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

A.E. Eissa,⇑ N.A. Tharwat, M.M. Zaki

Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
Department of Botany, Faculty of Science, Cairo University, Giza, Egypt
Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

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 dermatropic 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).
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 (Scophthalmus maximus), and free-living flat-fish (Pleuronectes platessa and Pleuronectes flesus) (Andlil 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 the presence 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 mycotope test, carbohydrate assimilation/fermentation, urea, citrate, pseudohyphae/blastoconidia 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
onto Mycosel Agar plates (Becton–Dickinson and Company) and Sabouraud Dextrose Agar (bioMérieux) supplemented with 0.5 g L\(^{-1}\) chloramphenicol (Sigma; St. Louis, USA). Further isolation and identification procedures were completed as stated before.

### 3. Results

#### 3.1. Case history and field visit

Emergent visit to the mass kills’ sites has revealed that large numbers of dead and surfacing fishes were seen along Mariotteya stream throughout the distance between Alharam and Albadrash-ein (Fig. 1). The water color appeared reddish brown\(^1\) along the distance from Shubramant till Aboseer (Fig. 2). The case was different in Sakara 7 drainage, where the water appeared bluish to bluish black in certain instance (Fig. 2).

Investigations indicated that fish mortalities were confined to the area of Shubramant and Aboul Noumros (North to Sakara 7 drainage). Larger portion of the mass kills was involving tilapia species while few instances were limited to catfish. The tilapia fingerlings were aggregating at the surface of water showing typical signs of asphyxia which include rapid opercular movements and gasping (Fig. 1). Further, larger size fishes were seen dying with unclosed operculum at different sites of the stream along the distance from Alharam through Shubramant till Aboseer. Some catfishes were found dead with overinflated post-cephalic region.

#### 3.2. Clinical picture

Most of tilapia fishes were suffering from typical form of asphyxia manifested by surfacing, piping and rapid opercular movement. External examination of the affected tilapias revealed the presence of multiple skin erosions along the lateral aspects of the fish with remarkable deep ulcer at the caudal peduncle area (Fig. 3). The ulcer was hyperemic at the periphery and whitish red at the center. Gills were congested with sloughing of some filaments and enlargements of others. Internally, internal organs were congested and friable in some cases.

On examination, catfishes were presenting severe form of dorsal skin ulcers which appeared creamy white in most of the dissected cases (Fig. 4). Gills were dark red in color with mud lodged in between the filaments. Accessory lungs were overinflated with muddy materials lodged within organ. In majority of the dissected catfishes, liver was pale brown to yellowish. Kidney and other internal organs were moderately congested.

#### 3.3. Water sample examination

The physical water measures was close to normal in the southern outlet of the Mariotteya stream toward the Sakara 7 drainage while cleaning procedures of the stream and its related drainages have increased the turbidity of the water column in both the heart of the Sakara 7 drainage and northern outlet of the stream toward Sakara 7 drainage. The water color of both heart of the Sakara 7 drainage and the northern outlet of the stream toward the drainage was dark to reddish brown. The odor of the water collected from the above mentioned sampling localities was ranging from fecal to ammonia like odor in some instances which is bad indicator of sewage pollution (Table 1).

In Sakara 7 drainage, the sharp increase in ammonia levels (210 mg L\(^{-1}\)) was associated high surge in pH (9.5) which is comparably high when compared with that of Northern outlet of the stream toward the drainage (32.4 mg L\(^{-1}\) for ammonia and 8.3 for pH). The dissolved oxygen concentrations was variable between normal values in southern outlet of the Mariotteya stream toward the Sakara 7 drainage with slight diminish in the northern outlet toward the drainage while concentrations were sharply declined in the heart of the drainage which is running parallel to sharp increase in ammonia and pH levels (Table 1).

Detectable levels of phenol and polycyclic aromatic hydrocarbons (PAHs) were reported in both Sakara 7 drainage core and northern outlet toward the drainage. The phenol levels were 5 ppm and 1.7 ppm in Sakara 7 drainage core and northern outlet.

