

Are The Water Quality of the Agricultural Drainage and Nile River Suitable for Tilapia Culture? A Case Study from Kafr El-Shaikh's Fish Farms, Egypt

Nashwa A. Shaaban^{1,*} , Samar Tawfik², Wael El-Tarras², Tamer El-Sayed Ali¹ 

¹ Alexandria University, Faculty of Science, Oceanography Department, Egypt

² Kafr El-shaikh University, Faculty of Aquatic and Fisheries Sciences, Aquaculture Department, Egypt

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Corresponding Author

Tel.: +2033922919

E-mail: nashwa.shaaban@alexu.edu.eg

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Abstract

Due to freshwater scarcity, the use of drainage water for aquaculture is a severe challenge in Egypt (one of the cultured tilapia producers worldwide). The present study was conducted, in the highest fish producer governorate (Kafr El-Sheikh). The study aims to assess the water quality of different fish farms fed with different water: agricultural drainage (ADW) and Nile River (Ri-N) water, and examine their suitability for fish culturing. Environmental parameters, nutrients, and metals in the water of fishponds and their sources, were measured. Results revealed that fish farms' waters were brackish (salinity <12 ppt), and slightly alkaline (pH of 8.5). The distribution of DO and BOD between farms was significantly different (p-value < 0.05). Metals varied from high Fe levels to below detection limits (Pb and Cd). Feeding water sources showed lower metal content. However, farm fed by Ri-N was enriched by nutrients and attend low metals contents relative to farms fed by ADW, which was verified by the significant statistical differences. Most studied parameters were lower than the acceptable guidelines for fisheries and aquatic life recommended by national and international standards. Overall, the water qualities of fish farms were in good condition and were suitable for tilapia culture.

Introduction

Fish farming has been developed significantly in response to the growing demand for aquatic products in developing countries (Gu et al., 2017). Fish production from the aquaculture sector exceeds that of natural fisheries resources (FAO, 2018), where aquaculture produces about 57% of the worldwide fish requirements (Ottinger et al., 2016).

The term water quality is used to express the physical and chemical characteristics of water (Chakraborty et al., 2017). Any deviation from the recommended acceptable levels of water parameters

(dissolved oxygen (DO), pH, salinity, nutrients salts, and metals) may lead to stress on farmed species and affect the immune system, rate of growth, and reproduction of fish (Shaaban et al., 2021a). In addition to the significant effects on the physiological activities of fish such as breathing, waste excretion, feeding, maintaining salt balance, and reproduction (Banjara et al., 2019), consequently cause serious diseases (Abu-elala et al., 2021; Nasr-Eldahan et al., 2021), and even causes a high rate of fish mortality with negative impacts on production, profit, and quality of the product.

Metals have a specific concern all over the world because of their environmental persistence (Jiang et al.,

2018) and their non-biodegradable ability (Abah et al., 2013). Metal concentrations are not necessary to be high in the body of the organism to cause harmfulness. Even in low concentrations, if the metal accumulation rate in the body organ is faster than the body's detoxification ability, metals gradually accumulated for a long time and can reach levels more than the permissible limits (Sardar et al., 2013). Cause modifications in physiological and morphological features of both cultured fish and humans (Chatta et al., 2016).

Accordingly, one of the serious challenges for fish farming is the water quality of the fish farm itself and feeding water sources (Henriksson et al., 2017), and their suitability for producing healthy fish with high growth performance.

The monitoring of water quality is a critical tool for assessing the current conditions and long-term trends for effective management of the fisheries and aquaculture systems (Oyeleke et al., 2019). Consequently, fish farmers must manage and improve the farm water quality to provide a relatively stress-free environment (Nasr-Eldahan et al., 2021).

Egypt ranks 9th in cultivated fish production and it is the 1st producer among African countries (Abu-elala et al., 2021). Aquaculture represents about 80% of total Egyptian fish production and the remaining from wild-fisheries resources. Aquaculture production increased two folds over the last 10 years (635 517 tonnes in 2007 to 1 451 841 tonnes in 2017 (GAFRD, 2017).

Kafr El-Shaikh governorate ranks the highest fish producer among all Egyptian governorates (Soliman and Yacout, 2016). It denotes nearly 50% of the total Egyptian aquaculture production (GAFRD, 2017). Moreover, the tilapia (aquatic chicken) production from the aquaculture sector constitutes about 73% of the total cultured species at Kafr El-Shaikh (Ghanem and Haggag, 2015). This could be attributed to the high tolerance of tilapia to the wide range of environmental conditions, short generation time, fast growth rates, omnivorous, and resistance to stress and diseases (Abu-elala et al., 2021; El-Sayed Ali et al., 2014), in addition to the production of cheap protein (Paz, 2004).

Fish farming in Egypt depends on the water of lakes, lagoons, and agricultural drainage water (ADW)

instead of fresh Nile water (Ri-N) as a feeding water source to preserve current freshwater (Shaaban et al., 2021b). The Egyptian Law 124 / 1983 banned the use of freshwater for fish cultivation (Ghanem and Haggag, 2015). Only governmental hatcheries and some governmental farms are allowed to use freshwater directly from the irrigated drains. Thus, about 90% of fish farms depend mainly on agricultural drainage waters (Soltan et al., 2016). These alternative water sources have different physicochemical characteristics. The ADW could receive domestic and industrial effluents in addition to agricultural wastewater. Therefore, the ADW is subjected to different organic and inorganic (nutrients and metals are the most important types) pollutants.

The current study constitutes a survey of the Egyptian farms of tilapia in the Kafr El-Sheikh district. To cover the research gap dealing with monitoring of inorganic residues in the water of fish farms and their impact on water quality and their suitability for fish farming. The present study aims to assess the water quality of different fish farms fed with different water sources: ADW, and irrigation river Nile water (Ri-N). In addition to examining their suitability for fish culturing. These objectives will be done by studying environmental parameters, nutrients, and some metals in the water of fishponds and their sources.

Material and Methods

Study Area

Three fish farms of Nile tilapia (*Oreochromis niloticus*) at Kafr El-Shaikh governorate were selected to evaluate the water quality of fish farms irrigated with different water types. For the first two farms, their water sources, were from agriculture drainage waters (ADW) of Drain-7. The former one is located at the upstream part of Drain-7, termed Upstream Farm (FU). The second one is situated at the downstream part of the same drain (Downstream Farm, FD), after receiving wastes from other fish farms situated along the drain, thus the feeding water source was previously subjected to agriculture, illegal domestic sewage discharge, and fish farms wastewaters. While the third fish farm is

Table 1. Comparison between the average levels of nutrients (mg/l) in three fish farms with their corresponding permissible limit (MPL) recommended by national and international guideline values

Farms	N-Forms				DIP	SiO ₄ - Si	References
	NH ₄ ⁺ -N	NO ₂ ⁻ -N	NO ₃ ⁻ -N	DIN			
Fish farms: -							
Upstream Fish Farm (FU)	0.57	0.06	0.79	1.43	0.11	0.50	Present study
Downstream Fish Farm (FD)	0.17	0.01	0.07	0.25	0.23	0.47	
Control Fish Farm (FC)	0.70	0.02	1.32	2.04	0.76	0.79	
Permissible limits: -							
Egyptian Law 48 of 1982	< 0.5	-	< 45	-	-	-	Mohamed et al., 2013
Acceptable range for aquaculture	0 - 0.05	0.02 - 2	0 - 100	-	0.01 - 3	-	Bhatnagar and Devi, 2013
Desirable range for aquaculture	0-0.025	< 0.02	-	-	-	-	Bhatnagar and Devi, 2013

located away from drainage water and its water source from the agricultural irrigation water of Nile water canals (Figure 1).

