Bioremediation of pollutant-contaminated water

Waseem A. Gad

ABSTRACT

Raw water resources in Kafr El-Sheikh Governorate are affected by many contaminants particularly during the winter season and low demand period of the Nile River, in which water level decreases and organic matter increases resulting in decreased dissolved oxygen, and increased ammonia, nitrite and bacterial load in the water. In this study, physicochemical and microbiological characteristics of water samples taken seasonally from selected sampling sites on seven main canals in Kafr El-Sheikh Governorate were tested and studied to monitor nitrification process occurrence and study its effect on water quality. The main results of the study included turbidity, conductivity, ammonia, nitrite and nitrate and showed increased values during the winter season. Heavy metals levels showed that iron increased in winter and aluminum increased in spring, while copper increased in winter and spring seasons. Total bacterial count, total coliform and algal count increased in the winter season. Activity of ammonia oxidizing bacteria also increased markedly during the winter season which could be explained by increased ammonia concentration during the same period which enhanced nitrification process occurrence. This study proved that nitrification in environments which provide unfavorable conditions for autotrophic nitrifying bacteria may result from the activity of heterotrophic microorganisms; *Bacillus amyloliquefaciens* was isolated and identified to be involved.

Key words | ammonia, ammonia oxidizing bacteria (AOB), *Bacillus amyloliquefaciens*, nitrification, total bacterial count, water pollution

Waseem A. Gad
Central Laboratory,
Kafr El-Sheikh Water and Wastewater Company,
Kafr El-Sheikh City,
Egypt
E-mail: *withyou_2010@yahoo.com*

INTRODUCTION

Water quality is now a major concern for all countries of the world, it depends on the location of the source and the state environmental protection in a given area. Therefore, the quality and the nature of water may be determined by physical and chemical characteristics. So, water resources are the critical factor affecting production, services, and sustainable development in Egypt. Egypt is facing four major constraints with respect to its water resources: (I) a fixed water supply and rocketing population growth; (II) difficulties in the country's relationship with the Nile Basin states; (III) independence of South Sudan declared in July 2011; and (IV) climate change and its hidden future. These four factors pose a number of questions related to the availability of water and the amount of supply that will be allocated for different consumptive and non-consumptive activities and development programs (International Center for Agricultural Research in the Dry Areas (ICARDA) 2011).

The total amount of wastewater discharged into the main stem of the River Nile has been estimated to be 2,628 million cubic metres per year, of which industrial wastewater constitutes 15%. On the other hand, the annual discharge of the river amounts to 55 billion (10^9) cubic metres per year, therefore, the contamination by industrial wastewater can be neglected due to the high dilution rate of the river. Another source of pollution in the River Nile is fish culture in floating cages at Kafr El-Sheikh Governorate; this type of fish culture is one of the reasons for deterioration of water quality because of

doi: 10.2166/aqua.2017.053

the potential negative effects of oxygen depletion, increasing poisonous ammonia, increasing the proportion of total dissolved solids (TDS), and chemical contamination resulting from the hormonal treatment of fish and nutritive materials and remnants of fish output. In addition, use of certain types of fish food, such as blood, meat, fish and poultry remnants would result in deterioration of river water quality, dry sludge and sewage leading to low water quality, transportation of these dangerous wastes into potable water, and transmission to humans, causing several health problems (Elewa 2010; Zaki *et al.* 2014).

Drinking water, according to hygienic rules, should not contain ammonia of organic origin. In case of ammonia of inorganic origin, the maximum acceptable concentration in drinking water is equal to 0.5 mg/dm^3 according to World Health Organization (WHO) Guidelines for Drinking Water and Egyptian Standards (WHO 2006; Jamil *et al.* 2013). Ammonia contamination of water bodies is a widespread environmental problem causing promotion of eutrophication which is fatal to fish and aquatic lives and a hindrance to the disinfection of water supplies, as well as having an offensive smell and carcinogenesis. The regulations on the amount of total nitrogen discharged to the environment have become stricter, especially for ammonia (Mandowara & Bhattacharya 2011). The ammonia in raw water can be removed or decomposed by several methods, such as air-stripping, breakpoint chlorination, biological nitrification and so on; however, none of these methods have been entirely satisfactory as yet. However, the biological remediation method needs continuous monitoring, such as pH control, addition of a carbon source, and temperature maintenance, and also requires the removal of byproducts such as nitrite (Li *et al.* 2009).

