Water and Fish Quality of Aquaculture Pond Adjacent to Intensive Pesticides Application Agro-System

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Abstract

Water and fish quality was specified for a private aquaculture pond localized at the north of Egypt during June-November 2017. The main traditional physicochemical parameters of water were evaluated. Moreover, the occurred levels of trace elements, polycyclic aromatic hydrocarbons (PAHs) and pesticide residues in water and fish of Nile Tilapia (Oreochromis niloticus) and grey Mullet (Mugil cephalus) were specified. The levels of total hardness, ammonia as N, sulfide, nitrite and phosphate were violated the water quality guidelines for the fish cultures. Trace metals of arsenic, cadmium, chromium, copper, nickel, lead and zinc were detected in all the water and fish tissue samples at remarkably high levels (but still below the maximum permissible levels established by WHO. From 15 PAHs analyzed, naphthalene, acenaphthene, phenanthrene, fluoranthene, anthracene, fluorene, benzo(a)anthracene and benzo(b)fluoranthene were detected in water at range of 0.05 µg/l and 0.71 µg/l, while phenanthenone, fluorene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, benzo(b)fluoranthene and dibenzo(a,h)anthracene detected in fish muscles at range of 0.18 µg/g-0.72 µg/g fresh wt. Residues of α- and Y-HCHs, endosulfan 1, endosulfan 2, endosulfan sulfate, heptachlor, heptachlor epoxide, p,p’-DDE, chlorpyrifos, cypermethrin, cyfluthrin and fenvalerate pesticides were detected between the permissible levels in water at the average of 0.12 ng/l-2.04 ng/l. The same compounds were also detected in the fish (except endosulfan sulfate, cyfluthrin and fenvalerate) at ranges of 0.87 ng/l-10.21 ng/g fresh wt. of Tilapia and 0.95 ng/l-8.32 ng/g fresh wt. of Mullet muscles. The potential occurrence of chlorpyrifos, cypermethrin, cyfluthrin and fenvalerate residues might be attributed mainly to its intensive current application on the vegetables and fruits around the fish pond, rather than the persistent organochlorines which still occurred as environmental contaminants from many years ago. The quantified levels of PAHs and pesticide residues in Tilapia and Mullet fish tissues analyzed were below its maximum permissible levels.

Keywords: Aquaculture; Fish; Metals; Pesticides; Polycyclic aromatic hydrocarbons; Water

Introduction

The aquaculture activity is exhibiting the strongest growth of any fisheries related activity in Egypt. It is considered as the only viable option for reducing the gap between production and consumption of fish. Most aquaculture activities are located in the Nile delta region. This sector is currently the largest single source of fish supply accounting for almost 65 percent of the total fish production of Egypt with over 99% produced from privately owned farms [1]. Egypt’s aquaculture industry ranks number 10 worldwide and number two in Africa. The total production of Tilapia in 2009, increased to 1.560 million tons in 2015 [2,3]. Currently, it’s a boom has been occurring where two major aquaculture projects were recently established, one is the Berket Pesticides Application Agro-System, whereas the pesticides residue levels reported in biota below FDA regulatory action levels [5-10].

Physicochemical characteristics of the fish cultures water play a vital role in primary productivity and growth of the ponds fish. Therefore, the water quality assessment is very important for implementation of the monitoring and remediation programs to minimize the risk promoted by hazardous substances in aquatic ecosystems [11]. Agricultural wastes mainly pesticides, synthetic fertilizers and human wastes released directly by underground and/or surface drained water constitute the major sources of pollutants of the aquaculture fish ponds [12]. The main water source for many fish aquaculture ponds located at the northern regions of Nile delta is the agricultural wastewater of intensive vegetable and fruit agricultural areas. Taking these considerations into account, the present investigation aimed to evaluate water quality of a represented private fish pond at the north of El-Behera governorate, Egypt by assessing its main traditional physicochemical parameters level including pH, dissolved oxygen, total dissolved solids, turbidity, total hardness, total organic matter, extractable organic matter, total of petroleum hydrocarbons, sulfide, sulfate, ammonia as N, nitrite, nitrate and phosphate. In addition to determining the potential occurred...
levels of trace metals, PAHs and pesticide residues in water and fish of the pond.

