World Journal of Fish and Marine Sciences 2 (5): 461-470, 2010
ISSN 2078-4589

Investigation of Mass Mortality Problem of *Oreochromis niloticus* in Mariotia Channel in Egypt

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Abstract: This study was designed to investigate the catastrophic mass mortality of *Oreochromis niloticus* (*O. niloticus*) in Marriotiah stream, an intrastate tributary of River Nile that crosses number of cities at Giza / October governorates. Mass kills as well as respiratory distressed fish were seen along 4 km distance at the Marriotiah stream. The disaster zone extended from the city of Shabramant till Aboseer and losses were estimated to approach several tons of tilapias. Clinically, the affected fish presented typical signs of asphyxia. It is worthy to mention that respiratory distressed fishes were mainly those of small sizes, while most of the mass mortalities were limited to large sized fish. Environmental records of such polluted stream have revealed that similar cases of mass kills have occurred 3 years ago, but the losses were much less. Analysis of the data extracted from the field and laboratory investigations of the disaster have lead us to conclude that the poorly treated organic and non organic chemical byproducts from Elhawamdia sugar factory into Marriotiah and other secondary drainage streams was the primary cause of this environmental adversity. Although the problem was multi-factorial, yet laboratory investigations have elaborately identified that the initial stimulus behind the case was the nasty chemical byproducts found in stream water. More comprehensively, the physical and chemical examination of water samples have revealed the presence of abnormal water colors, high levels of ammonia, low levels of dissolved oxygen (DO) and also characteristic abnormal findings of phenol and polycyclic aromatic hydrocarbons were found. So, we concluded that the presence of interactive abnormal chemical findings in the stream water was biologically translated into an intense case of respiratory distressed Nile tilapias with consequent mass mortalities.

Key words: Nile tilapia % Ammonia toxicity % Decreased dissolved oxygen % Polycyclic aromatic hydrocarbons

INTRODUCTION

Aquatic ecosystems serve as the ultimate sink for many environmental pollutants, which accumulate in fish species. Fish are among the most sensitive species to the toxicity of chemicals, particularly at early developmental stages [1-4].

Increased environmental pollution can be attributed to a variety of factors resulting from different industrial and agricultural technologies. The aquatic environment becomes the final point for these contaminants. Many classes of pollutants can be uptaken in fish from water, food, sediments and suspended particulate material, namely the persistent contaminants of agricultural origin insecticides and herbicides [5].

The environmental disaster of mass mortalities among fish populations in the natural water resources have been repeatedly encountered in the past few years due to number of environmental problems, such as acute toxicities with some pollutants [6] illegal use of cyanide salts in fishing, viral, mycotic and bacterial infections [7-9], red tides, abrupt thermal changes, extreme reduction in dissolved oxygen and high ammonia levels above the aquatic species tolerance threshold [10].

Viral causes for mass mortality were recorded in many circumstances; herpesvirus was isolated from adult koi, *Cyprinus carpio*, suffering mass mortality [11]. Also, mass mortalities of seven band grouper *Epinephelus septemfasciatus*, red drum, *Sciaenops ocellatus* and Asian sea bass *Lates calcarifer* caused by

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a fish nodavirus were recorded by Fukuda *et al*. [12]; Myung-Joo [13] and Parameswaran *et al*. [14]. Moreover, Minamoto *et al.* [15] observed catastrophic damages to cultivated and natural carp populations, caused by cyprinid herpesvirus 3 (CyHV-3).

Mass mortalities due to bacterial agents were also recorded [16]. A bloom of *Hemidiscus hardmannianus* (Bacillariophyceae) was associated with heavy mortality of fishes. Also, fish mass mortality due to cyanobacteria in Spain water basins was recorded [9]. *Streptococcus agalactiae* could be a cause of mass mortalties in *Oreochromis niloticus* worldwide [17]. Several cases of acute mass mortalities among the production stages of brook trout, brown trout and rainbow trout from intensive fish cultures were reported in Michigan, USA [8].

Mortalities due to fungal causes were reported in January 2001 following severe cold weather, in which an episode of mass mortality caused by *Saprolegnia parasitica* has occurred among the *Oreochromis niloticus* fingerlings at a semi intensive farm in Nile delta, Egypt [7, 18]. Controversially, several cases of mass mortalities of unknown etiology to date have been happening [19, 20].

Patterns of fish mortality due to physicochemical factors were registered in many parts of the world because of low dissolved oxygen, high un-ionized ammonia [21] and an abrupt temperature fall [22]. In addition, Mass mortalities might be attributed to the presence of saponin, a hemolytic glycoside in environmental water bodies [23].

Ammonia toxicity is closely correlated with pH and, to a lesser extent, by water temperature and DO. At high pH a greater percentage of total ammonia converts to the toxic un-ionized form. Ammonia toxicity may increase as a result of increasing ammonia levels and decreasing oxygen concentration following crashes of phytoplankton populations [24]. Moreover, under reduced oxygen level most of the mortality occurs within a few hours, apparently as a consequence of an additive toxic effect [25].

Polycyclic aromatic hydrocarbon (PAH), family of compounds, is widely distributed in both fresh water and coastal marine ecosystems whereas they have been found to bioaccumulate in several aquatic species. They also represent one of the most significant classes of organic pollution due to their carcinogenic and mutagenic potentials [26]. It is a nearly ubiquitous environmental contaminant and found in coal tar and byproducts of incomplete carbonaceous matter combustion. It inhibits both cell-mediated and humeral immune responses [27].