It is worthy to mention that Drain-7 collects fish farms effluents from the surrounding areas (El-Amier et al., 2017), in addition to agricultural, industrial, and sewage drainage water from Kafr El-Shaikh and other governorates, via three other big drains (namely, Nasser, Nasr, and Tirah drains) and finally the Drain-7 discharge its water into the south-eastern part of Burullus Lagoon (the 2nd largest northern Egyptian coastal lagoons, El-Zeiny and El-Kafrawy, 2017).

Sampling Sites

The sampling strategy was designed to collect a total of 33 subsurface water samples. The sampling was performed once during the fish harvesting month (October 2018). To cover the different fish farms and their feeding water sources the study area was divided into 6 subareas. The first three groups were including the three farms (FU, FD, and FC, respectively); from each farm, 6 sampling stations were selected covering two representative ponds (out of total 4 ponds/each farm), in addition to 2 stations representing the inlets water of the two ponds and 2 other stations for the outlets. Besides 3 stations representing three sources of feeding

water (the last three groups), one station for the upstream of Drain-7 (D7-U), and another station for the downstream of Drain-7 (D7-D), in addition to the last station for irrigation of fresh Nile water (Ri-N).

Methods of Analysis

The water quality parameters including water temperature, salinity, and pH were determined *in situ*, using the temperature sensor of HANNA HI-9146, salinometer (model. Hylotele portable Handheld ATC), and pH-meter (model. AD11 Waterproof pH-TEMP), respectively. The dissolved oxygen (DO) concentration was measured according to the conventional Winkler’s method (APHA, 1995), using BOD bottles. In which DO was fixed using the manganous solution and alkaline azaid solution, then the developed brownish precipitate of the higher valence state of manganic (hydroxide) was acidified by sulfuric acid (50%v/v). After the dissolution of all the precipitates, the liberated equivalent iodine was titrated against a pre-standardized thiosulfate solution using starch as an indicator. The biochemical oxygen demand (BOD) was examined by following APHA (1995), where two water samples were collected in BOD bottles. DO was measured immediately in one sample in-situ using HANNA HI-9146. In the laboratory, the other sample was immediately incubated for 5 days,

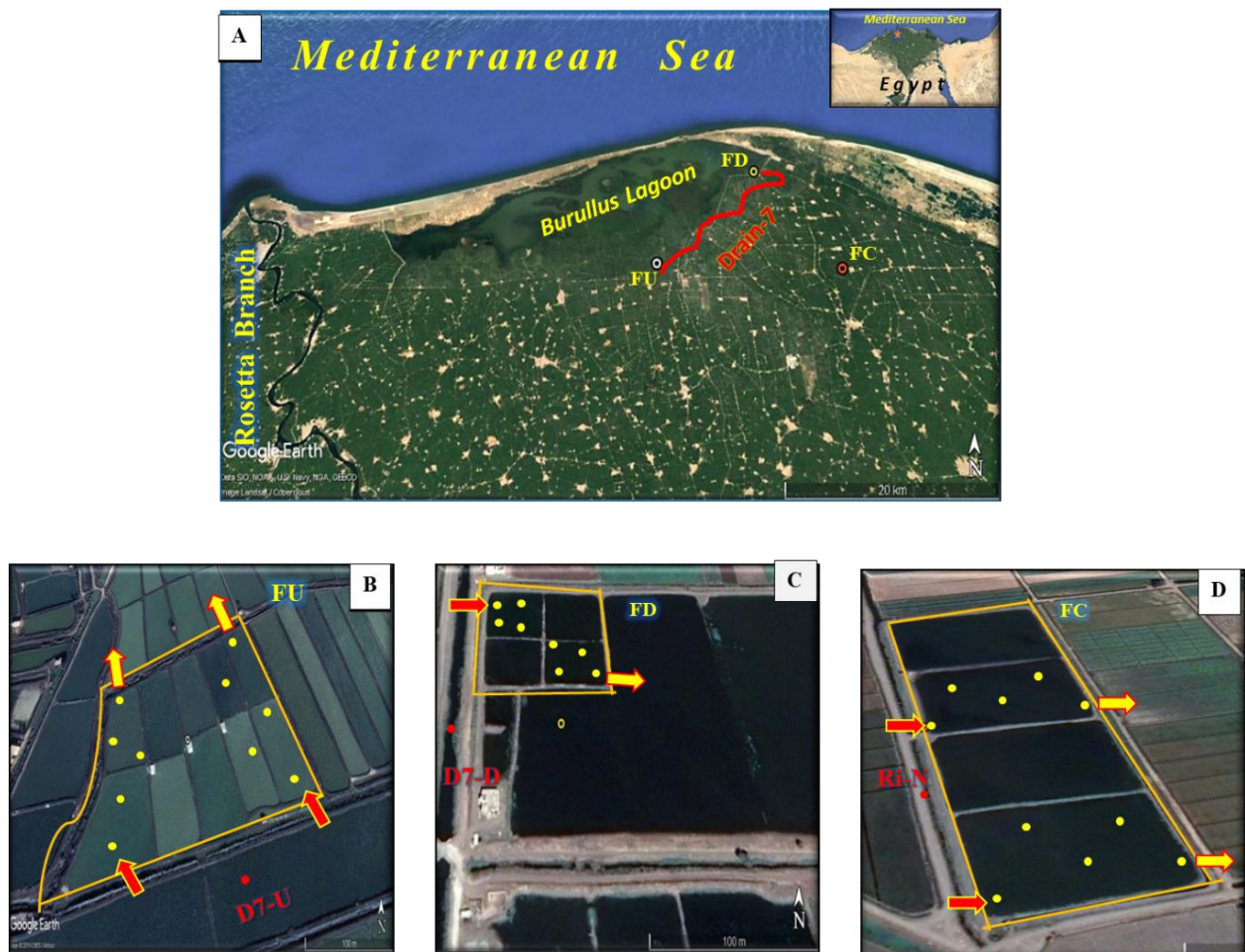


Figure 1. Satellite images showing the three selected fish farms; (A) three farms locations; (B) the upstream farm (FU), (C) downstream farm (FD), and (D) the control farm (FC), and location of the sampling stations.

then DO was measured in mg/l using the same instrument. BOD was calculated according to the following formula:

$$\text{BOD (mg/l)} = \text{DO}_{(\text{in-situ})} - \text{DO}_{(\text{after 5 days})}$$

For nutrients [ammonia (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), dissolved inorganic phosphorus (DIP), and reactive silicates] measurements, water samples were collected in acid-washed containers, followed by filtration through a 0.45 μm membrane filter and kept frozen until analysis. Concentrations of nutrient salts were estimated following Strickland and Parsons (1972) methods using Shimadzu UV-VIS spectrophotometer (model UV-1201) as described in detail in supplementary materials.

The dissolved and total essential metals (including Cu, Zn, Mn, and Fe) and non-essential metals (including Cr, Cd, and Pb) were measured according to EPA (1994). Dissolved metals were determined by filtration of about 20 ml of water sample through dried and pre-acid washed membrane filter of pore size 0.45 μm , followed by adding 0.4 ml nitric acid (HNO_3 (1% v/v)). While total metal concentrations were determined by digesting 100 ml of unfiltered water sample with 2 ml HNO_3 and 1 ml hydrochloric acid, HCl was followed by gentle heating to no more than 85 °C on the hot plate until the sample size was reduced to about 20 ml. After cooling, the residue was transferred to a 50 ml volumetric flask then the volume of the flask was made up with DDW. Mixing and centrifuging were applied. The measurements of dissolved and total metals were performed using Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES 5100 vdv).

Statistical Analysis

The water quality, nutrients, and metals results of the three fish farms were tested for spatial significant differences by operating one-way ANOVA analysis and Tukey multiple range tests, using IBM SPSS Statistics (version 25). In addition to applying the Pearson's correlation coefficient analysis to measure the significant relationship between the studied parameters, using STATISTICA 7 program. Lastly, the cluster analysis was performed, to identify correlations between the metals and categorize them into a group with the same origins or similar transformation processes (Hao et al., 2019), using STATISTICA 7 program. The statistical significance was reported at the $P < 0.05$ level.