Materials and Methods

Study area and sampling procedures

The study was carried out at a private fish aquaculture pond laid at the west of Rosetta, El-Behera governorate. The pond area is 4146 m² supplied with agricultural wastewater from the adjacent vegetable and fruit sandy fields. Duplicate water samples (2.5 L each) were collected monthly using empty organic solvent glass bottles. About one kg (8-18/kg)/sample of Nile Tilapia (O. niloticus) and grey Mullet (M. cephalus) fish were collected every two months from the pond during June to November 2017.

Reference standards

The reference standards of the tested metals; arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn)). Polycyclic aromatic hydrocarbons (PAHs): Naphthalene, acenaphthylene, acenaphthene, phenanthrene, fluorene, anthracene, pyrene, chrysene (internal standard (i.s)), benzo(a)anthracene, fluoranthene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene. Pesticides standard of organochlorines (α, β), endosulfan sulfate, aldrin, dieldrin, endrin, heptachlor epoxide, organophosphorus (chlorpyrifos) and pyrethroids (cypermethrin, cyfluthrin and fenvalerate) were purchased from Supelco (USA).

Samples preparation and analysis

Physicochemical parameters analysis of water: Water quality of the fish aquaculture pond under investigation was characterized by determining levels of its common physicochemical parameters. Measuring the levels of pH, dissolved oxygen (DO), total suspended solids (TSS), turbidity, total hardness, total organic matter (TOC), extractable organic matter (EOM), total petroleum hydrocarbons (TPHs), sulfide, sulfate, ammonia as N, nitrite, nitrate and phosphate were carried out according to the methods of APHA and US-EPA [13-15].

Determination of trace metals in water and fish: The represented water and muscle tissues of O. niloticus and M. cephalus fish samples were acid digested according to standard methods of US-EPA [16,17]. The digested filtrate samples were subjected to metals analysis using Inductively Coupled Plasma Optical Emission Spectroscopy (Agilent ICP-OES 5100 VDV).

Determination of polycyclic aromatic hydrocarbons and pesticides in water: Water sample (2L) was extracted with methylene chloride according to US-EPA methods [18,19] for PAHs and pesticides, respectively. The extract was dried and concentrated to a volume of 1 ml, then divided into two 0.5 ml. The first 0.5 ml final extract was subjected to PAHs analysis using gas chromatograph equipped with flame ionization detector (GC-FID) and mass spectrometer (thermo-scientific MS-I5Q, 2009). Injection port was 250°C with splitless injection for 3 min, then split mode at a split ratio of 1:10. TG-1MS columns (100% dimethyl polysiloxane 30 m, 0.32 mm i.d., 0.25 film thickness) and helium as a carrier gas (1 ml/min) were used. Oven temperature program: 80°C initial temperature to 240°C with increasing rate of 7°C min^-1 and then to 300°C at the rate of 3°C min^-1, and held for 5 min at 300°C was applied for FID and MS detector lines. FID temperature; 300°C, mass transfer line temperature; 300°C and ion source temperature at 220°C. The mass spectrometer-electron ionization mode with electron energy of 70 eV and quadrupole mass analyzer at mass range of 50 amu-500 amu was applied. NIST library 2.0 was used for compound identification depending on the fragmentation pattern matching with the given mass spectrum.

For qualitative and quantitative analysis of the tested pesticides, 1 μl-2 μl of the second 0.5 ml final extract was injected using thermo scientific tripuls auto sampler at splitless mode (3 min) then, split at ratio of 1:10. The flow of nitrogen as the carrier gas was 1 ml/min. injector temperature was 250°C. TG 5 MS column (95% dimethyl polysiloxane, 5% diphenyl polysiloxane, 30 m, 0.32 mm i.d., 0.25 μm film thickness) was used under the following temperature program: GC oven was started at initial temperature 60°C, increased with rate of 4°C min^-1 to the final temperature at 300°C and hold for 3 min. Micro-electron capture detector (63 Ni-µECD) temperatures were 300°C and 310°C for ECD base and cell, respectively, with 35 ml N2 as make up gas.