Pseudomonas fluorescence represents an ideal model for pathogenic as well as saprophytic bacteria which directly / indirectly impact vulnerable fishes through bacterial invasion [28]. *Pseudomonas florescence* can induce typical form of acute septicemia which is manifested by the appearance of severe fin rot, skin ulcers, nervous manifestation and finally acute episodes of deaths in immune-compromised fishes [28-30].

Liver in fish plays an important role in several vital functions on basic metabolism [31] and it is also the major organ of accumulation, biotransformation and excretion of contaminants [32,33]. The study of histological changes in fish organs has become an important tool for monitoring environmental exposure of fish to contaminants in laboratory and field studies [34, 35].

The aim of this study was to discuss the historical environmental back ground behind the case; determine the possible causes of the mass mortality in Marriotiah stream and determining the most practical methods to prevent the re-occurrence of such problem in the future.

MATERIALS AND METHODS

Case History and Field Visit: The eruption of the catastrophic mass mortalities has quickly triggered us to perform an emergency visit to the mortalities scene. The emergent visit to the mortalities scene along the Mariottiah stream has enabled us to obtain the history of the problem, record mortality patterns, determine species affected and record clinical findings / abnormal behavioral changes in affected fishes.

Fish Samples: A total of 30 clinically affected juvenile tilapia *Oreochromis niloticus* with average weights of 10 - 50 g was obtained from the Marriotiah stream and brought to the wet lab of the Department of Fish Diseases and Management, Cairo University. The fish were kept in well-aerated glass aquaria at 25°C. Fish were examined for any abnormal external signs and gross lesions.

Water Quality Parameters: A total of 3 water samples was aseptically collected, one represent the drainage Sakara 7, the second taken from Marriotiah stream south to the drainage Sakara 7 and the third represent the Marriotiah stream north to the drainage

Sakara 7. All samples were obtained in sterile plastic bottles and stored according to Standard Methods described by APHA [36], then physico-chemically analyzed. The Physical parameters including, color, odor and turbidity were detected. Water temperature and pH were measured using a waterproof digital combo pH meter and thermometer (HI 98127 (pHep 4) - Hanna instruments Inc., RI, USA). Dissolved oxygen concentrations were measured using a digital dissolved oxygen meter (HI 9142 - Hanna instruments Inc., RI, USA). Total ammonia nitrogen (TAN mg/L) was determined following the method described by Chattopadhyay [37]. The water samples were also tested for the presence of some chemical pollutants with direct relation to the history of the problem such as phenol and polycyclic aromatic hydrocarbons using High Performance Liquid Chromatography (HPLC) [Jasco, Model UV-2075 Plus] (USEPA SW-846 Method 8310) [38].

Bacteriological Examination: Loopfuls from gills and the internal organs aliquot (liver, kidney, spleen and from lesions on the skin of some fish) were inoculated into Trypticase soy broth (Becton, Dickinson and Company -BD, NJ – USA) and then incubated at 25ºC for 18-24 hours. The inoculated broths were checked for the required turbidity degree by matching against McFarland standard turbidity tubes. Then, inoculated in Triptic Soya Agar (TSA) and incubated at 25ºC for 24-48 hours. Purified isolates were identified using phenotypic and biochemical test according to Austin and Austin [28].

Histopathological Examination: Gills, liver, brain and kidney tissue samples of the clinically affected *Oreochromis niloticus* were fixed in 10% neutral buffered formalin solution. Formalin fixed tissues were then processed and embedded in paraffin. Five-micron sections of tissue samples were stained with hematoxylin and eosin (H & E) using methods described by Bancroft *et al.* [39].

RESULTS

Case History and Field Visit: Analysis of the case history and field visit indicated that the mortalities and clinically affected fish were observed along Marriotiah stream throughout a distance about 4 km which extend from Shabramant till Aboseer.

The color of water was brownish red at the area extended from Shabramant till Aboseer (Plate 1 (2)), whereas mortalities and signs were predominant. Normal color of water in Marriotiah stream was found after Aboseer, while dark bluish or blackish coloration of water was observed in drainage Sakara 7 (Plate 1 (1)).

The mortalities and signs were only found in *O. niloticus* as they showed signs of asphyxia which include accumulation of fish on the surface with gasping of air and increased opercular movement (Plate 1 (3 - 4)). The signs of asphyxia involved different sizes of *O. niloticus* (fingerlings and large fish), while the mortalities were mostly limited to large sized fish. It is worthy to mention that the mortalities and signs of asphyxia among fish have disappeared in the Marriotiah stream after Aboseer, south to the outlet of the drainage Sakara 7.

The total mortalities approached several tons. The history revealed that this disaster has previously occurred 3 years ago, but on small scale. The case history refers to the drainage from the sugar factory in Elhawamdia into the Sakara 7drainage and then into the Marriotiah stream to be the cause of this disaster.

Water Sample Parameters: The results of physical and chemical examination of important parameters in association with detection of some pollutants were recorded in Table 1.

Clinical and Post-Mortem Examination: The examination revealed that different sized fish have suffered from tail and fin rot, dark body coloration and dwarfism in many of the affected fish (small size in relation to the length).

