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Field assessment of the mid winter mass kills of trophic fishes at Mariotteya stream, Egypt: Chemical and biological pollution synergistic model

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HIGHLIGHTS

- Environmental pollutants have synergized to produce catastrophic fish mass kills.
- Fish immunity was jeopardized with phenol, PAHs and heavy metals.
- Mutual biological invasion would have triggered an intense dermal damage.

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ABSTRACT

Pathogenic *Candida albicans* was isolated from water and fish samples collected during an emergent event of mass mortalities among the juvenile Nile tilapia (*Oreochromis niloticus*), Sharp toothed catfish (*Clarias gariepinus*) along the stream of Mariotteya drainage. Investigations indicated that fish mortalities were confined to the area of Shubramant and Aboul Noumros (North to Sakara 7 drainage). *C. albicans* was isolated from the lesions associated with multiple skin ulcers in both Nile tilapia juveniles and Sharp toothed catfish. Assessment of the field and laboratory data has indicated that Mariotteya environmental disaster was a multifactorial problem. The fish mass kills were initially flared up through the dumping of the improperly treated nasty organic and inorganic chemicals from Elhawamdia sugar factory and municipal sewage. The physical stagnation of the stream, high levels of ammonia, phenol and polycyclic aromatic hydrocarbons (PAHs) and low levels of dissolved oxygen (DO) were all incriminated as the initial stimulus behind biological invasion of pathogenic bacteria (*Pseudomonas fluorescence*) and yeast (*C. albicans*). Pathologically, fishes were dying from both respiratory and osmoregulatory failure induced by the severe damage of both gills and skin. It has been implied that such environmental pollutants have direct damaging effects on gills, skin and fins with consequent suppression of the skin's natural innate components. The adversely confronted immunological barriers were further exacerbated by the possible synergistic interactions of *P. fluorescence* dermotropic toxins followed by the secondary invasion of the pathogenic *C. albicans*.

1. Introduction

The causes of mass mortalities among wild fish populations are numerous and interacting. Non-infectious causes might include variable numbers of environmental pollutants. Mercury, cadmium and lead are the most prevalent heavy metals with direct impact on the aquatic animals and their natural environments (Moore and Ramamoorthy, 1984; Ullrich et al., 2001). Pesticides are the most dangerous chemicals running through the agricultural drainage water, which is mandated by law of irrigation/1984 to be the only source of water for aquaculture in Egypt (Ferrando et al., 1992; Khan, 1977). Illegal fishing by cyanide salts is another dangerous unethical mean by which millions of fish seeds are captured while containing variable levels of toxins (Leduc et al., 1982; Barber et al., 2002). Infectious agents are potential primary causes of mass kills among cultured and wild fish populations. Septicemic bacterial pathogens such as *Pseudomonas fluorescence* (*P. fluorescence*), *Aeromonas hydrophila*, *Streptococcus iniae* and many others are usually isolated from fish and environmental samples associated with mass mortalities in the wild aquatic environments (Wakabayashi and Egusa, 1972; Foo et al., 1985; Nyman, 1986). Mass kills of mycotic origin are relatively uncommon. However, winter kills (Saprolegniosis) have been reported among cultured Nile tilapias in winter of 2001 (Mohamed and Mahmoud, 2004).

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Branchiomycosis was incriminated in disastrous cases of summer kills in both earthen pond reared fishes and lake fish populations worldwide (Ramaiah, 2006).

Aquatic fungi and fungus-like organisms can be found in water reservoirs where they exist on leaves, coastal grasses, floating plants and decomposed aquatic animals. Some fungi act as parasites of plants, animals, and humans. Others, which live as saprophytes, in favorable conditions attain pathogenic invasive properties, and may acts as potential source of infection (Kiziewicz, 2004). Yeasts are microorganisms that represent a huge sector of the microbiota in all natural environments. They can survive for extended period of time in aquatic environment including marine and fresh water (Valdéz-Collazo et al., 1987). Many authors have reported the incidence of yeasts in diverse ecosystems such as soils, plants, animals and other organic matter in aquatic and marine environments including not only surface water but also demersal waters and sediments (Kiziewicz, 2004).

Candida albicans (*C. albicans*) is frequently found in the digestive tract and mucosal regions of mammals and birds. *C. albicans* is isolated occasionally from other habitats, including aquatic habitats receiving city sewage effluents (Cook and Schlitzer, 1981). Isolations from the above mentioned habitats appear to be due to recent pollution with animal and human wastes (Ahearn et al., 1968; Cook, 1970). *C. albicans* can survive for long periods as a single culture in sterile water or seawater (Jamieson et al., 1976). Also, it has been proposed that predation by protozoa reduces the population of yeasts in sewage (Cooke, 1965). Therefore, *C. albicans* can be used as an indicator of recent fecal pollution. Host wise, *C. albicans* were isolated from the intestine of farmed rainbow trout (*Salmo gairdneri*), turbot (*Scophtalmus maximus*), and free-living flat-fish (*Pleuronectes platessa* and *Pleuronectes flesus*) (Andlid et al., 1995). Yeasts were also isolated from different type of aquatic substrates, from seawater to endemic animals as *Rimicaris exoculata* shrimps, *Bathymodiolus azoricus* mussels and even deep-sea corals (Burgaud et al., 2010).

