows the average concentrations of several heavy metals in the Rosetta Branch of the Nile (1). It was found that in the summer the concentrations of Fe, Zn, Cu, Mn, and Cd in water at the selected stations ranged from (0.173-0.775), (0.093-0.124), (0.008-0.113), (0.066-0.078), and (0.016-0.078), while in the winter, they varied from (0.223-0.952), (0.111-0.193), (0.022-0.177), (0.040-0.171), and (0. During the sample period, heavy metal levels were greatest at station (3) Kafr El-Zayat and lowest at station (1) El-Qanater El-Khayria. In terms of concentration, Fe was found to have the greatest value (0.173-0.952 mg/l) while Cd was found to have the lowest value (0.011-0.028 mg/l) during the course of the research. In addition, the three stations' water Fe, Zn, Cu, Mn, and Cd contents were higher in winter than in summer.

Table (1): Concentrations of heavy metals (mg/L) in the Rosetta Branch of the Nile River

Heavy metal	Season	Station I	Station II	Station III	(Mean±S.E)	Law48/1982 Decree92/2013
Fe	**S** **W**	0.173 0.223	0.603 0.772	0.775 0.952	0.517±0.18 0.65±0.22	1.0*
Zn	**S** **W**	0.093 0.111	0.119 0.147	0.124 0.193	0.112±0.01 0.15±0.02	1.0*
Cu	**S** **W**	0.008 0.022	0.011 0.143	0.133 0.177	0.05±0.03 0.11±0.04	1.0*
Mn	**S** **W**	0.016 0.049	0.062 0.144	0.078 0.171	0.052±0.02 0.121±0.04	0.5*
Cd	**S** **W**	0.011 0.016	0.019 0.024	0.021 0.028	0.017±0.003 0.023±0.004	0.01*

(I) El-Qanater El-Khyria. (II) Al Qata. (III) Kafr El-Zayat. S: summer W: winter

5.2 O. niloticus tissue heavy metal and BAF concentrations:

Results in Table (2) showed that the mean concentrations of heavy metals such as iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and cadmium (Cd) in the muscles of O. niloticus during the summer were 29.2, 4.97, 1.21, 1.05, 0.81, 0.81, 0.80, and 0.0950.05 mg/kg dry wt., respectively, while they were 60.5, 11.1, 6.4, 1.4, 3.56 There were significant amounts of Fe, Zn, Cu and Mn in O. niloticus muscles at station III in winter and low quantities at station I in summer of these elements. At station II in July, Cd concentrations were as low as 0.095 mg/kg dry weight, while this concentration was not found at station I throughout the investigation. Most heavy metal concentrations in O. niloticus muscles were found to be lowest at station II, whereas station III was the highest (2).

Table (2): Muscles of O. niloticus obtained from the Rosetta Branch of the River Nile have amounts of heavy metals (mg/kg dry wt.)

Heavy metal	Season	Station I	Station II	Station III	(Mean±S.E)	FAO (1983)
Fe	**S** **W**	8.25 15.55	34.35 62.3	45.16 103.75	29.2± 11.1 60.5 ± 25.9	30
Zn	**S** **W**	2.97 6.01	4.84 11.21	7.11 16.13	4.97 ± 1.21 11.1 ± 3.1	30
Cu	**S** **W**	0.047 0.69	1.19 5.74	2.1 7.62	1.1 ± 0.51 4.6 ± 1.7	30
Mn	**S** **W**	0.22 0.62	1.02 4.11	1.21 5.94	0.817 ±0.30 3.56 ±1.6	---
Cd	**S** **W**	ND ND	0.095 0.28	0.19 0.36	0.095 ± 0.05 0.32 ± 0.03	0.5

ND: not detected. (I) El-Qanater El-Khyria. (II) Al Qata. (III) Kafr El-Zayat. S: summer. W: winter.

When it comes to O. niloticus muscles' BAF of metals such as Fe (108.9, 83.4, 43.1, 34.5 and 9.2) and Zn (108.9, 83.4, 43.1, 34.5 and 9.2), station III in winter had the highest and station I in summer had the lowest, respectively. At station II in July, the lowest BAF of Cd in O. niloticus muscles was 4.1. Heavy metals in O. niloticus muscles were found to have the greatest BAF in winter at station III Table, whereas Fe was found to have the lowest BAF (Table 3).