Table 1: Physical and chemical examination of water with detection of some pollutants

	Physical characters of water				Chemical characters of water			Water pollutants	
Location	Color	Odor	Turbidity	Temp	pH	Dissolved O_2	Ammonia	Phenol	Polycyclic Aromatic Hydrocarbons
Sakara 7 drainge	Dark brown	Bad odor (sewage)	Highly turbid	21	9.5	3 ppm	210 mg/L	5 ppm	102.8 ppm
Mariotia channel (south to sakara 7drainge)	Normal color	Normal	Normal	20.7	7.2	5 ppm	0.01 mg/L	No detectable	3.2 ppm
Mariotia channel (north to sakara 7drainge)	Brownish red	Fishy odor	High due to side irrigation	20	8.3	4.5 ppm	32.4 mg/L	1.7 ppm	23.8 Ppm

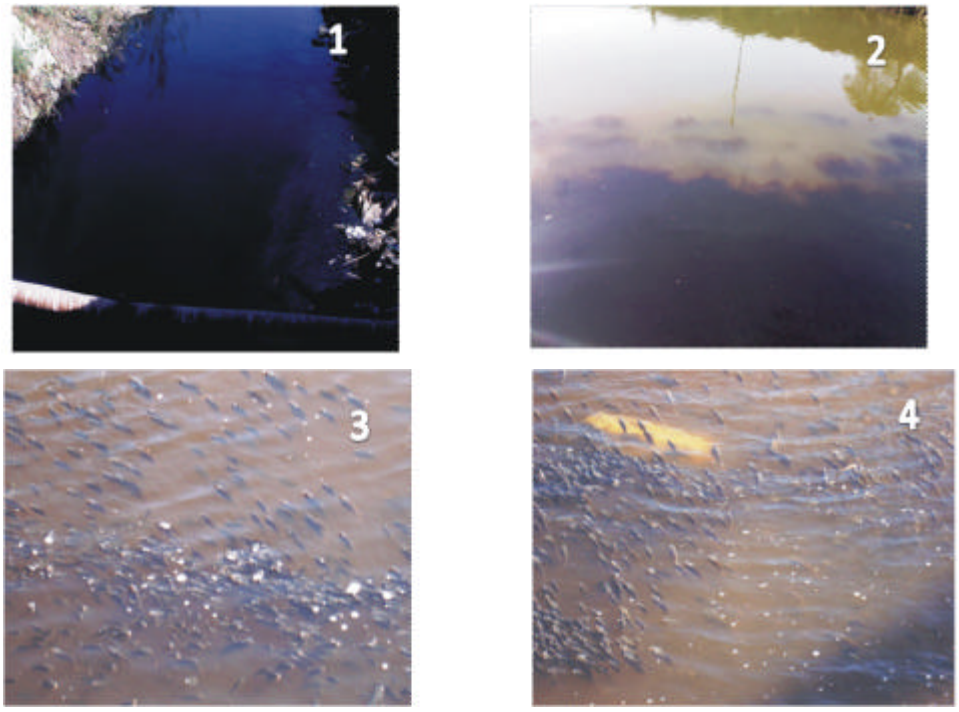

Plate 1: Fig. (1): Showing water of the drainage Sakara 7; Fig. (2): Showing the water of Mariotia channel at the insertion of drainage Sakara 7; Fig. (3): Showing accumulation of large number of *Oreochromis niloticus* on the surface of water; Fig. (4): Showing signs of asphyxia ad distress

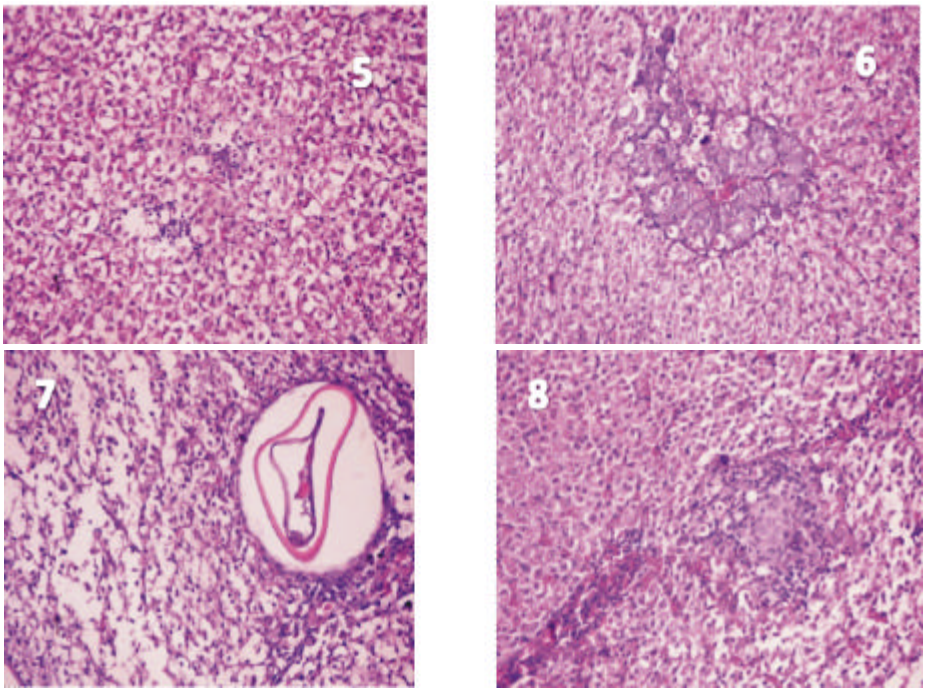

Plate 2: Fig. (5): Liver of Oreochromis niloticus showed marked vacuolation of hepatocytes (H&E X 200); Fig. (6): Liver of Oreochromis niloticus showed vacuolations and necrosis of hepatopancreatic tissue (H&E X 200); Fig. (7): Liver of Oreochromis niloticus showed vacuolar degeneration of hepatocytes and parasitic cyst (H&E X 200); Fig. (8): Liver of Oreochromis niloticus showed focal hepatic necrosis associated with leucocytic cell infiltration (H&E X 200).