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\(^1\) For interpretation of color in Figs. 1 and 4, the reader is referred to the web version of this article.
toward the drainage respectively. The Sakara drainage water PAHs levels were much higher (102.8 ppm) than that of the northern outlet toward the drainage (23.8 ppm). It is worthy to mention that phenol and PAHs levels within the southern outlet of the stream toward the drainage were negligible (Table 1).

3.4. Bacteriological examination

The results of bacterial isolation and biochemical identification (API 20 E) have revealed that *P. fluorescence* a specific fish pathogen was identified from water and fish samples of the Sakara 7 drainage and the northern outlet of the Mariotteya stream toward the same drainage. Isolates retrieved from the kidneys of tilapias and catfishes were both associated with clinical form of septicemia which is typical for such pathogen.

3.5. Mycological examination

The results of isolation trials and biochemical identification have revealed the isolation of *C. albicans*, opportunistic yeast. The Candida was isolated from both water and fish samples of the Sakara 7 drainage. Isolates retrieved from the skin lesions of tilapias and catfishes were both associated with clinical form of severe dermatitis manifested by dorsal skin ulcers, dermal hemorrhages, fin and gill rot which are typical signs for cutaneous Candidosis in fish (Figs. 3 and 4). The isolates were presumptively identified as *C. albicans* after performing number of morpho-chemical tests. After 48 h of incubation, mature form of the fungus was noticed on Mycosel agar/SDA. The retrieved colonies were typically whitish to creamy, smooth and glistening (Fig. 5). The growth of such fungal colonies in the presence of cycloheximide (Actidione) present in the Mycosel agar media is indicative for *C. albicans* whereas most other yeasts, including *Cryptococcus neoformans*, are inhibited when grown on such media. Microscopic examination of a wet preparation made from colonies of the yeast grown on SDA has revealed round to oval budding yeast-like cells 4–6 by 5–8 μm. On
Gram staining the suspect yeast cells retained crystal violet (Table 2). On SDA-Tween 80 agar incubated at 25 °C for 72 h, pseudohyphae with clusters of round blastoconidia at the septa were found. Further, semi-automated biochemical identification of the fungal isolates was performed using the API 20 C system (bioMérieux). According to its carbohydrate assimilation profile, the yeast was confirmed as *C. albicans* (Table 2).

### 4. Discussion

Mass fish kills is a frequent syndrome that emerge due to the interaction of number of environmental and biological factors that belong to both natural water habitat encircling fish and the biology of the fish species itself (U.S. Environmental Protection Agency, 2000). Chemical pollution was incriminated in several incidents of mass fish deaths worldwide (Dou Abdul et al., 1997; Abdelaziz and Zaki, 2010). In our current research, mass mortalities among the tilapia and catfish populations along the Mariotteya stream were mainly attributed to a complicated form of chemical pollution that has incorporated diverse varieties of pollutants.

Among the three sampled points in the current study (Sakara 7 drainage, Outlet south to Sakara 7 drainage and outlet north to Sakara 7 drainage) the Sakara 7 drainage has presented the worst case. The fact that Sakara 7 drainage was the worst case could be attributed to the highly shooting levels of organic and inorganic pollutants.

Table 1

<table>
<thead>
<tr>
<th>Location</th>
<th>Physical measures</th>
<th>Chemical measures</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Color</td>
<td>Odor</td>
</tr>
<tr>
<td>Sakara 7 drainage</td>
<td>Dark brown</td>
<td>Fecal odor</td>
</tr>
<tr>
<td>Outlet south to Sakara 7 drainage</td>
<td>Normal color</td>
<td>Normal</td>
</tr>
<tr>
<td>Outlet north to Sakara 7 drainage</td>
<td>Brownish red</td>
<td>Ammonia like odor</td>
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Fig. 5. *Candida albicans* colonies on SDA/chloramphenicol agar plates.