Results and Discussion

Environmental Parameters

The environmental parameters including water temperature, pH, dissolved oxygen (DO), salinity, and BOD were represented in Figure 2. Focusing on the

water temperature of the three fish farms and their suitability for the aquaculture purposes, it was noticed that the average water temperatures (24.4°C, Figure 2a) were within the acceptable ideal range for tilapia is 20°C–35°C (Girard, 2011; Bhatnagar and Devi, 2013). In addition, there was no statistically significant difference between the fish farms (P -value > 0.05, Figure 2a).

The pH values were varied between 8.1 and 8.9, which is acceptable for fish farming (Figure 2b), and in agreement with corresponding values obtained in Abbassa fish farm (Al-Kenawy and Aly, 2015) and Sharkia fish farms (Soltan et al., 2016). The results of one-way ANOVA showed significant differences between fish farms (P -value < 0.05), which attributed to the significant mean difference of 0.25 between FU and FC (P -value < 0.01), as the maximum mean pH value was 8.78 ± 0.04 at FC.

Another factor, affecting the density and development of aquatic animals' populations, is salinity. The salinity of FD water was as double as the other studied farms (11 and 5 ppt, respectively). Feeding water sources showed the same salinity content as the farms, as shown in Figure 2c. The elevated salinity of the FD farm may be due to its location at the end of Drain-7 closing to Burullus Lagoon. When the water level in Drain-7 becomes lower than Burullus, water is pushed up from the lagoon to the drain and raises its water salinity. This observation is confirmed by the present study, where the downstream part of Drain-7 attained elevated salinity than its upstream part (Figure 2c).

The DO is considered one of the most vital parameters that regulate aquatic organisms' activities and is used as an index for water quality (Eghomwanre et al., 2019). In the present study, DO values were significantly varied between three farms (P -values < 0.01), with mean values of 11.5, 9.1, and 6.4 mg/l for FD, FC, and FU, respectively, (Figure 2d). The high DO levels were accompanied by relatively low BOD content (1.6, 4.9, and 7.1 mg/l, respectively, Figure 2e). Moreover, Peterman (2011) discussed the relation between fish stock densities and DO, which was verified in the present study, as FD farm had the lowest fish density from 2 to 3 fish per m^2 relative to the other two fish farms. Fish wastes can produce a possible rise in BOD values in water (Nzeagwu et al., 2017). However, FD and FC waters were supersaturated (with a percentage of oxygen saturation of 135% and 110%, respectively), while FU water was under saturation (65%). It is worthy to mention however FD attained high DO concentrations, its cultured fish was under stress. Bhatnagar and Devi (2013) stated the DO level > 8 mg/l is undesired for fish farming.

Excluding FU, the results obtained agreed with the recommended limits (< 6 mg/l) according to Egyptian Law 48 of 1982. In addition to the recommended limits mentioned by Bhatnagar and Devi, (2013) who cited that the acceptable range of BOD levels is 3–6 mg/l and the desirable range is from 1–2 mg/l. If BOD levels are higher than 10 mg/l, it is considered stress for aquatic

organisms (Bhatnagar and Devi, 2013). So, the area of the study recorded relatively suitable BOD levels for reared fish.

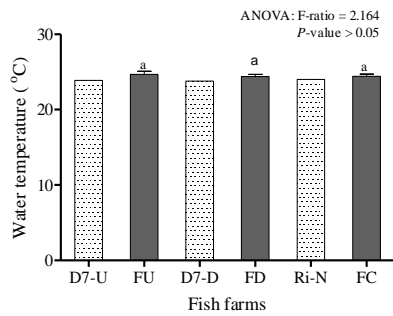
Nutrient Levels

Nutrients levels of dissolved inorganic nitrogen (DIN) forms, including ammonia, nitrite, and nitrate, in addition to the dissolved inorganic phosphorus (DIP) and reactive silicates, were represented in Figure 3. The results indicated statistically significant differences between fish farms (P -value<0.05). FC displayed the highest concentrations of nutrient salts relative to those

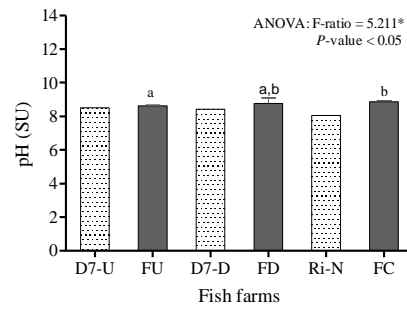
fish farms located at Drain-7 (with mean values of 96.1, 25.3, and 28.8 μ M, for DIN, DIP, and reactive silicates, respectively), as illustrated in Figure 3, and Table S2a and S2b.

Dissolved Inorganic Nitrogen (DIN)

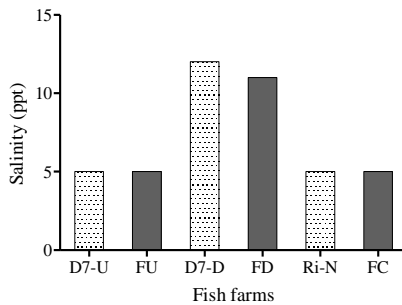
The average values of ammonia were 41, 12, and 50 μ M in FU, FD, and FC, respectively (Figure 3a). Higher levels of NH_4^+ -N were observed in FC compared with the other farms. This can be attributed to higher fish density, which gives a higher amount of NH_4^+ -N as a result of the breakdown of solid fish wastes, as a by-



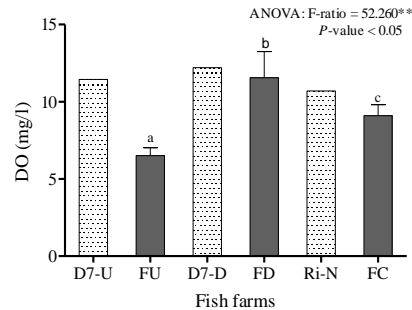
(b) Water temperature



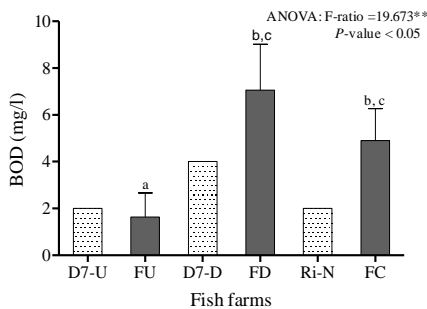
(a) pH



(d) Salinity



(c) Dissolved oxygen



(e) Biochemical oxygen demand (BOD)

Fish farms: -
 FU: Upstream fish farm
 FD: Downstream fish farm
 FC: Control fish farm
 Feeding water sources: -
 D7-U: upstream part of Drain-7
 D7-D: Downstream part of Drain-7
 Ri-N: River Nile irrigation canal
 Means with the same letter within the same histogram for each metal are not significantly different ($P < 0.05$).

product of metabolism, and uneaten feed can give large amounts of ammonia-nitrogen (Peterman, 2011; Ude et al., 2018). The protein content in fish feed has 16% nitrogen and it is the main source of ammonia that exists in the pond system.

Although ammonia content was low in FD, it was the predominant form of DIN representing about 74% of DIN. In contrast, the percentage of NH₄⁺-N to DIN in FU and FC was less than 55% which coincided with a higher nitrate percent. This observation was previously observed by Sipaúba-Tavares et al. (2016). Excluding FD, the current results were slightly higher than most recommended and acceptable ranges for aquacultures stated by the Egyptian Law 48/1982, Table 1. Moreover, if NH₄⁺-N levels are higher than 21.4 µM, it is considered stress for aquatic organisms (Bhatnagar and Devi, 2013). The current results are close to the lower poisonous limit of NH₄⁺-N for fish (42.8 -142 µM) according to (Yones et al., 2013).