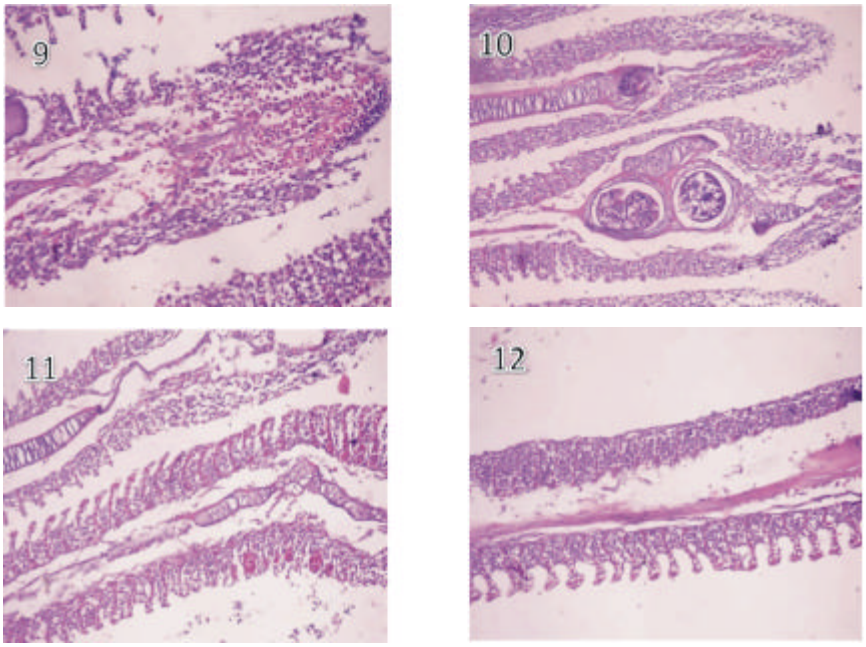

Plate 3: Fig. (9): Gills of Oreochromis niloticus showed necrosis of epithelial cells of secondary gill lamellae together with Focal haemorrage (H&E X 200); Fig. (10): Gills of Oreochromis niloticus showed multiple parasitic cyst, congestion and lamellar fusion (H&E X 100); Fig. (11): Gills of Oreochromis niloticus showed marked congestion associated with focal necrosis (H&E X 200); Fig. (12): Gills of Oreochromis niloticus showed congestion, lamellar fusion and hyperplasia (H&E X 200).

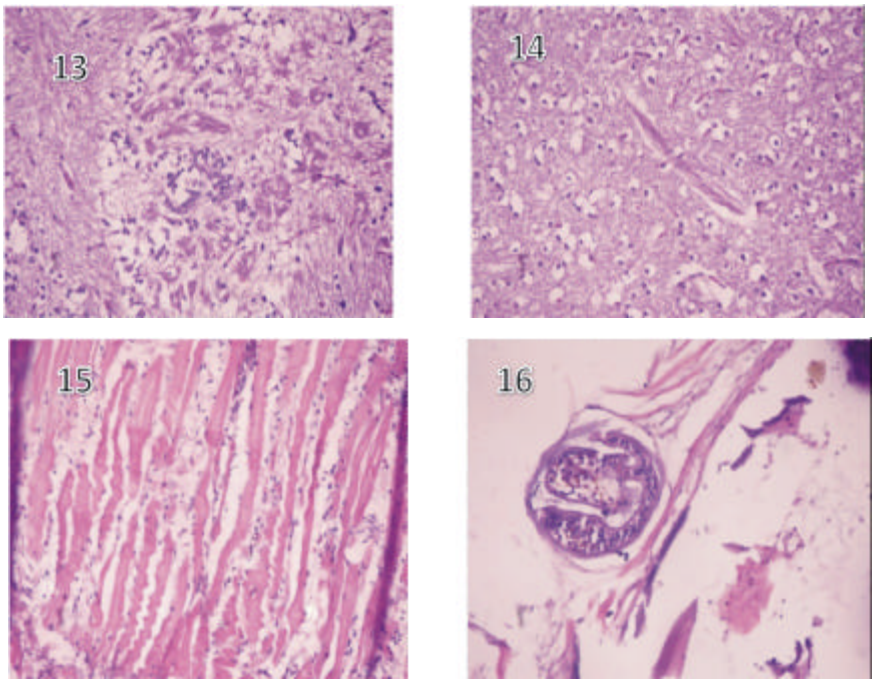

Plate 4: Fig. (13): Brain of Oreochromis niloticus showed focal necrosis associated with gliosis (H&E X 200); Fig. (14): Brain of Oreochromis niloticus showed brain edema (H&E X 200); Fig. (15) Muscles of Oreochromis niloticus showed inter-muscular edema (H&E X 200); Fig. (16) Muscles of Oreochromis niloticus showe parasitic cyst (H&E X 200).

Erosions and hemorrhages were observed on the skin of some fish with hyperemic gills in association with gill rot. Also abnormal discoloration of liver and internal body organs was observed.

Bacteriological Examination: The results of bacterial isolation and biochemical identification (API 20 E) have revealed that *Pseudomonas fluorescence* a specific fish pathogen was identified from kidney samples of the examined fish.

Histopathological Examination: Histopathological examination of samples from liver, gills, brain and muscles from affected *O. niloticus* revealed that the liver showed marked degrees of vacuolation of hepatocytes to marked necrosis of hepatopancreatic tissue (Plate 2 (5-6)). Some fish livers showed vacuolar degeneration of hepatocytes and parasitic cysts (Plate 2 (7)) while others showed focal hepatic necrosis associated with leucocytic cell infiltration (Plate 2 (8)).