Determination of polycyclic aromatic hydrocarbons in fish: The blended free powder homogenate using anhydrous sodium sulfate (ACS reagent grade) of the Tilapia and Mullet muscles (20 g fresh wt. each) was extracted and cleaned up based on the method of Yunker et al. [20] with some modifications. N-hexane used (to extract PAHs after lipid saponification with 50% KOH methanol solution) and 1:1 n-hexane-dichloromethane mixture (for cleanup elution process through silica gel and alumina column chromatography). Qualitative and quantitative analysis of the PAHs were carried out using GC-FID and GC-MS as mentioned above for water analysis [21].

Determination of pesticides in fish: The dry homogenate of fish muscle was subjected for ultrasonic extraction method using sonicator water bath (Selecta, Spain), with pesticide residue grade solvents; acetonitrile: n-hexane (1:1) mixture for extraction of the three pesticide groups (OCs, OPs and pyrethroids) [22-24]. Final concentrate of water or fish extracts was subjected for estimation of the extractable organic matter (EOM) gravimetrically followed by clean-up procedure through silica gel and alumina column chromatography. Qualitative and quantitative analysis of the tested pesticides was carried out using GC/63Ni-µECD as mentioned above for water analysis [25].

Quality assurance procedure: The quality control procedures for the determination of trace metals, PAHs and OCPs in water and fish tissue samples were carried out by analysis of matrix spikes, duplicates, laboratory blanks. Instrumental external calibration of ICP-OES, GC-FID, GC-MS and GC-µECD were performed prior to and in between the analysis of each batch of samples by using working standard solutions of the tested metals and organic components mixture within the appropriate concentration range for its detection. Internal calibration for the chromatographic analysis was also applied using internal standards of 40 pg/μl δ-HCH, and 20 ng/μl chrysene. Determination of the recovery percentages was performed for all target metals and organic compounds in order to assess the efficiency of the analytical operating procedures applied. These percentages were found at ranges of 85%-97%, 72%-88% and 64%-93% for the tested metals, PAHs and pesticides, respectively. Moreover, method detection limits (MDLs) of the tested parameters were determined and calculated at ranges: for metals; 0.01 mg/l-0.03 mg/l water and 0.04 mg/kg-0.07 mg/kg.
mg/kg fresh wt. fish. PAHs: 1.5 µg/l-4.5 µg/l water and 0.28 µg/g-0.58 µg/g fresh wt. fish. Pesticides; 1.0 ng/l-3.4 ng/l water and 3.7 ng/g-6.5 ng/g fresh wt. fish.

**Results and Discussion**

**Physico-chemical characteristics of water**

Water quality of the investigated aquaculture pond was specified monthly from June to November 2017 by measuring its common physicochemical parameters including pH, turbidity, dissolved oxygen (DO), total dissolved solids (TDS), total hardness, total organic matter (TOM), extractable organic matter (EOM), total of petroleum hydrocarbons (TPHs), sulfide, sulfate, ammonia as N, nitrite, nitrate and phosphate. The obtained results listed in Table 1 found that the levels of most physical and chemical parameters determined were not violated the water quality guidelines for the fish pond culture. Whereas some of the parameters specified namely; total hardness, ammonia as N, nitrate and phosphate were beyond permissible limits of the water quality guidelines [26-28].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>Desirable limits [26-28]</th>
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<td>8.1</td>
<td>7.96</td>
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<td>8.11</td>
<td>6.5-9</td>
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<td>18.1</td>
<td>15.1</td>
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<tr>
<td>DO</td>
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<td>6.64</td>
<td>6.81</td>
<td>5.76</td>
<td>5.94</td>
<td>5.6</td>
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<td>655</td>
<td>629</td>
<td>692</td>
<td>720</td>
<td>716</td>
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<tr>
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<td>147</td>
<td>161</td>
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<td>163</td>
<td>160</td>
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<td>20.1</td>
<td>22.5</td>
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<td>1.11</td>
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<td>1.33</td>
<td>2.17</td>
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<td>1.07</td>
<td>1.15</td>
<td>0.96</td>
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<td>124</td>
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<td>109</td>
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</tr>
<tr>
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<td>5.97</td>
<td>6.25</td>
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<td>4.78</td>
<td>5.15</td>
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</tr>
<tr>
<td>Phosphate</td>
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<td>0.97</td>
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<td>0.85</td>
<td>0.85</td>
<td>0.64</td>
<td>&lt;0.05-0.1</td>
</tr>
</tbody>
</table>