Nitrite is an intermediate product of the aerobic nitrification process (Chang et al., 2019). The NO₂⁻-N resembled a very low percent where it was on average 2% of DIN in the study area. The average NO₂⁻-N concentrations were 4.4, 0.3, and 1.1 µM in FU, FD, and FC, respectively (Figure 3a). The results of the current study (Table 1) were within the acceptable range for aquaculture according to Bhatnagar and Devi (2013).

In the present study, there was a wide variation in NO₃⁻-N values between the studied farms. NO₃⁻-N in FD was extremely low regarding other farms. The distribution of NO₃⁻-N levels showed the following pattern: FD (5 µM) < FU (57 µM) < FC (94 µM). Excluding FD, nitrate content resembled the dominant form of DIN, (Figure 3a). NO₃⁻-N/ DIN percent was high in FC where it was about 64%. Also, FU recorded NO₃⁻-N percent on average 50%. Concentrations of NO₃⁻-N are

normally higher in many stations than NH₄⁺-N and NO₂⁻-N (Nzeagwu et al., 2017) where it is the most stable inorganic nitrogen form in natural water (Emara et al., 2016; Sipaúba-Tavares et al. 2016). Nitrate values were within the acceptable range for fish farming (Bhatnagar and Devi, 2013), as shown in Table 1.

It is worthy to mention that a noticeable positive significant correlation between nitrate and ammonia (r=0.77 and P-value<0.05, Table S1 in supplementary materials) was recorded, where the increase of NO₃⁻-N levels was parallel with the increase of NH₄⁺-N levels. This may be because NO₃⁻-N is the product of the nitrification process in the natural water, so it has the same distribution as NH₄⁺-N in water samples.

Moreover, a significant negative correlation between N-forms and DO (r>-0.6, P-value<0.05, Table S1 in supplementary materials) and a positive correlation between most of N-Forms and BOD content (r>0.6, P-value<0.05, Table S1) indicated that amounts of DO content were consumed for the nitrification process, these observations were recorded previously by Khatita et al. (2017). Additionally, significant negative correlations were also recorded between N-forms and salinity (r>-0.7, P-value<0.05) where higher salinity water has lower N-forms. This observation was recorded previously by Bach and Shin (2011). Increasing salinity affects the metabolic rate of fish which subsequently decreases the ammonia produced from metabolic activities (Kir and Öz, 2015).

Dissolved Inorganic-P (DIP)

Like DIN, the DIP content of the control farm (FC) showed the highest values (25.5 µM) relative to farms located at Drain-7 (Figure 3b). This may be because FC has a higher fish density where phosphorus is

Table 2. Comparison between the average levels of metals (µg/l) in three fish farms with their corresponding permissible limit (MPL) recommended by national and international guideline values

	Area of study	Essential metals								Non-Essential metals				References				
		Fe		Zn		Cu		Mn		Cr		Pb			Cd			
		T-	D-	T-	D-	T-	D-	T-	D-	T-	D-	T-	D-		T-	D-		
Fish Farms	FU	68.3	16.8	29.3	4.8	7.3	5.3	17.8	5.7	0.6	BDL	1.2	1.2	BDL	Present study			
	FD	94.6	13.7	40.3	4.7	22	5.2	22.9	17.7	2.4	BDL	4.5	0.7	BDL				
	FC	83.3	14.7	29.6	2.8	5.3	3.3	13	6.3	6.9	BDL	12.2	1.3	BDL				
	Drain-7	Upstream	40.5	19	28.5	4	3.5	3	2.5	1	BDL	BDL	BDL	BDL				
Downstream		139.5	58	27	4	5.5	5	19	14	BDL	BDL	BDL						
Nile River	Canal	25	14	24.5	2	4.5	2	2	1	9	6	6	BDL					
Guidelines	Water quality criteria for aquaculture		<150		<5		10- 30		<10		-		< 20			0.5- 5		Timmons and Ebeling, 2007
	Egyptian Law 48 of 1982		< 1000		< 1000		< 1000		< 500		< 50		< 5			< 10		
	Water guidelines for fisheries and aquatic life	Canada	300		30		2 – 4		-		2-20		1- 7			0.02- 1.8		Chapman, 1996
		Russia	100		10		1		10		5-20		0.1			5		
U.S. Environmental Protection Agency (US-UPA) standards		300		5000		1000		500		-		5		10		Badr et al., 2014		

BDL = Below Detection limit (for Cr < 0.002, Cd < 0.012 and Pb < 0.003) = not mentioned D-= dissolved T-= total
 FU= the farm at Drain-7 upstream FD= the farm at Drain-7 downstream FC= control farm

considered one of the main nutrients in wastes from aquaculture (El Zokm et al., 2018). Fish feed is normally having phosphorus levels that reach sometimes 17 μM . So, phosphorus levels in the pond are associated with fish density (Osman et al., 2010). It was noticed that the second pond of each farm was enriched with nutrient salts than the first pond which related to the water circulation. The phosphorus levels of the three fish farms were within the recommended limits for fish culture, as shown in Table 1.

Reactive Silicates

As nitrogen and phosphorus, the reactive silicates values (Figure 3c) were elevated in FC (28 μM) when compared with other farms (with an average of 17 μM). Sites of higher silicate were found to have higher DIP and $\text{NO}_3\text{-N}$ which was verified by the positive significant correlations between SiO_4 and DIP ($r=0.65$, $P\text{-value}<0.05$) and between SiO_4 and $\text{NO}_3\text{-N}$ ($r=0.61$,

$P\text{-value}<0.05$). This indicated that silicate is one of the parameters related to productivity, as mentioned by Abe and Watanabe (1992). Also, Calandra et al., (2016) and Khatita et al. (2017) found locations that have higher silicate contain higher $\text{NO}_3\text{-N}$ and DIP levels.

Metals

From a biological point of view, metals were classified into essential and non-essential. Cu, Zn, Fe, and Mn were grouped as essential metals due to their role in enzymes and several biological processes, development, reproduction, hemoglobin formation, and vitamin synthesis in all living species (Shaaban et al., 2021a). Those metals become toxic when the organism intakes them at excessive times at high concentrations. Whereas Cd, Cr, and Pb are classified as non-essential metals as they have no biological importance for living organisms. In addition, they are toxic even in traces (Ju et al., 2017; and Jiang et al., 2018).