The histopathological examination of gills of examined *O. niloticus* showed secondary gill lamellar necrosis together with focal hemorrhage (Plate 3 (9)). In some fish, the gills showed multiple parasitic cyst, congestion and lamellar fusion (Plate 3 (10)). Also some gills showed marked congestion associated with focal necrosis (Plate 3 (11)) while others showed congestion, lamellar fusion and hyperplasia (Plate 3 (12)).

Brain of examined *O. niloticus* showed focal necrosis associated with gliosis (Plate 4 (13)). Other fish brain showed edema (Plate 4 (14)). Further, examination of muscles showed inter-muscular edema (Plate 4(15)) and some revealed the presence of parasitic cyst (Plate 4(16)).

DISCUSSION

Case history data and field visit evaluation revealed that the mortalities and signs of asphyxia which appeared in the affected fish were observed along the Marriotiah stream in a distance about 4 km extending from Aboseer at the south till Shabramant at the north. The investigation of this environmental crisis is linked to the fact that the outlet of the drainage Sakara 7 into the Marriotiah stream at Aboseer is the location whereas mortalities and signs have started, then extended till Shabramant with the direction of the water current.

The mortalities and signs have only involved *O. niloticus*, which could be due to the fact that *O. niloticus* is currently the predominant trophic species in the river Nile tributaries. However, other cohabitating fish species are present such as sharp toothed catfish (*Clarias gariepinus)* which is a demersal species with high resistance to the bad water quality and environmental pollutants [40].

Signs of asphyxia have been presented by different sizes of fish, while the mortalities were mostly limited to large sized fish. The history revealed that this emerging case has previously occurred 3 years ago among slightly low number of fish compared to our current disaster. The extracted case history data mainly refer to the drainage from the sugar factory in Elhawamdia, which pour its industrial effluents into the drainage Sakara 7 which then passes into the Marriotiah stream, to be the primary cause of the environmental catastrophe.

The results of physical and chemical examination of important water quality parameters together with the detection of some environmental pollutants have revealed relative similarity between those of Marriotiah stream at Aboseer till Shabramant (main mortality scene) and that of the drainage Sakara 7. The marked shift of water color from the apparently classic appearance in Marriotiah stream south to the outlet of drainage Sakara 7 directs the attention that the water from Sakara 7 could be on of the suspect causes of this problem.

The marked abnormal water quality parameters recorded for the water samples taken from drainage Sakara 7 and from Marriotiah stream north to the outlet of the drainage Sakara 7 compared to those recorded for the sample collected from the Matriotia stream south to the outlet of the drainage Sakara 7 could also point out to the role of Sakara 7 drainage in the emergence of this environmental crisis. The fluent move of water toward the northern parts of the stream explains why the disaster was mainly restricted to the north than to the south. Water examination for the 3 diverse water samples has revealed marked abnormal results of drainage Sakara 7, which then markedly reduced due to dilution of the Marriotiah stream north to the outlet of the drainage till reach the apparently normal levels at the south of the outlet of the drainage Sakara 7.

The toxicity of ammonia to aquatic organisms has been reported since long time and it is principally due to the unionized form of ammonia which may predispose to inferior growth rate, asphyxia and mortality [10, 41, 42].

Massive mortality of tilapia occurs when water unionized ammonia concentrations was greater than 2 mg/l. Prolonged exposure (several weeks) to unionized ammonia concentration greater than 1 mg/l causes losses, especially among fry and juveniles in water with low DO. It is therefore, surprising that the chemical condition was acute enough to cause mass mortalities in fish [10].

The high levels of phenol and ammonia in conjugation with low levels of dissolved oxygen in the area of the problem explicitly explain the signs of asphyxia observed among different sized *O. niloticus.* Also, it could explain the mortalities recorded in the large sized fish.

The toxic action of phenol has been previously attributed its effect effects on the nervous system, causing paralysis and convulsions. The main toxic effect is the interference with the respiratory system resulting in asphyxia which was clearly seen in affected *O. niloticus* in this study. The results were in accordance with that reported by Saha [43] who revealed that phenol and ammonia were additive in their action even though the toxic mechanisms were probably different. The high levels of phenol which have been found may be due to the fact that these compounds are ubiquitous water pollutants which come to the natural resources from the effluents of a variety of chemical industries [43].

At the same time the high levels of polycyclic aromatic hydrocarbons of the examined water samples hypothesized that this pollutant is one of the most widespread organic environmental pollutants that are known to be carcinogenic and immunotoxic [44,45]. These compounds have been found to exhibit toxic and hazardous properties, as a consequence, the U.S. Environmental Protection Agency has listed 16 PAH compounds, including naphthalene and acenaphthene, as priority pollutants to be monitored in industrial effluents [38].

Regarding the clinical and postmortem finding, the examination revealed that different sized fish were suffered from tail and fin rot, dark body coloration which may be related to the poor water quality and pseudomonas florescence infection (fin rot disease) [46]. Environmental factors may act as stressors and can predispose a fish to bacterial diseases. The increased values of Ammonia, pH and decreased DO in addition to the possible synergistic interactions of other cultural parameters may result in stressing and immuno-compromising fish as well as enhancing environmental selection of certain type of microorganisms [28-30].