*N.A=not available.

**Table 1:** Physico-chemical characteristics of water of the fish aquaculture pond localized between vegetable and fruit fields, June-November, 2017.

The results obtained are potentially attributed to the intensive application of organic manure, chemical fertilizers (e.g., urea (46% N), ammonium nitrate (33.5% N) and phosphate (12.5% P₂O₅)), especially during growing seasons (spring and summer) of the vegetables and fruits. The discharge of these fertilizer residues into the agricultural wastewater (as the main water feeding source for aquaculture ponds) can be leading to fluctuating the parameters measured in water at different levels through the sampling period. Moreover, the study area is located near to Rosetta Mediterranean coast. This allows for seeping of the underground seawater toward the agricultural fields, mainly during the shortage of irrigation intervals in this area after fruits harvesting period. This might reflect the increase of water TDS from 585 mg/l in early summer to 720 mg/l during the autumn season.

**Trace metals**

Certain essential and non-essential elements were detected in the all water and fish samples analyzed as shown in Figures 1-3. The detected levels in water of As, Cd, Cr, Cu, Ni, Pb and Zn were at ranges of (0.19-0.28) µg/l, (0.27-0.40) µg/l, (1.18-1.50) µg/l, (128-165) µg/l, (1.34-1.92) µg/l, (1.14-1.70) µg/l and 351-406 µg/l. These metals were at levels of (0.36-0.52) µg/g, (0.70-0.85) µg/g, (1.33-2.18) µg/g, (2.81-4.02) µg/g, (0.36-0.43) µg/g, (0.65-0.77) µg/g and (7.72-10.45) µg/g fresh wt. of Tilapia fish tissues, whereas in Mullet at ranges of (0.24-0.28) µg/g, (0.57-0.76) µg/g, (1.21-2.34) µg/g, (3.34-3.66) µg/g, (0.31-0.38) µg/g, (0.55-0.90) µg/g and (6.15-8.95) µg/g fresh wt., respectively.
The study reveals that the Zn was found at higher levels in Tilapia than in Mullet fish muscles analyzed. The variations for metal levels through the different months of sampling period might be attributed to a fluctuation of the agricultural wastewater quality concerning levels of certain soil and foliar chemical fertilizers, fungicides containing metals as well as mineralized organic matter discharged into the pond studied.

In spite of the potential sources of many types of chemicals related to the agricultural practices around this pond, the levels of all elements in water and fish samples analyzed were not violated the maximum permissible levels established by WHO and US EPA regarding water used for fish cultured pond at (100, <1.1, 100, 2000, <100, <3.2, 5000) µg/l for As, Cd, Cr, Cu, Ni, Pb and Zn as well as for freshwater fish muscles at (1.0, 30, 2.0, 0.5-1.0, 100) µg/g wet wt. for Cd, Cu, Ni, Pb and Zn, respectively [29-31]. No data available for the maximum acceptable levels of As and Cr in fish [5].
Polycyclic aromatic hydrocarbons