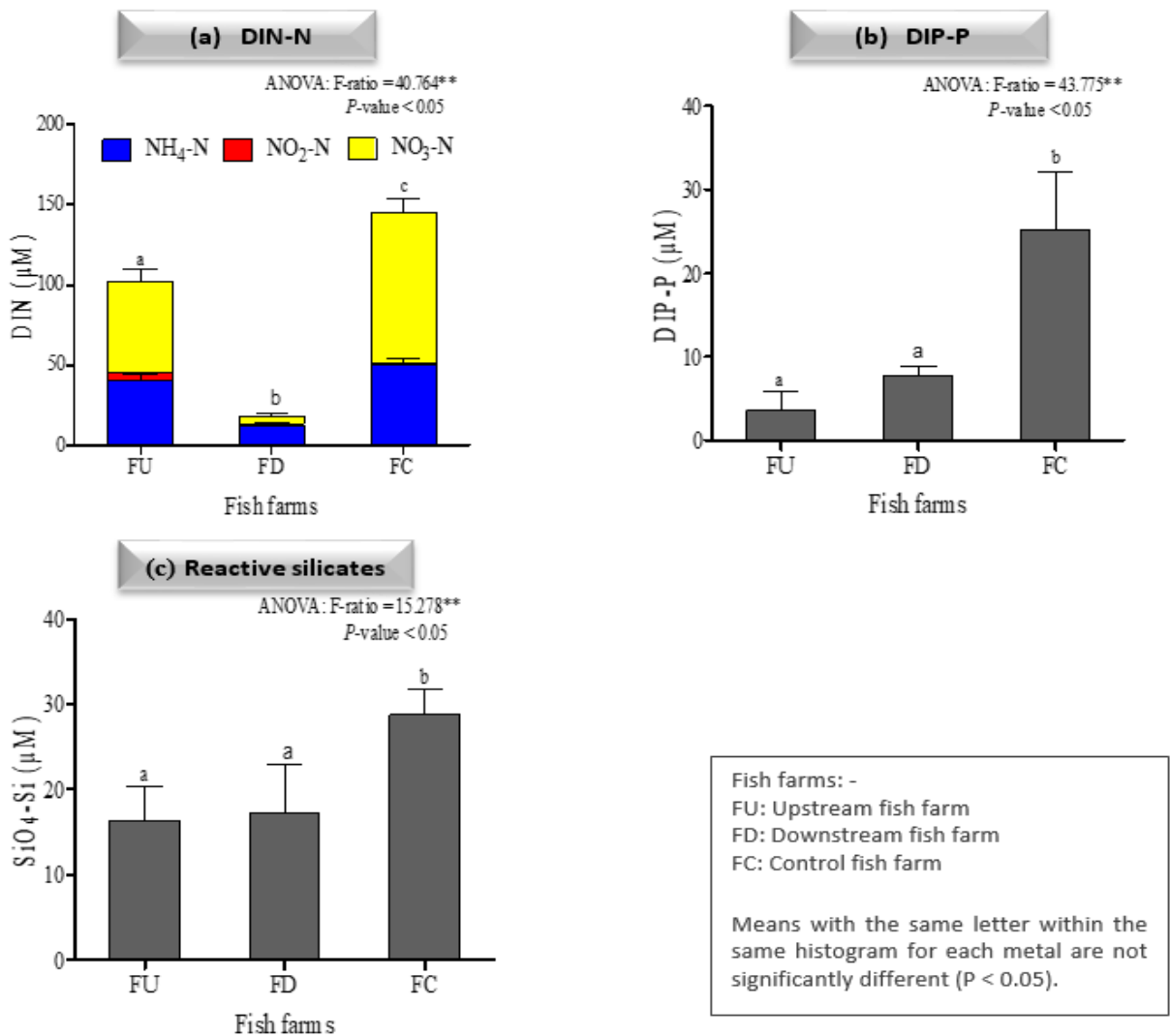


Figure 3. The mean concentration values of nutrient salts include (a) dissolved inorganic nitrogen (DIN), (b) dissolved inorganic phosphorus (DIP), (c) reactive silicates from the three fish farms.

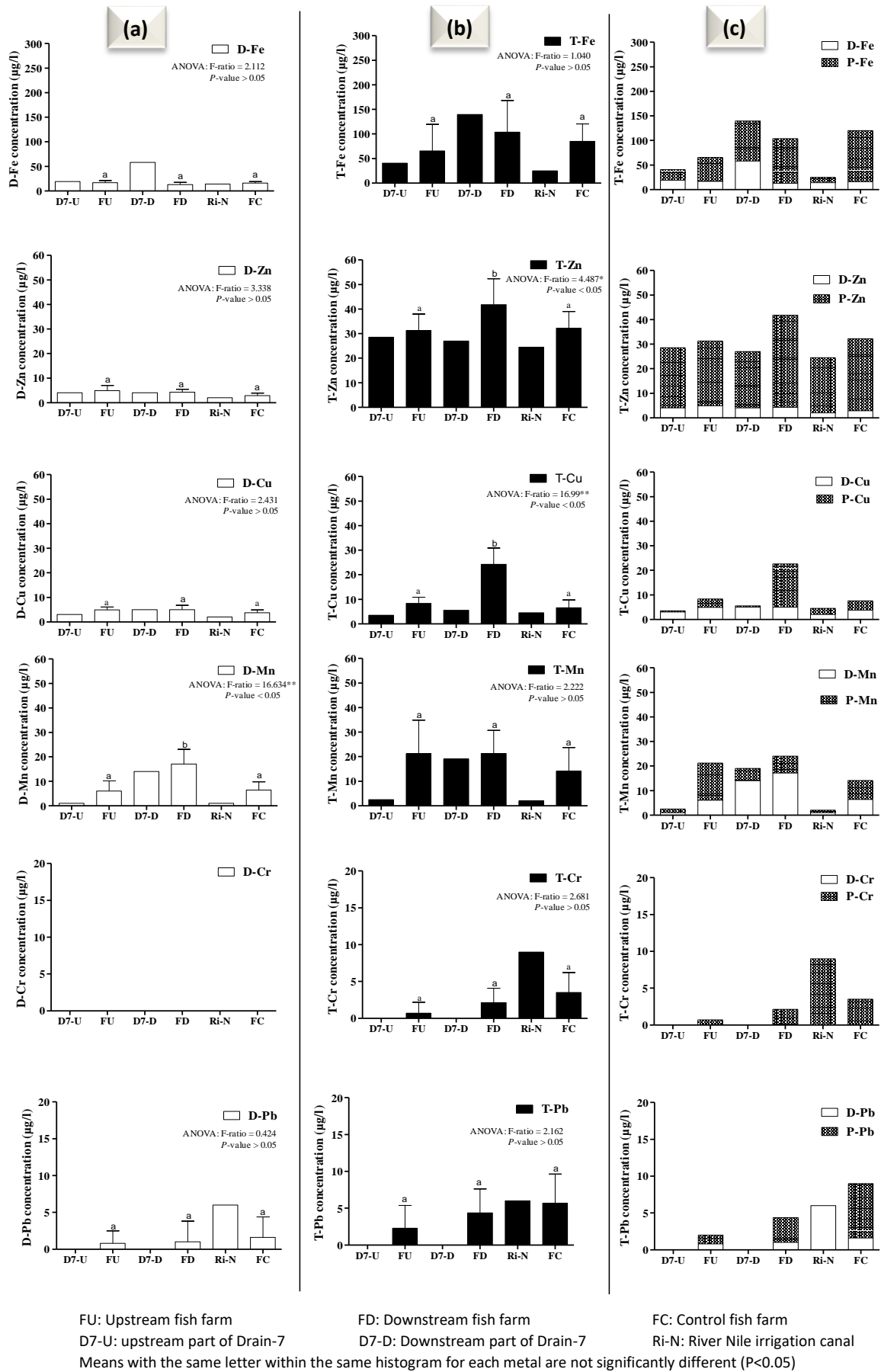


Figure 4. The mean concentration values of metals including (a) dissolved, D- and (b) total, T- metals (c) metals speciation from the three fish farms.

The concentrations of essential metals (Cu, Zn, Fe, and Mn) and non-essential metals (Cr, Cd, and Pb) in waters of fish farms (FU, FD, and FC) are represented in µg/l (Figure 4).

Generally, the order of abundance of the total and dissolved metals according to their concentration levels was Fe > Zn > Mn > Pb > Cu > Cr > Cd and Fe > Mn > Pb > Cu > Zn > Cr = Cd, respectively. The Fe (T- and D-) showed extremely high concentrations relative to the other detected metals. Statistically, Fe showed a single cluster (A) with dissimilarities 100% of other metals as shown in Figure 5. On the other hand, there were observable statistical differences in levels of each of T-Zn, T- Cu, T- Cr, and D-Mn between the studied farm (P < 0.05), in contrast to T-Fe, T-Mn, D-Fe, D-Cu (P>0.05).