The dwarfism reflects the slow growth rate of fish which is also related to the poor water quality, especially high ammonia [10, 41]. The hyperemic gills in association with gill rot may be due to the high ammonia and low dissolved oxygen. Also, abnormal coloration of liver and internal body organs may be due to the presence of toxic pollutants of water. The abnormal clinical findings and mortalities may be due to the fact that phenol affects the metabolism, survival, growth and reproductive behavior of fish despite the active potential and detoxification of phenol in fish. Also, the sub-lethal doses of phenol interfere with various enzyme activities and can produce many unpredictable changes in fish [43].

The histopathological examination of gills showed necrosis of epithelial cells of secondary gill lamellae together with focal hemorrhage. Some gills showed multiple parasitic cyst, congestion and lamellar fusion. In other fish the gills showed marked congestion associated with focal necrosis, lamellar fusion and hyperplasia. The necrosis of epithelial cells of secondary gill lamellae together with hemorrhage may be due to the toxic action of phenol, that sub-lethal concentration have been shown to give rise to a series of pathological effects including necrosis of the gills and increased mucus production [47].

The histopathological examination of brain of examined *O. niloticus* showed focal necrosis associated with gliosis and sometimes edema. This could explain the toxic action of phenol which has been attributed to its effects on the nervous system causing paralysis and convulsions [48]. The presence of parasitic cysts in liver, gills and muscles may be due to the poor water quality and dwarfism which render the fish susceptible to parasites. The histopathological findings, especially those of gills, liver and internal body cavity agree with Mitrovic *et al.* [47] who reported that fish was killed within a few hours of exposure to phenol and they revealed some gross at autopsy gross changes which included inflammation and necrosis of the pharynx and gills, internal hemorrhages, with blood in the body cavity and swelling of the spleen. Severe damage involving the gall-bladder, liver and kidney has occurred in fish surviving for 7 days in the lower concentrations. Gill damage, following the initial inflammatory response, consisted of the stripping of the epithelium from the secondary lamellae, leaving only the naked pillar cells and from the filament.

The effect of phenol, regarding the metabolic changes in oxygen consumption which decreased significantly and histopathological findings in the tissues of liver, gills, muscle and brain, agreed with Ravichandran and Anantharaj *et al*. [48] who revealed that following sub-lethal exposure to phenol oxygen

consumption total protein, total carbohydrate and total lipids in the tissues of liver, gills, muscle and brain of the fish decreased greatly. The histopathological findings in liver, muscle and brain explain the general decrease of the activity of phosphomonoesterases and adenosine triphosphatase in such organs which have been reported by Ravichandran and Anantharaj [48].

Although the mortality of fish can be attributed with certainty to changes in the water volume with increased concentration of chemical pollutants, the exact cause of death of the fishes is more difficult to ascertain. Two major potential causes of the mortality are the oxygen depletion and the chemical poising by reduced substances including ammonia and other contaminants [24, 49-51].

From this study, it could be concluded that the drainage Sakara 7 with its high abnormal water quality findings of phenol, polycyclic aromatic hydrocarbones and ammonia has negatively affected the condition of water at Marriotiah stream. The problem extends for about 4 km in Marriotiah stream till dilution occurred. From the history, water analysis, clinical, post mortem, bacteriological and histopathological findings, it could be attributed that phenol and ammonia could be responsible for the signs of asphyxia and mortalities which appeared on affected *O. niloticus*. The release of new water from Gizawia stream (other tributary of river Nile) into Marriotiah stream for 3 successive days have corrected the condition and lead to the cessation of mortalities and associated signs.

ACKNOWLEDGMENTS

Authors are very grateful to Dr. Alaa Eldin Eissa, assistant professor of fish Diseases and Dr. Mohamed Moustafa Mohamed, professor of fish diseases at faculty of Veterinary Medicine, Cairo University for their valuable support during the sampling process, result interpretation and data analysis.

REFERENCES

1. Walker, M.K. and R.E. Peterson, 1991. Potencies of polychlorinated dibenzop-dioxin, dibenzofuran and biphenyl congeners, relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin for producing early life stage mortality in rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicolol., 21: 219-238.
2. Walker, M.K., J.r. Hufnagle, M.K. Clayton and R.E. Peterson, 1992. An egg injection method for assessing early life stage mortality of polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls in rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicolol., 22: 15-38.
3. Dong, W., H. Teraoka, K. Yamazaki, S. Tsukiyama, S. Imani, T. Imagawa, J.J. Stegeman, R.E. Peterson and T. Hiraga, 2002. 2, 3, 7, 8- tetrachlorodibenzo- p-dioxin toxicity in the Zebrafish embryo: Local circulation failure in the dorsal midbrain is associated with increased apoptosis. Toxicological Sci., 69: 191-201.
4. Carney, S.A., A.L. Prasch, W. Heideman and R.E. Peterson, 2006. Understanding dioxin developmental toxicity using the Zebrafish model. Birth Defects Research A., 76: 7-18.
5. Hardersen, S. and S.D. Wratten, 1998. The effects of carbaryl exposure of the penultimate larval instars of Xathocnemis zealandica on emergence and fluctuating asymmetry. Ecotoxicol., 7: 297-304.
6. Sawako, H., H. Daisuke, T. Shin, I. Toshiyuki, K. Michio and S. Hinsuke, 2009. Mass mortality and trace element residues in Isaza *(Gymnogobius isaza)* collected from lake Biwa Japan. Interdisciplinary studies on environmental chemistry - Env. Research in Asia Eds, Yobashi and Others, pp: 177-183.
7. Mohamed, A. and A.M. Mahmoud, 2004. Seasonal study, histopathological and treatment trial on saprolegniosis in some fish farms. In the proceeding of the 2004 first scientific conference of veterinary Medicine. Moshtohor, Banha, pp: 1-4.
8. Eissa, A.E., 2005. Bacterial kidney disease (BKD) in Michigan salmonids. Ph.D. Dissertation. Michigan State University, East Lansing, Michigan, pp: 210.
9. Lopez-Rodas, V., E. Maneiro, M.P. Lanzarot, N. Perdigones and E. Costas, 2008. Mass wildlife mortality due to cyanobacteria in the Donana National Park. Spain Veterinary Record, 162: 317-8.
10. Harris, J.O., B.M. Greg, E. Stephen and M.H. Stephen, 1998. Effect Of ammonia on the growth rate and oxygen consumption of juvenile greenlip abalone, *Haliotis laevigata* Donovan. Aquaculture, 3: 259-272.