Fig. 6. A map showing different sampling sites along Mariotteya drain. HR: (Alharam) Harranyah urban, Shubramant urban, HW: Elhawamdia, Saqqara (Sakkara) urban, BDR: Albadrashein urban. It is worthy to mention that the distance between mortalities start point at Harranyah urban (Alharam) and end point at Albadrashein urban is 4 km.
that triggered mass kills in most of the instances (Luther et al., 2004; Mandic et al., 2009).

Catfishes were suffering from remarkably less mortalities and respiratory distresses than tilapias. This observation could be explained by the natural biological capability of catfishes to live in the absence of dissolved oxygen for a prolonged period (24–72 h) using their gill-gifted accessory lung (dendiritic organ) which keeps atmospheric oxygen for longer periods of time (Dunham et al., 1983). The bottom living nature of the catfishes would accommodate them to tolerate the physical dynamic impacts of higher levels of suspended particles/sludge as well as enhance their natural tolerance to higher levels of organic/inorganic chemicals by a highly regulated process of bioaccumulation (Murphy and Spiegel, 1983).

High levels of toxic ammonia (NH₃), phenols (Saha et al., 1999; Ibrahim, 2011), high alkaline pH, high levels of combined organic matters (Bagarinao and Lantin-Olaguer, 1999) and PAHs are well known to have different degrees of damaging effects on epithelial tissues of skin, gills, nostrils and cornea of majority of fishes (De Maagd and Vethaak, 1998). Most critically, systemic and mucosal immunity of fish are usually deterred by the highly immunosuppressive effects of phenols (Wester et al., 1994) and PAHs (Reynaud and Deschaux, 2006). The PAHs are suggested to suppress macrophage activity (Reynaud and Deschaux, 2006), antagonize T Lymphocytic proliferation (Faisal et al., 1991a,b), suppress the tumourytic activity of the anterior kidney of fishes exposed to concentrated levels of PAHs at open water (Faisal and Huggett, 1993). The widely publicized hypotheses about the multi-potential immunosuppressive effects of the above mentioned environmental pollutants have proportionally derived us to assume that consequent secondary biological invasion of the fish skin/gills were predictable.

P. florescence and C. albicans were the only pathogenic biological agents isolated from the fish and stream water during the environmental catastrophe. The ubiquitous and opportunistic nature of both pathogens would provide another detrimental factor that had worsen the condition by invading the severely impacted skin/gill natural barriers. The isolation of P. florescence from kidneys of both fishes (systemic form) is suggestive for high bacterial load through the entire water column. Clinical signs were much restricted to fins, skin which could be due to the well documented pathogenicity mechanism induced by the P. florescence heat stable proteases (Thune et al., 1993; Austin and Austin, 2007). Such potent proteases are effective proteolytic agents that directly liquefy the proteinaceous material (hyaluronic acid and collagen) in the cement substance that links cells together with an ultimate result of skin ulcers and fin rot (Thune et al., 1993; Austin and Austin, 2007).

The integumentary/branchial health status of the immunologically exhausted fishes was further jeopardized by the ubiquitous existence of higher dermotropic pathogenic yeasts such C. albicans. The successful damage of the fish skin epidermal/gill lamellar layers by the corrosive effects of some environmental pollutants (phenol, ammonia) and proteolytic nature of P. florescence toxins would explain how simple was the process of secondary fungal invasion by C. albicans. The potential disposal of the municipal sewage and farm animal wastes into Mariotteya stream and its associated drainages would provide an eminent source of C. albicans to various cohabitating aquatic species (fish, amphibians, water reptiles and aquatic plants). Several worldwide publications have declared similar findings (Ahearn et al., 1968; Cook, 1970).

The opportunistic C. albicans is a normal saprophyte of various animals' digestive tracts (Odds, 1988). In mammalian species, Candidosis is a common problem of immunosuppressive diseases such as AIDS and IBIOLA viral infection (Korting et al., 1988; Calderone, 1989; Anees et al., 2010). Most critically, physical damage to the epidermal layer would have enabled C. albicans to invade and

### Table 2

Morphochemical tests for Candida albicans isolates retrieved from water and fish samples of Mariotteya stream and its related drainages.