Essential Metals

Regarding T-Fe values, the FD waters showed the highest concentration (94.6 µg/l), while the average levels of T-Fe at FU and FC were 68.6 and 83.3 µg/l, respectively (Figure 4). The D-Fe values were close at all studied farms (with an average of 14 µg/l) as shown in Figure 4. The elevated levels of Fe in water were attributed to Fe one of the most abundant constituents in the earth's crust (Khattaby, 2015). But its increase in the farms located on Drain-7 maybe because inorganic fertilizers are applied in agriculture practices as discussed by Emara et al., (2016) who stated that inorganic fertilizers increase the levels of Fe in agricultural drainage water.

The T-Zn concentrations were higher in FD (40.3 µg/l) than in the other two farms (29.4 µg/l). D-Zn values, the water of the control farm (FC) showed the lowest Zn levels with an average of 2.8 µg/l. In addition, FD and FU waters contained the same values of D-Zn with a mean of >4.5 µg/l, (Figure 4).

Similarly, T-Cu levels were extremely low in FC (average of 5.3 µg/l) compared with relatively high levels at FD (average of 22.0 µg/l). The distribution pattern of T-Cu concentrations was FC<FU<FD, (Figure 4). Which coincided with the slightly low T-Cu levels at the feeding water source of FC (4.5 µg/l). In the study area, Cu concentrations were low in the farm depending on Nile water (FC) in comparison with other studied farms. The average level of D-Cu at FC was 3.3 µg/l while corresponding average values at FU and FD were, close to each other, about 5.2 µg/l.

The control farm (FC) showed the lowest T-Mn concentration (13 ±3 µg/l), and this might be because of lower Mn in Nile water, followed by FU (18 ±7 µg/l) and the highest T- Mn average concentration was recorded

in FD with an average value of 23 ±5 µg/l, (Figure 4). The D-Mn concentrations at the farm located at the downstream part of Drain-7 (FD) were, 17.7 µg/l, a threefold magnitude higher than the corresponding values of FU and FC (<6.5 µg/l).

Non-essential Metals

Generally, the order of abundance of T-Cr in the selected farms, according to their concentration levels was FU (0.6 µg/l) < FD (2.4 µg/l) < FC (6.9 µg/l). While D-Cr concentrations were below the detection limit (< 0.002 µg/l) in all water samples, (Figure 4).

Regarding to T-Pb content, the FC was extremely high (with a mean of 12.2 µg/l) relative to the other farms (means<5 µg/l). There was a wide range of variation in D-Pb concentrations (with a coefficient of variation >200%), and D-Pb levels fluctuated from <0.003 µg/l at FU to 8.0 µg/l at the inlet of the control farm (FC). Levels of Cd (D- and T-) were below the detection limit (< 0.012 µg/l) in the water of three fish farms (Figure 4).

It was noticeable that the levels of the majority of metals (essential and non-essential) at most outlet stations showed high values compared with the stations at the inlet and center of fish’s ponds. This was because of wastes of fish and fish feed residue inside the pond, that observation was previously noticed by Osman et al. (2010) who found the richness of ponds outlets water with Fe than inlets in El-Serw Fish Farm. Moreover, most metals (especially in their dissolved form) were lower in the control farm which depends on the River Nile water.

All recorded levels of metals (excluding elevated Pb concentration at FC) at different fish farms and their water sources were below the recommended levels stated by the Egyptian Environmental Affairs Agency (EEAA) and U.S. Environmental Protection Agency (US-EPA) as shown in Table 2. The additional comparison of metals content in waters of fish farms with Canadian guidelines and Russian guidelines (Chapman, 1996), reveals their suitability for fisheries and aquatic life. However, Cu and Mn levels slightly exceeded the permissible limits. Moreover, the water metals content at different fish farms was suitable for aquaculture purposes according to guidelines stated by Timmons and Ebeling, (2007).

Comparing the results of T- and D- metals between fish farms, using a one-way ANOVA test, revealed that there was no statistically significant difference according to most measured metals (P-value>0.05). In contrast, fish farms showed significant variations in the distribution of D-Mn, T-Zn, and T-Cu (P-value<0.05).

Table 3. The Q- values and overall water quality index, NSF-WQI (based on 6 parameters) in the studied farms.

	DO Sat.	pH	BOD	Temperature Change	DIP	NO ₃ .N	Overall WQI
FU	66	66	46	92	96	96	76
FD	81	56	88	92	89	97	83
FC	96	56	56	92	48	96	75

FD: Farm at Drain-7 Downstream, FU; Farm at Drain-7 Upstream, FC: The Control Farm

These differences in mean values have been attributed to the variation observed by a fish farm located at and irrigated by downstream water of the agriculture drain D-7.

Feeding Water Sources

Generally, almost all studied metals were relatively found in lower levels in the water of irrigation sources than on farms. Not all irrigation water had the same metals levels where Nile water source (feeding water source of FC) showed lower metals levels than Drain-7 water (feeding water source of FU and FD). Moreover, the downstream part of Drain-7 attained elevated metals content (T- and D-forms) relative to the lower ones at the upstream part. This was approved by Ibrahim et al. (2013) who recorded higher levels of Fe and Cu in drainage water than in Nile river water.

The values of T-Fe levels were 139 and 58 µg/l and D-Fe contents were 40.5 and 19.0 µg/l at downstream and upstream parts, respectively. The current results

agree with (Yones et al., 2013) who found the Fe level in drains (Drain-7) was 50-137 µg/l. The current Nile water source (feeding FC) showed extremely low Fe content (25 and 14 µg/l for T- and D-Fe, respectively). The Fe content in the Nile water source agreed with the values reported for unpolluted rivers worldwide (0 - 50 µg/l) (Chapman, 1996).

The average concentration values of D- and T- Zn at Drain-7 were 4 and 28 µg/l, respectively. While it ranged between 2 and 25 µg/l for D- and T- Zn, respectively, at the Nile water source. The variation of Zn levels at feeding water sources was previously documented by Ibrahim et al., (2013) who recorded higher Zn levels in drainage water of El-Rahawy Drain than Nile water at El-Kanater El-Khyria (60 and 16 µg/l, respectively).

The D-Cu values were 3.0 and 5.0 µg/l at upstream and downstream parts of Drain-7, respectively. Similarly, the downstream part attained a higher T-Cu level (5.5 µg/l) than its upstream part (3.5 µg/l).