11. Hedrick, R.P., O. Gilad, S. Yun, J.V. Spangenberg, G.D. Marty, R.W. Nordhausen, M. Kebu, J.H. Bercovier and A. Eldar, 2000. A Herpesvirus Associated with Mass Mortality of Juvenile and Adult Koi, a Strain of Common Carp. J. Aquatic Animal Health, 12: 44-57.
12. Fukuda, Y., H.D. Nguyen, M. Furuhashi and T. Nakai, 1996. Mass Mortality of Cultured Sevenband Grouper, Epinephelus septemfasciatus, associated with Viral Nervous Necrosis. Fish Patholol., 31: 165-170.
13. Myung-Joo, O.H., J. Sung-Ju, K. Suk-R, K.V. Rajendran, K. Young, J.C. Tae-Jin,K. Hyeung-R and K. Jin-D, 2002. A fish nodavirus associated with mass mortality in hatchery-reared red drum, *Sciaenops ocellatus*. Aquaculture, 211: 1-7.
14. Parameswaran, V., S.R. Kumar, A.V.P. Ishaq and H.A.S. Sahul, 2008. A fish nodavirus associated with mass mortality in hatchery-reared Asian Sea bass, *Lates calcarifer*. Aquaculture, 275: 366-369.
15. Minamoto, T., M.N. Honjo, K. Uchii, H. Yamanaka, A.A. Suzuki, Y. Kohmatsu, T. Iida and Z. Kawabata, 2009. Detection of cyprinid herpesvirus 3 DNA in river water during and after an outbreak. Veterinary Microbiolol., 135: 261-6.
16. Subramanian and A. Purushothaman, 1985. Mass Mortality of Fish and Invertebrates Associated with a Bloom of Hemidiscus hardmannianus (Bacillariophyceae) in Parangipettai (Southern India). Limnolology and Oceanography, 30: 910-911.
17. Mian, G.F., D.T. Godoy, C.A. Leal, T.Y. Yuhara, G.M. Costa and H.C. Figueiredo, 2009. Aspects of the natural history and virulence of S. agalactiae infection in Nile tilapia. Veterinary Microbiolol., 136: 180-3.
18. Munchan, C., O. Kurata, K. Hatai, N. Hashiba, N. Nippon, N. Nakaoka and H. Kawakami, 2006. Mass mortality of young striped jack Pseudocaranx dentex caused by a fungus *Ochroconis humicola*. Fish Patholol., 41: 179-182.
19. Shih-Ling, H., C. Wei-Cheng, S. Mei-Chuan, L. I-Chiu and C. Shiu-Nan, 1999. Studies on Epizootiology and Pathogenicity of *Staphylococcus epidermidis* in Tilapia (*Oreochromis* spp.) cultured in Taiwan. Zoolological Studies, 38: 178-188.
20. Belvin, S., R. Tremblay, M. Roussy and S.E. MCGladdery, 2008. Inoculation Experiments to Understand Mass Mortalities in Sea Scallop, *Placopecten magellanicus*. J. Shellfish Res., 27: 251-260.
21. Sargent, C.J. and L. David, 2002. Galat fish mortality and physicochemistry in a managed floodplain wetland. Wetlands Ecology and Management, 10(2): 1-5.
22. Economids, P.S. and V.P. Vogiatzis, 2009. Mass mortality of Sardinella aurita Valenciennes (Pisces, Clupeidae) in Thessaloniki Bay (Macedonia, Greece). J. Fish Bioliol., 41(1): 147-149.
23. Grib, V., N. Goncharenko and D. Voytyshina, 2006. Saponin as a Factor of Mass Fish Mortality in the Rivers of Ukraine. Hydrobiol., 42: 61-71.
24. Wajsbrot, N., A. Gasith, M.D. Krom and D.M. Popper, 1991. Acute toxicity of ammonia to juvenile gilthead seabreamSparus aurata under reduced oxygen levels. Aquaculture, 92: 277-288.
25. Diana, J.S., J.P. Szyper, T.R. Balterson, C.E. Boyd and R.H. Piedrahita, 1997. Water quality in ponds, In: H.S. Egna and C.E. Boyd, (Eds.), Dynamics of Pond Aquaculture. Lewis publishers in an imprint of CRC Press, New York, USA, pp: 53-71.
26. Hassanin, A.A.I., Y. Kaminishi, M.M. Osman, Z.H. Abdel-Wahad, M.A.H. El-Kady and T. Itakura, 2009. Cloning and sequence analysis of benzo-a-pyreneinducible cytochrome P450 1A in Nile tilapia (*Oreochromis niloticus*). African J. Biotechnol., 8: 2545-2553.
27. Smith, D.A., 1998. The development and application of a hemolytic plaque forming cell assay (pfc) and a cytotoxic t-lymphocyte assay (ctl) in tilapia *(oreochromis niloticus)* for immunotoxicity risk assessment of environmental contaminants. M.S. Thesis, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University in Partial fulfillment of the requirements.
28. Austin, B. and D.A. Austin, 2007. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. Chichester: Praxis Publishing Ltd.
29. Ahne, W., W. Popp and R. Haffman, 1982. *Pseudomnas fluorescence* as a pathogen of tench (Tinca tinca). Bulletin of European Association of Fish Pathologistic, 4: 56-57.
30. Fernandez, A.I.G., L.A. Rodriguea and T.P. Nieto, 1990. Characterization *Pseudomonas* strains producing Septicemia in rainbow trout cultures in the North West of Spain. Bulletin of European Association of Fish Pathologistic, 10: 133-134.