Figure 4 illustrates the detected levels of the tested polycyclic aromatic hydrocarbons (PAHs) in water of fish aquaculture pond localized between vegetable and fruit fields, June-November, 2017. Naphthalene, acenaphthene, phenanthrene, fluorine, anthracene, fluoranthene, benzo(a)anthracene and benzo(b)fluoranthene were detected and ranged between 0.05 µg/l and 0.71 µg/l. The other tested PAHs; Pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, indeno(1,2,3-cd) pyrene and dibenzo(a,h)anthracene were below the method detection limits (0.15 µg/l-0.45 µg/l) at all months during the sampling period. Residual levels of PAHs were also determined in Nile Tilapia and grey Mullet fish muscles of the aquaculture pond. Figure 5 shows that some of PAHs tested namely; Phenanthrene, fluorene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, benzo(b)fluoranthene, and dibenzo(a,h)anthracene were detected at a range of 0.18 µg/g-0.72 µg/g fresh wt. fish muscles, with no remarkable difference for the PAH levels between the two types of fish. Naphthalene, acenaphthylene, acenaphthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene and indeno(123-cd) pyrene were below the detection limits (<DLs) which ranged from 0.65 µg/g to 5.53 µg/g fresh wt.). Generally, medium molecular weight PAHs with 3-5 benzene rings were predominant in the two types of fish compared with those in water samples analyzed (3-4 rings). The obtained levels of PAHs in all water analyzed were below the water quality guidelines [32] which specify the permissible accepted levels for naphthalene, acenaphthene, phenanthrene, fluorine, fluoranthene and benzo(a)anthracene in fresh water at (1, 6, 0.3, 12, 4 and 0.1) µg/l, respectively. It must also be noted that although benzo(a)pyrene was below the method detection limit in this study, the European Union determined only the permissible limit of benzo(a)pyrene (0.002 ppm in fish) due to its serious public health significance among all the PAHs compounds [33]. A previous research was carried out on Tilapia and Mullet fish samples collected from different markets at Sharkia Governorate, Egypt. Eleven PAHs namely; acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene were detected in the fish tissues. No significant difference between the mean total PAHs residues in the two examined fish species. Benzo (a) pyrene levels were exceeded the permissible limits in 15% and 55% of Tilapia and Mullet samples, respectively [8].

Figure 3: Trace elements (µg/g fresh wt.) in Nile Tilapia and grey Mullet fish muscles of aquaculture pond, June-November, 2017.
Figure 4: Residual levels (µg/l) of polycyclic aromatic hydrocarbons (PAHs) in water of fish aquaculture pond, June-November, 2017.

Figure 5: Polycyclic aromatic hydrocarbon levels (µg/g fresh wt.) in Nile Tilapia and grey Mullet fish muscles of aquaculture pond, June-November 2017.

Pesticide residues

Figure 6 shows the detected levels of organochlorine, organophosphorus and pyrethroid pesticides in water of fish aquaculture pond surrounded by vegetable and fruit fields, June-November, 2017. Trace levels of α-HCH, Y-HCH, endosulfan 1, endosulfan II, endosulfan sulfate, heptachlor, heptachlor epoxide, p,p’-DDE, chlorpyrifos, cypermethrin and cyfluthrin were detected in water with the averages of (0.77-0.114, 1.15-1.26, 0.78-1.31, 0.80-1.44,
0.15-1.04, 0.91-1.24, 0.84-1.44, 0.12-1.03, 0.26-1.04 and 0.11-0.23) ng/l, respectively. The same compounds were also detected in the two types of fish analyzed (except endosulfan sulfate, cyfluthrin and fenvalerate below the minimum detection limits (3.7 ng/g-6.5 ng/g fresh wt.) at ranges of 0.87 ng/g-10.21 ng/g fresh wt. of Tilapia and 0.95 ng/g-8.32 ng/g fresh wt. of Mullet muscles (Figure 7). p,p′-DDE was a predominate residue quantified in the two types of fish.