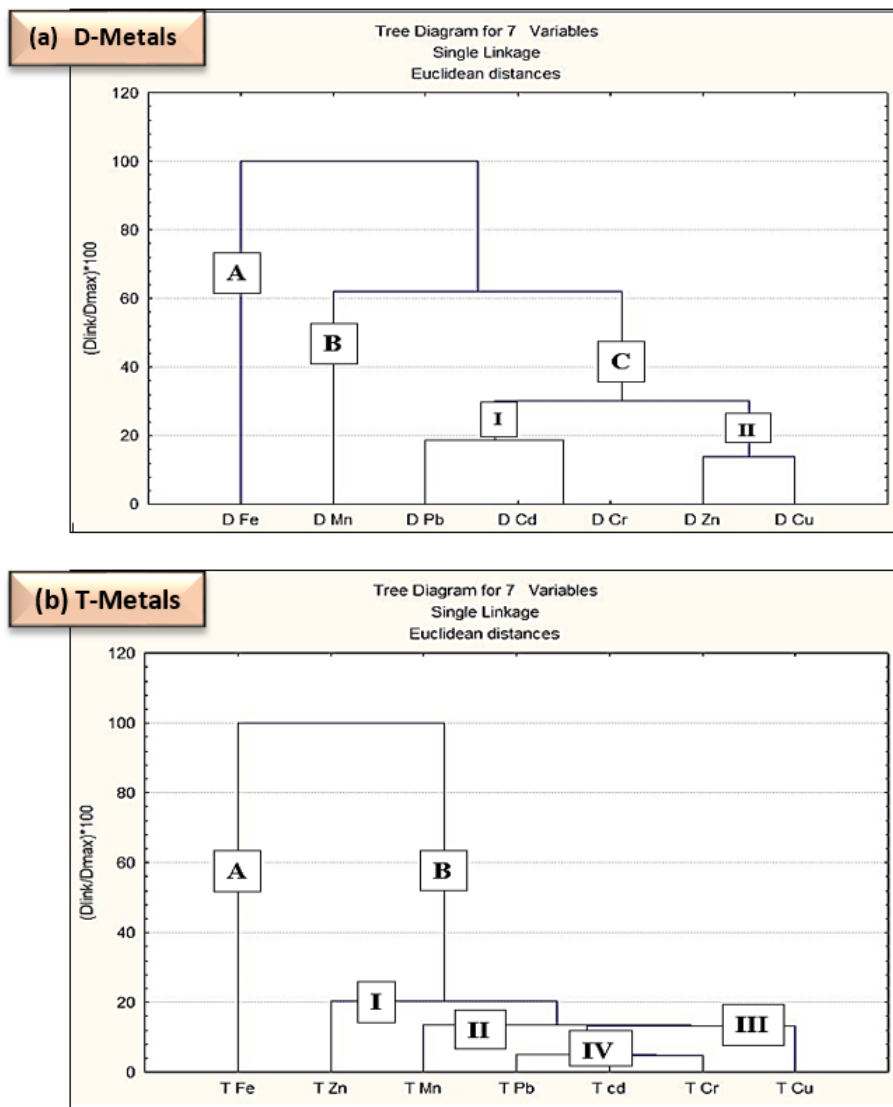


Figure 5. Dendrogram obtained by cluster analysis for (a) dissolved, D- and (b) total, T- metals in water of the study area.

The T- and D- Mn values at the downstream part of Drain-7 were extremely high (>14 µg/l) relative to low values (<2 µg/l) at the upstream part of Drain-7 and the Nile water source. Which coincided with the elevated levels of Mn content at FD. Ahmed (2013) found Mn levels in the same ranges in drainage water of Bahr Terra which connects with Drain-7 at its end were 17 µg/l and 27 µg/l in Burullus Lagoon in Kafr El-Shaikh.

Regarding non-essential metals including Cr and Pb, they were below the detection limit (<0.002 and <0.003 µg/l, respectively) at Drain-7. Alternatively, the Nile water source showed relatively high concentrations of T-Cr and T-Pb (9.0 and 6.0 µg/l, respectively) which were responsible for the enrichment of FC waters with Cr and Pb. Additionally, the toxic Cd was not detected in feeding water sources. However, the concentrations of most studied metals at Drain-7 waters -in the present study- were within or slightly higher than the permissible limits for aquaculture purposes when compared with Egyptian Environmental laws, EEAA No.48 of 1994, and both Canadian and Russian water guidelines for fisheries and aquatic life.

Metal Bioavailability and Statistical Analysis

Bioavailability is known as the limit to which metals in a water-soluble form are readily uptake and assimilated by living organisms (Shaaban 2015; Asgedom et al., 2012). It is used as an indicator of the toxicity of metals to aquatic species (Desta and Weldemariam, 2013).

The bioavailability of metals statuses health hazards to living organisms and humans who may interact with contaminated locations (Mohammed et al., 2011). It is acknowledged that the metal bioaccumulation pattern in fish has a link to

bioavailability (Achary et al., 2017; Ju et al., 2017; Mziray and Kimirei, 2016). The bioavailability of each metal can be calculated using the following equation:

$$\text{Bioavailability (\%)} = \frac{\text{dissolved metal concentration}}{\text{total metal concentration}} \times 10$$

In the present study, there was a wide range of bioavailability values, and the bioavailability of metals showed different patterns between farms and even between ponds of the same farm.

Most essential metals showed lower bioavailability percent (<50%). Zn and Fe were found in higher particulate form than dissolved. Dissolved per total ratio (D-/T-) of Fe at the study area was close between farms (< 25%). Similarly, the D-/T-Zn ratio was lower than 20% in all farms (Figure 4c). The dissolved percent of Cu was close in FU and FC (lower than 50%). In contrast FD, D/T Cu percent was about 20%. Excluding FD which recorded high Mn bioavailability (80%), the D/T ratio of Mn was lower than 50% in other farms.

For non-essential metals, Cr bioavailability percent was 100%. This is due to D- Cr below detection limits. D/T ratio of Pb in FU was 100% due to being D-Pb lower than the detection limit. The values of D/T of Pb in other farms were close to <15%, as illustrated in Figure 4c.

The metal bioavailability and accumulation in aquatic are influenced by chemical and physical water properties (Mendis et al., 2015). In the present study, there were negative correlations between NH₄⁺-N and most of the studied metals, such as T-Cu, T-Zn, T- Mn, and D-Mn (-0.87, -0.72, -0.76, -0.83, respectively). NH₄⁺-N levels were lower at stations of higher metals levels. In presence of ammonia, metal ions combine with the ammonia in an aqueous medium forming a stronger complex, (Kowenje et al., 2010).

Table S1. Pearson correlation coefficient (r- values) between dissolved and total studied metals concentrations and water parameters in Kafr El-Shaikh. The significant value is in the bold letters, P-value<0.05

Variables	Environmental parameters						Metals						Nutrients					
	Temp.	pH	DO	Salinity	BOD	D-Cu	T-Cu	D-Zn	T-Zn	D-Mn	T-Mn	D-Fe	T-Fe	NH ₄ -N	NO ₂ -N	NO ₃ -N	DIP-P	SiO ₄ -Si
Environmental parameters	Temp.	1																
	pH	-0.01	1															
	DO	-0.11	0.44	1														
	Salinity	0.05	0.35	0.83	1													
	BOD	-0.22	0.46	0.98	0.76	1												
Metals	D-Cu	0.35	-0.28	-0.2	0.31	-0.29	1											
	T-Cu	0.13	0.46	0.59	0.87	0.51	0.36	1										
	D-Zn	0.44	-0.28	0.02	0.09	0	0.43	-0.05	1									
	T-Zn	-0.02	0.35	0.59	0.79	0.53	0.43	0.74	0.22	1								
	D-Mn	0.15	0.15	0.71	0.81	0.67	0.23	0.59	0.35	0.45	1							
	T-Mn	0.44	-0.1	0.29	0.61	0.21	0.57	0.61	0.52	0.49	0.71	1						
	D-Fe	0.36	-0.4	-0.71	-0.68	-0.74	0	-0.51	0.02	-0.42	-0.7	-0.18	1					
	T-Fe	0.05	0.12	-0.27	0.05	-0.33	0.32	0.35	-0.61	0.12	-0.27	-0.01	0.09	1				
Nutrients	NH ₄ -N	-0.18	-0.29	-0.67	-0.94	0.58	-0.46	-0.87	-0.21	-0.72	-0.83	-0.76	0.58	-0.06	1			
	NO ₂ -N	0.12	-0.54	-0.73	-0.69	0.72	0.11	-0.59	0.45	-0.44	-0.47	-0.19	0.6	-0.32	0.54	1		
	NO ₃ -N	-0.19	-0.07	-0.63	-0.86	-0.55	-0.38	-0.80	-0.14	-0.68	-0.67	-0.56	0.54	-0.19	0.77	0.46	1	
	DIP-P	-0.49	0.38	0.07	-0.27	0.2	-0.6	-0.34	-0.44	-0.16	-0.29	-0.46	-0.06	-0.03	0.43	-0.35	0.54	1
	SiO ₄ -Si	-0.26	0.26	-0.45	-0.43	-0.41	-0.14	-0.25	-0.65	-0.23	-0.61	-0.42	0.32	0.56	0.42	-0.16	0.61	0.65

*Bold number refers to the statistically significant correlation

Moreover, it was observed that sites with high silicate levels showed low metal concentrations, which verified by the negative correlations were mostly significant in dissolved metals as D-Mn ($r=-0.61$) and D-Zn ($r=-0.65$). These reverse relations could be attributed to the high combination between metals and silicates (Adele, 2016), where silicate is used for metal reduction (Lu et al., 2017).