31. Moon, T.W., P.J. Walsh and T.P. Mommsen, 1985. Fish hepatocytes: a model metabolic system. Canadian J. Fisheries and Aquatic Sci., 42: 1772-1782.
32. Triebskorn, R., H.R. Kohler, J. Flemming, T. Braunbeck, R.D. Negele and H. Rahmann, 1994. Evaluation of bis(tri-n-butyltin)oxide (TBTO) neurotoxicity in rainbow trout Oncorhynchus mykiss). I. Behaviour, weight increase and tin contents. Aquatic Toxicol., 30: 189-197.
33. Triebskorn, R., H.R. Kohler, W. Honnen, M. Schramm and S.M. Adams, 1997. Induction of heat shock proteins, changes in liver ultrastructure andalterations of fish behaviour: are these biomarkers related and are they useful to reflect the state of pollution in the field?. J. Aquatic Ecosystem Stress and Recovery, 6: 57-73.
34. Hinton, D.E. and D.J. Laure´n, 1990. Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure, In: J.F. McCarthy and L.R. Shugart, (Eds.), Biomarkers of Environmental Contamination, Lewis, Boca Raton, pp: 17-57.
35. Biagianti-Risbourg, S., 1997. Les perturbations (ultra) structurales du foie des poissons utilise´es comme biomarqueurs de la qualite´ sanitaire des milieux aquatiques, In: L. Lagadic, (Ed.), Utilisation de Biomarqueurs en Ecotoxicologie, Aspects Fondamentaux, Masson Publications, pp: 355-391.
36. APHA, 1995. Standard methods for the examination of water and waste water. 17th Edition. USA.
37. Chattopadhyay, G.N., 1998. Chemical Analysis of Fish Pond Soil and Water. Daya Publishing House, New Delhi, India.
38. James, R., Mihelcic and R.G. Luthy, 1988. Degradation of polycyclic aromatic hydrocarbon compounds under various redox conditions in soil-water systems. Applied and environmental microbiol., 54: 1182-1187.
39. Bancroft, G.D. and A. Stevens, 1996. Theory and Practice of Histological Techniques. Fourth edition. Churchill Livingstone. New York.
40. Randall, D.J. and T.K.N. Tsui, 2002. Ammonia toxicity in fish. Marine Pollution Bulletin, 45: 17-23.
41. Richard, W.S., B.F. John and H.R. Schmittou, 1983. Effects of Ammonia on Growth and Survival of Rainbow Trout in Intensive Static-Water Culture. Trans-actions of the American Fisheries Society, 112: 448-451.
42. John, W.A., W.W. Corlis, N.A. Kathleen and F.H. Steven, 1987. Seasonal toxicity of ammonia to five fish and nine invertebrate species. Bulletin of Environmental Contamination and Toxicol., 38: 324- 331.
43. Saha, N.C., F. Bhunia and A. Kaviraj, 1999. Toxicity of Phenol to Fish and Aquatic Ecosystems. Bulletin of Environmental Contamination and Toxicol., 63: 195-20.
44. Reynaud, S. and P. Deschaux, 2006. The effects of olycyclic aromatic hydrocarbons on the immune system of fish. Aquatic Toxicol., 77: 229 -38.
45. Renata, G., S. Martin, F. Ana, M. Marta and D. Correia, 2008. The joint effect of polycyclic aromatic hydrocarbons on fish behavior. Environmental Res., 108: 205-213.
46. Miyazaki, T., S.K. Kubota and T. Miyashita, 1984. A histopathological study of *Pseudomonas fluorescence* infection in tilapia. Fish Patholol., 19: 161-166.
47. Mitrovic, V.V., V.M. Brown, D.G. Shurben and M.H. Berryman, 1968. Some Pathological effects of sub-acute and acute poisoning of rainbow Trout by phenol in hard water. Water Res., 2: 249-252.
48. Ravichandran, S. and A. Bernice, 1984. Effect of phenol on the phosphomonoesterases and ATPase activity in the fish *Sarotherodon mossambicus* (Peters) in saline waters. Water Res., 93: 557-563.
49. Baird, R., J. Bottomley and H. Taitz, 1979. Ammonia toxicity and pH control in fish toxicity bioassays of treated wastewater. Water Res., 13: 181-184.
50. Zhao, J.H., T.J. Lam and J.Y. Guo, 1997. Acute toxicity of ammonia to the early stage larvae and juveniles of Eriocheir sinensis H. Milne-Edwards, 1853 (Decapoda: Grapsidae) reared in the laboratory. Aquac Res., 28: 517-525.
51. Pillay, T.V.R., 1992. Aquaculture and the Environment, 1st ed. University Press, Cambridge, pp: 189.