The organochlorines in fish tissues of El Sharkia and El Menofiya governorates, Egypt were mostly detected in the safe limits admitted by FAO and WHO except for chlordane [7,34]. In the present study, the occurrence of organophosphorus (chlorpyrifos) and pyrethroid (cypermethrin, cyfluthrin and fenvalerate) insecticides mainly attributed to its intensive current application during the growing and fruiting periods of vegetables and fruits around the fish aquaculture pond investigated, while the occurrence of the other detected pesticide residues (organochlorine compounds) not due to its direct application on crops but expected to reach the water and fish studied as environmental contaminants transported and accumulated via different food chains same as the other persistent organic pollutants (POPs). Residue levels of these pollutants and their bioaccumulation have been intensively monitored by many of other researchers. For example in the ecosystem of lake Temsah ecosystem, Ismailia. The OCP levels reported in biota were below FDA regulatory action levels of 0.3 μg/g. For biota, bioaccumulation factors (BAF) of OCPs were at 23.7-560 much higher than those of PAHs (0.1-1.2), with heptachlor epoxide showing the highest mean BAF [9]. Another study on the distribution pattern of certain POPs was evaluated in water, sediment and aquatic biota of O. niloticus and Donax trunculus at the Rosetta Nile branch estuary. The results found that α-HCH, p,p′-DDE and PCBs were the predominant compounds detected at ranges of 0.54 ng/g-4.90 ng/l water, 0.75 ng/g-2.41 ng/g, d. wt. sediment and 2.19 ng/g-28.11 ng/g, fresh wt. biota, whereas β and γ-HCHs, endosulfan compounds, heptachlor and heptachlor epoxide detected at low frequencies. The total POPs were found at high levels and abundances in Donax spp. than in Tilapia spp., and both at below its tolerable residue levels [10].

**Figure 6:** Residual levels (ng/l) of pesticides in water of fish aquaculture pond, June-November, 2017.

**Figure 7:** Residual levels (μg/kg fresh wt.) of pesticides in Nile Tilapia and grey Mullet fish of the aquaculture pond, June-November, 2017.
Based on the data listed in Table 2 concerning freshwater quality criteria for the acute and chronic levels of organochlorine, organophosphorus and pyrethroid pesticides, the obtained levels of the tested pesticides in water and fish tissues of the pond investigated were within the permissible limits, as well as, within the acceptable daily intake (ADI) levels of pesticide residues established by the FAO/WHO, and consequently, below its tolerable average residue levels (TARLs) in muscle tissues of Nile Tilapia and grey Mullet fish analyzed were [35].

<table>
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<tr>
<th>Pesticides</th>
<th>* Freshwater-quality criteria (ng/l)</th>
<th>Acceptable daily intake in fish (mg/kg bw/day)</th>
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<tr>
<td></td>
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<td>Chronic</td>
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</tr>
<tr>
<td>Y-HCH</td>
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<tr>
<td>Heptachlor Epoxide</td>
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<td>Cyfluthrin</td>
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</table>

*EPA ambient freshwater-quality criteria of acute and chronic levels for aquatic organisms.

** N.A: Not available.

*** Sum of DDTs.

Table 2: Freshwater and fish quality criteria for the tested pesticides.

In spite of some physicochemical parameters measured at levels violated the water quality guidelines for the management of pond fish culture, it can negatively affect the fish growth and quality of such aquaculture ponds. Fortunately, the Tilapia and for somewhat Mullet fish are easy to tolerate a wide range of environmental conditions [36,37]. At the level of human health, the study recommends that regular monitoring is therefore required to control the levels of different environmental contaminants such as PAHs and pesticide residues in different aquatic ecosystem including fish aquaculture ponds. Also, the private aquaculture fish ponds must be subjected to good aquaculture practices supervised by the responsible food safety authority.

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References

ORASCOM Company, El-Gouna resort, Red Sea Governorate, Egypt.
Cairo, Aquaculture Consultant
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15. USEPA Method 1664 (2010) Revision B: n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material by Extraction and Gravimetry.