Table (3): What is the BAF of O. niloticus muscles from the Rosetta Branch of River Nile?

Heavy metal	Season	Station I	Station II	Station III
Fe	**S**	47.7	56.9	58.2
	W	69.7	80.7	108.9
Zn	**S**	31.1	40.6	57.1
	W	53.9	76.2	83.4
Cu	**S**	5.9	10.8	14.7
	W	31.7	40.1	43.1
Mn	**S**	8.14	15.4	15.6
	W	12.6	28.4	34.5
Cd	**S**	----	4.1	6.8
	W	----	8.2	9.2

ND: not detected. (I) El-Qanater El-Khyria. (II) Al Qata. (III) Kafr El-Zayat. S: summer W: winter

5.3 Biochemical parameters:

O. niloticus's liver and white muscles' mean SOD activity increased significantly (P 0.01) at station III when compared to the corresponding control values (site I). White muscle and liver increased by 36.5 and 49.7 percent and 41.1 and 62.9 percent, respectively, throughout the two sample seasons in winter. Each sample location saw a 33.9 percent rise in white muscles and a 54.4 percent increase in liver during the summer months. At stations II and III, the CAT enzyme activity increased significantly (P 0.01) in the liver and white muscles over the winter, by 39.6 and 59.1 percent. Both white muscles and liver were found to be 41.71 and 57.1 percent, respectively, at each sample location. Each sample site showed a 33.8 percent rise in white muscle CAT enzymes and a 35.5 percent increase in liver CAT enzymes throughout the summer months.

At stations II and III, GPx activity in white muscles and liver rose 1.4 and 1.6-fold, respectively, in winter. At each sample point, the GPx enzyme increased by 1.3 and 1.6-fold in white muscles and by 1.8 and 2-fold in liver over the summer. Station III's winter GST activity in O. niloticus white muscles and liver was considerably higher than at reference station I, with the liver showing the highest level of significance (P 0.01). At stations II and III, GST activity increases 1.8 and 2.1-fold in white muscles, and 1.9 and 2.4-fold in liver, respectively, in the winter. In the summer, the GST enzyme increased by 1.2 and 1.7-fold in white muscles and by 1.5 and 2.1 fold in liver, respectively, at each sample site. O. niloticus white muscles and liver GR mean activity increased significantly (P 0.05) at station III. It was found that white muscle activity increased by 1.2 and 1.5-fold in the summer, whereas liver activity increased by 1.4 and 2-fold. During the winter months, GST enzyme activity in white muscles and liver increased 1.4 and 1.6-fold, respectively, according to the corresponding sampling stations. At station III, MDA levels in the liver rose 2.1 and 2-fold, whereas white muscle MDA increased 1.6 and 1.5-fold in the summer and winter, respectively. In white muscles, MDA levels rose 1.6 and 2 times in summer, whereas in liver they rose 1.7 and 2.1 times. Each test location saw an increase in MDA concentrations of 1.5 to 1.8 times in white muscles and 1.7 to 2 times in the liver over the winter. MDA levels in liver and white muscles, as well as enzyme activity, rose from summer lows to winter highs throughout the course of the research, with station III showing the greatest rise in both enzymes' activity and MDA levels when compared to stations I and II

6. Discussion

As ferrous ions are liberated from sediments and industrial waste effluent in the Kafr El-Zayat industrial region, Fe concentrations in water rise. As a result of this oxidation process, Fe (OH3) precipitates in the sediment of oxygenated water, which may explain why the minimum levels of Fe are lower than they should be.

The greatest concentrations of zinc (Zn) were found in the study area's water samples throughout the winter. As water levels fall during winter, zinc absorption decreases, and clay containing zinc ions dissolving in the water gives the water a high zinc concentration. humic and organic matter have a propensity to form complexes with copper, which reduces the number of free ions that may enter water, which has resulted in 90% of the water's copper being complexed by dissolved organic materials and suspended matter. Table: Manganese concentrations within acceptable limits in water samples taken from Rosetta Branch (1). The level of cadmium in the water used in this study was higher than the allowable limit.

According to the results of this investigation, the following heavy metals were found in the highest amounts in the water samples: Fe, Zn, Mn, copper, and lead (Cd). Additionally, water tests demonstrated seasonal fluctuations in heavy metal concentrations. As high as possible during the winter, and as low as possible during the summer (summer). This is in accordance with the findings of El Bouraie et al. (2010) and Islam et al. (2015b), who found that heavy metal concentrations rose in the winter.