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Retrieved isolates</th>
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<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td></td>
<td>O. niloticus</td>
</tr>
<tr>
<td></td>
<td>C. gariepinus</td>
</tr>
<tr>
<td>Gram staining</td>
<td></td>
</tr>
<tr>
<td>On SDA-Tween 80 agar incubated at 25 C for 72 h</td>
<td>Blue purple budding cells</td>
</tr>
<tr>
<td>Cyclomeximide resistance</td>
<td></td>
</tr>
<tr>
<td>(growth on Mycosel agar media)</td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate fermentation/assimilation</td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td>+/</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+/</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+/</td>
</tr>
<tr>
<td>Lactose</td>
<td>+/</td>
</tr>
<tr>
<td>Maltose</td>
<td>+/</td>
</tr>
<tr>
<td>Galactose</td>
<td>+/</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+/</td>
</tr>
<tr>
<td>Inositol</td>
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colonize the epidermis with consequent multiple ulcerations throughout the fish skin. This assumption coincides with Odds, 1988 who reported similar hypothetical mechanism in infected human skin.

In most of aquatic and terrestrial animal species, the attachment mechanism of C. albicans is mainly based on the capability of adhering to plastic surfaces and to cells in various target tissues. This mechanism has widely been accepted as a first step in the pathogenesis of Candidosis and can be considered as an important virulence factor of C. albicans compared to other Candida species (Kennedy, 1988; Calderone, 1989; Fukayama and Calderone, 1991; Anees et al., 2010). Ollert et al. (1993) have suggested multiple molecular mechanisms such as protein–protein, lectin-carbohydrate, and yeast–yeast co-aggregational interactions that are responsible for optimal C. albicans attachment to cultured skin cells. The most confirmed virulence factors of C. albicans are dimorphism, adherence to the epithelia, thigmotropism, cell surface hydrophobicity, and the activity of hydrolytic enzymes (Lukaszuk et al., 2005; Thiele et al., 2008).

Tissue wise, the expression of histolytic enzymes was identified as being one of the most important factors for the development of symptomatic ulcerative Candidosis (Lukaszuk et al., 2005; Thiele et al., 2008; Anees et al., 2010). The above mentioned attachment theory could be applied to the catfish Candidosis because of its scalyless nature, which is relatively close to the mammalian skin/intestinal epithelia. The quick attachment, invasion and proliferation of the C. albicans onto the damaged skin layers would have been initiated by the exposure of the dermal soft proteinacious tissues (plastic surface) to C. albicans rich sewage water which further hydrolyzed by the histolytic enzymes secreted from candida cells with an ultimate production of severe ulcers (Anees et al., 2010). In case of tilapia Candidosis, physical sloughing of scales with direct effect to such huge amounts of environmental and biological pollutants would have set the nucleus for the candida opportunistic adherence, invasion and proliferation with subsequent release of huge amounts of histolytic enzymes onto the invaded skin/gill tissues. Dramatically, candida histolytic enzymes will digest the most exposed epithelial layers of skin/gills ending with deep ulcerative syndromes.

Obviously, most of the skin ulcers of both tilapias and catfish collected from the scene of the environmental catastrophe were restricted to tail, fin, lateral/dorsal aspects of fishes. These anatomical regions are the most dynamic skin parts in the fish body (Stoskoff, 1993; Colgate and Lynch, 2004). Thus, they are very likely susceptible to physical damage, physiological stress with accumulation of some endogenous toxic metabolites (Lindsey, 1978; Webb et al., 1984; Reidy et al., 1995, 2000; Sfakiotakis et al., 1999). Further, being physically damaged, physiologically stressed and immunologically compromised would present the fish as prime target for dermotropic bacteria such as P. fluorescens and consequently possible opportunistic C. albicans invasion.

To sum up, in our environmental catastrophe, numbers of interacting factors including municipal sewage, temporal stagnation of the stream water, seasonal turnover of the stream banks, immunosuppressive industrial chemicals and primary bacterial invasion (P. fluorescence), would have synergized to produce an obvious sort of physical skin/gill damage which consequently invaded with the highly opportunistic C. albicans.

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