Thus, the current paper investigates the possible consequences of environmental deterioration of Mariotteya water body and subsequent invasion of the major trophic fishes with some opportunistic fungi such as *C. albicans*.

2. Materials and methods

2.1. Case history and field visit

On January 4th 2010, an erupting episode of mass mortalities among the trophic fish stages of the Mariotteya water stream was publicized through the Egyptian media. The magnitude of mass kills has approached several thousands of dead and dying fishes with typical respiratory signs. Visiting the mortality scene along the Mariotteya water stream and its collateral drainages was an essential demand for the investigation team to uncover the history behind the environmental crisis (Fig. 6). The emergent visit to the mortalities sites along the Mariotteya water stream and some of its collateral drainages has covered the distance between Alharam and Albadrashein (Fig. 6). Such investigatory visit has enabled us to depict mortality patterns, determine affected species, record clinical findings and abnormal behavioral changes among affected fishes.

2.2. Water samples

Water samples were collected under complete aseptic condition from certain sampling points along the Mariotteya stream at Shubramant and Aboul Noumros localities at northern and southern direction till the outlet of Sakara drainage into the stream. A total of three water samples were taken, one represent the drainage Sakara 7, the second taken from Mariotteya stream south to the drainage Sakara 7 and the third represent the Mariotteya stream north to the drainage Sakara 7. Water samples were physically examined for color, odor, turbidity and temperature. As major chemical water parameters, the hydrogen ion concentration (pH), dissolved oxygen and ammonia were all determined according to APHA, 1989. Water samples at Elhawamdia locality were also examined for the presence of some organic intermediate compounds such as phenol and polycyclic aromatic hydrocarbons.

2.3. Fish samples

A total of 50 clinically affected *O. niloticus* (average weight of 40 g) and 25 sharp tooth catfish (average weight of 150 g) were taken from the above mentioned mortality scenes along the Mariotteya stream. Fish samples were transferred into plastic container supplied with battery aerator and transported alive to the Fish Diseases and Management Laboratory (FDML) till submitted for different clinical and laboratory examinations.

2.4. Clinical examination

Collected fishes were examined while in water for the presence of any abnormal behavioral changes. Outside water, fishes were clinically examined for the presence of any lesions involving skin, fins, gills, internal organs and abdominal cavity.

2.5. Bacteriological examination

Water samples were aliquoted into several 15 ml sterile disposable centrifuge tubes. Tubes were centrifuged at 4000 rpm for 10 min then sediments were spread onto Trypticase soy agar (TSA) (Becton, Dickinson and Company -BD, NJ – USA) and then incubated at 25 °C for 18–24 h. Grown colonies were further purified and morphologically examined for their cultural characteristics and Gram staining criteria according to Austin and Austin (2007). The retrieved isolates were identified using API 20 NE semi-automated kit (bioMérieux Inc., NC, USA). Results were interpreted at 24–48 h according to the manufacturer's instructions.

Loopfuls from skin and kidneys of some clinically affected fishes were spread onto TSA media then incubated at 25 °C for 18–24 h. Isolation and identification procedures were proceeded as described before.

2.6. Mycological examination

Water samples were aliquoted into several 15 ml sterile disposable centrifuge tubes. Tubes were centrifuged at 4000 rpm for 10 min then sediments were spread onto Mycosel Agar plates (Becton–Dickinson and Company, Maryland, USA) and Sabouraud Dextrose Agar (bioMérieux) supplemented with 0.5 g/L chloramphenicol (Sigma, St. Louis, USA). Plates were incubated at 25–28 °C for 48–72 h. Culture plates were visually inspected for colonial growth of any possible fungal growths on the bases of fungal shape, color and size over the plate (cultural characteristics). Single colony of the retrieved isolates were stained with Gram stain and examined under the microscope. Yeast colonies selected at random were identified by mycotube test, carbohydrate assimilation/fermentation, urea, citrate, pseudohyphae/blastconidia formation on SDA-Tween 80 agar incubated at 25 °C for 72 h, cycloheximide resistance, and other tests according to Lodder (1970) with the use of rapid tests and alternative methods (Joshi et al., 1973).

Fish were sterilized with 70% alcohol then superficial layers of the skin lesions were trimmed to get rid of the surface contaminants. Loopfuls from the deep layers of the skin ulcers were spread