Heavy metal content has decreased in the current study during warmer seasons, which may be due to phytoplankton development that absorbs huge quantities of heavy metals from the water. Organic matter and clays, which contain surface functional groups (negatively charged) that bind heavy metals (positively charged) and settle down in sediments, are more likely to hold heavy metals than other materials. river water's diluting impact on rainfall (Islam et al., 2015a). On the other hand, we believe that the higher concentrations of heavy metals found in water samples during the winter months may be related to: (1) decreased water levels caused by the closure period, leading to an increase in metal concentrations (Abdel-Moati and El-Sammak, 1997). agricultural drainage water fluctuations, sewage effluent fluctuations, industrial waste fluctuations (Zyadah, 1995). Heavy metal concentrations (Fe, Zn, Cu, Mn, and Cd) were found to be higher in water samples taken from Rosetta Branch at stations II and III than at station I. This could be because of the impact of pollution sources in these locations, such as sewage, domestic wastes, and other pollutants discharged into El-Rahawy drain and industrial effluents from the Kafr El-Zayat industrial area that flowed directly into the branch water without treatment (2013).

6.1 Concentrations of heavy metals in fish muscle

Because of the rapid pace of industrialization, there has been a dramatic rise in the amount of water polluted with hazardous elements. Trace elements have been proven to have harmful effects on fish, changing physiological activity and biochemical markers in blood and tissues. There are several studies showing the detrimental effects of metals on aquatic creatures, which in turn affects human health due to the biomagnification of these metals over time.

As a result, determining the number of deposited metals in fish is crucial for reasons related to human health. Heavy metals were mostly absorbed by fish via their meals, sediments, and the surrounding waterways, resulting in tolerable concentrations. Furthermore, the buildup of heavy metals in fish has a significant impact on the fish's development rate, physiological and biochemical state, and flesh quality. At station III (Kafr El-Zayat), iron (Fe) concentrations in muscles of O. niloticus Table (2), were the greatest, whereas the lowest amounts were found at station I. (relatively unpolluted site). This is because water with a lot of iron is what causes the most (Fe) buildup in fish muscles. In the winter, O. niloticus muscles had greater concentrations of (Fe) than in the summer. In the Kafr El-Zayat industrial area station III, the release of ferrous ions from sediments and industrial wastes effluents is the primary cause of the increase in (Fe) in water, but the lowest values in site (1) may be due to the oxidation of $Fe2^{+}$ to $Fe3^{+}$, resulting in the precipitation of $Fe(OH)_3$ on sediment. Data collected by Gad and Yacoub in the same location and by Gad and Mohamed in O. niloticus and lates niloticus gathered from the Damietta branch of the River Nile are in agreement with our findings. Research at Stations II and III found elevated amounts of iron in O. niloticus muscles taken from the Rosetta Branch of the Nile

7. Conclusion

According to the findings in O. niloticus white muscles and liver from polluted Rosetta Branch of River Nile, enzymatic responses to heavy metal exposures result in significant increases in antioxidant defence system enzymes (SOD, CAT, GPx, GST, and GR) and the lipid peroxidation indicator MDA. This study concluded. Station II (Al-Qata) had the greatest concentration of heavy metals in the current investigation, due to the long-term detrimental impact of El-Rahawy drain; station III (Kafr ElZayat) had the lowest concentration, due to industrial effluents. In biomonitoring systems, these enzymatic responses may serve as biomarkers for early identification of pollution. Analysis of both field and laboratory data is critical for determining the validity of both sets of findings. Even from an economic standpoint, fish health management is a vital part of fish agriculture and production. Farmers have faced disease outbreaks in the previous five years that have sometimes resulted in the death of all fish in ponds, leading to significant financial losses. The quality, quantity, and price of fish on the market are also affected by diseases. Furthermore, the presence of agricultural, industrial, municipal, and drain discharges along the Nile River's path contributes to poor water quality. Heavy metals in particular should be given extra care when polluting the Nile without remediation. Antioxidants (such as vitamins A, C, E, and beta-carotene) have been shown to reduce the risk of sickness and increase fish health and survival rates. Since free radicals are thought to be a contributing factor in the development of many immunological diseases and organ dysfunctions.

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