Water Quality Index (WQI) of Fish Farms

The water quality index (WQI) is excellent management and administrative mean of cooperating water quality data. Many organizations use the WQI to make a primary evaluation of the state of a water body since it is a fast and simple tool of assessment, (Shreadah et al., 2020). The National Sanitation Foundation Water Quality Index (NSF-WQI) was proposed for different water types. The NSF-WQI combines the results of six water quality parameters (including DO, pH, BOD, temperature change, NO₃-N, and DIP) into a single number, by using the program of Wilkes University, Centre for Environment Quality Environmental Engineering and Earth Sciences, for calculating the Q-value and NSF WQI according to the following formula:

$$NSF -WQI = \sum_{i=1}^p W_i \cdot I_i$$

Where: *i*: the quality parameter, *p*: the number of water quality parameters, *I_i*: the sub-index for *i*th water quality parameter, *W_i*: the weight associated with the parameter (DO=0.17, pH=0.11, BOD=0.11, temperature

change=0.10, NO₃-N=0.10, and DIP=0.10). The classification criteria standards based on NSF-WQI was excellent (A)=91-100, good (B)=71 – 90, medium (C)=51 – 70, bad (D)=26 – 50, and very bad (E)=0 - 25.

Results of the Q-value and NSF WQI of the present study were illustrated in Table (3). Although the recorded water parameters were different between the studied farms, the overall WQI values of the three studied farms were close to each other 75 and 83. Indicating the FU, FD and FC had good water quality where they were all found in category (B) according to NFC-WQI.

Conclusion

The water characteristics of the aquatic environment is the critical aspect controlling the growth of healthy fish (cultured and wild). According to laws and legislations, the majority of farms in Egypt use drainage water as feeding water sources. Thus the continuous monitoring of water quality parameters of fish farms and their water sources is a must.

The suitability of fish farms irrigated by ADW was examined and compared with the fish farm irrigated by Nile water (Ri-N). The results indicated that most of the studied environmental parameters and nutrients salts of the three fish farms were suitable for aquatic life. According to NFC-WQI, the overall water quality was in good condition. However, the statistically significant differences between farms irrigated by ADW and Ri-N were observed for pH, DO, BOD, nutrients salts, D-Mn, T-Zn, and T-Cr, the distribution of the rest studied water quality parameters did not show statistical variations.

Table S2a. Mean, standard deviation, F-values of three fish farms from the one-way ANOVA test of significant studied parameters, (P<0.05)

Dependant parameter		Mean	Std. Deviation	F	Sig.	Dependant parameter		Mean	Std. Deviation	F	Sig.	
pH	FU	8.626	0.040	5.211	0.013	DIP-P	FU	3.5600	2.27058	43.775	0.000	
	FD	8.769	0.323				FD	7.7567	1.13750			
	FC	8.878	0.047				FC	25.2333	6.91961			
DO	FU	6.512	0.519	52.260	0.000	SiO4-Si	FU	16.3733	3.99304	15.278	0.000	
	FD	11.558	1.698				FD	17.2283	5.62203			
	FC	9.090	0.716				FC	28.7600	2.97149			
BOD	FU	1.6417	1.01954	19.673	0.000	D-Mn	FU	0.0061	0.0041	16.634	0.000	
	FD	7.0533	1.96763				FD	0.0171	0.0060			
	FC	4.9000	1.37142				FC	0.0064	0.0034			
NH ₄ -N	FU	0.5700	0.12426	36.991	0.000	T-Zn	FU	0.0313	0.0067	4.487	0.022	
	FD	0.1733	0.05465				FD	0.0418	0.0106			
	FC	0.7000	0.13491				FC	0.0322	0.0068			
NO ₂ -N	FU	4.3800	1.30005	41.214	0.000	T-Cu	FU	0.0082	0.0027	16.999	0.000	
	FD	0.3233	0.25272				FD	0.0226	0.0086			
	FC	1.0917	0.52343				FC	0.0074	0.0062			
NO ₃ -N	FU	56.7033	20.31637	40.877	0.000							
	FD	5.0317	4.07268									
	FC	94.3750	21.36770									
DIN-N	FU	61.6550	20.78623	40.764	0.000							
	FD	5.5283	3.87249									
	FC	96.1650	21.84054									

Focusing on levels and distribution of non-biodegradable and potentially hazardous metals, it revealed that the order of abundance of the total and dissolved metals according to their concentration levels was Fe > Zn > Mn > Pb > Cu > Cr > Cd and Fe > Mn > Pb > Cu > Zn > Cr = Cd, respectively. Almost studied metals were relatively found in lower levels in the water of feeding sources than those corresponding levels of fish farms. The outlet fishpond showed a higher level of metals than inlets. Ri-N water source showed lower metals levels than Drain-7 water, consequently the control farm (FC) displayed the minimum values for most metals (especially in their dissolved form), relative to other farms irrigated by Drain-7 water. All metals (except Pb) were lower than the permissible limits cited by the Egyptian Law 48 of 1982 and U.S. Environmental Protection Agency (US-EPA) standards. Also, most metals were lower than the water guidelines for fisheries and aquatic life recommended by national and international organizations.

Finally, it was concluded that using of ADW is suitable as using the usage of Nile river water (Ri-N). The continued assessment of drainage water characteristics is a must, as a control measure of water pollution.

Ethical Statement

Ethics required are approved, by the Ethical Committee of Alexandria University and Kafr Elsheikh University.

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Author Contribution

Nashwa A. Shaaban, Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft, - Review & Editing, Visualization, Supervision. Samar Tawfik, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - Original Draft, Visualization. Wael El-Tarras, Investigation. Tamer El-Sayed Ali Conceptualization, Investigation, Data Curation, Writing - Original Draft, - Review & Editing, Supervision, Project administration.

Conflict of Interest

The authors declare that they have no conflict of interest to declare

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Table S2b. Tukey Duncan's multiple range results of significant parameters.

Dependent Variable			Mean Difference	Sig.	Dependent Variable			Mean Difference	Sig.
pH	FD	FU	0.14275	0.21773	DIP-P	FD	FU	4.19667	0.235
	FC	FU	0.25200*	0.00956		FC	FU	21.67333*	0.000
		FD	0.10925	0.39962		FD	FD	17.47667*	0.000
DO	FD	FU	5.04550*	0.00000	SiO ₄ -Si	FD	FU	0.85500	0.938
		FC	2.46750*	0.00011		FC	FU	12.38667*	0.000
	FC	FU	2.57800*	0.00003		FD	FD	11.53167*	0.001
BOD	FD	FU	5.41167*	0.000	D-Mn	FD	FU	.01103*	0.0001
		FC	2.15333	0.062		FC	FC	.01073*	0.0001
	FC	FU	3.25833*	0.005		FC	FU	0.0003	0.9879
NH ₄ -N	FU	FD	.39667*	0.000	T-Zn	FD	FU	.01045*	0.0283
	FC	FU	0.13000	0.137		FC	FC	.00955*	0.0476
		FD	.52667*	0.000		FC	FU	0.0009	0.9658
NO ₂ -N	FU	FD	4.05667*	0.000	T-Cu	FU	FC	0.0008	0.9536
		FC	3.28833*	0.000		FD	FU	.01443*	0.0001
	FC	FD	0.76833	0.269		FC	FD	.01523*	0.0001
NO ₃ -N	FU	FD	51.67167*	0.000					
	FC	FU	37.67167*	0.005					
		FD	89.34333*	0.000					
DIN-N	FU	FD	56.12667*	0.000					
	FC	FU	34.51000*	0.010					
		FD	90.63667*	0.000					

* The mean difference is significant at the 0.05 level.

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