Nitrification is a two-step process, first ammonia is oxidized to nitrite by ammonia oxidizing bacteria (AOB), and then nitrite is further oxidized to nitrate by nitrite oxidizing bacteria (NOB). Nitrification in environments which provide unfavorable conditions for autotrophic nitrifying bacteria may result from the activity of heterotrophic microorganisms. The phenomenon of heterotrophic nitrification was first described in 1894 for a fungus (Stutzer & Hartleb 1894). Since then, numerous reports have demonstrated unequivocally that nitrite/nitrate production is not restricted to autotrophic ammonia oxidizers (e.g. Nitrosomonas) or nitrite oxidizers (e.g. Nitrobacter) but is a widespread phenomenon among different genera of fungi and heterotrophic bacteria (Robertson *et al.* 1995). Furthermore, there is no selective enrichment or isolation method for heterotrophic nitrifying microorganisms (Li *et al.* 2009). With the advancement of research, more and more heterotrophic nitrification strains have been isolated and characterized such as Bacillus sp. strains (Yang *et al.* 2011).

MATERIAL AND METHODS

Water sampling

Raw water samples were collected seasonally between December 2013 and October 2014 from selected sites located on the seven main canals of Kafr El-Sheikh Governorate according to the study plan (Eaton *et al.* 2005).

Physical and chemical analyses of water samples

The quality of resource water samples was determined after some measurements such as pH (2510 platinum electrode), turbidity as nephelometric turbidity units (NTU) (2130), conductivity and TDS measured by Analytical unit (WTW Model InoLab cond 720, WTW, Germany) fitted with conductivity probe, total alkalinity (mg/l) (2320B titration method), total hardness (mg/l) (2340B EDTA titration method) and chloride (mg/l) (4500 argentometric method), nitrogen forms as free ammonia (mg/l) (Nesslerization method), nitrite (4500B colorimetric method) and nitrate ($4500-NO_3^-$B ultraviolet spectrophotometric screening method) and heavy metals (3111B metals by flame atomic absorption spectrometry). All the physicochemical analyses were in duplicate and determined by the procedures recommended in *Standard Methods for the Examination of Water and Wastewater* (Eaton *et al.* 2005).

Microbiological analysis of water samples

Total bacterial count (heterotrophic plate count method), total coliforms, fecal coliforms and fecal streptococci (membrane filter technique) were measured (Eaton *et al.* 2005).

Ammonia oxidizing bacteria

Detection of nitrifying bacteria (9245 the multiple-tube method) and identification of involved bacteria (using conventional polymerase chain reaction (PCR) and BIOLOG GEN III Microplate system) were carried out (Eaton *et al.* 2005).

Statistical analyses

Correlation (predictive statistics) was carried out using statistical software (SPSS Version 17, SPSS INC, Chicago, IL, USA). The correlation coefficients are considered significant at the 95% confidence level ($p \leq 0.05$) as shown by Ali *et al.* (2014).

Water quality index

In this study Water Quality Indices of National Sanitation Foundation (NSF WQI) were used to investigate the raw water quality index (WQI) (Roy *et al.* 2017).

RESULTS

Concerning chemical and physical characteristics of water, during the whole sampling period, it was noticed that the maximum increase in pH values was recorded in the summer season followed by the autumn season and began to decrease during winter and spring seasons. The highest value of pH was recorded in Al-Qudaba canal during the summer season (August 2014) while the lowest pH value was recorded in Rewina canal during the winter season (December 2013) (Figure 1). The maximum conductivity values were recorded during the winter season and began to decrease through the spring season; the lowest conductivity values were recorded during the summer season and began to increase again through the autumn season. The highest value of conductivity was recorded in Al-Rashidia canal during the winter season (December 2013) while the lowest conductivity value was recorded in Rewina canal during the summer season (June 2014) (Figure 1). The maximum turbidity values were recorded during winter and spring seasons; the lowest turbidity values were recorded during the summer season and began to increase again through the autumn season. The highest value of turbidity was recorded in Al-Rashidia canal during the winter season (December 2013) while the lowest turbidity value was recorded in Bahr Nashart canal during the summer season (June 2014) (Figure 1). The maximum values of chlorides were recorded during the winter season and began to decrease through the spring season; while the lowest values were recorded during summer and autumn seasons. The highest value of chlorides was recorded in Al-Rashidia canal during the winter season (December 2013) while the lowest chlorides value was recorded in Bahr Tirra canal and Al-Qudaba canal during the summer season (June and August 2014 respectively).

Concerning nitrogenous compounds, the maximum values of ammonia were recorded during winter and autumn seasons; while the lowest values were recorded during summer and spring seasons. The highest value of ammonia was recorded in Al-Rashidia canal during the winter season (December 2013) while the lowest ammonia value was recorded in Met Yazid canal during the summer season (June 2014) (Figure 2). The maximum values of nitrite were recorded during winter and autumn seasons; while the lowest values were recorded during summer and spring seasons. The highest value of nitrite was recorded in Al-Rashidia canal during the winter season (December 2013) while the lowest nitrite value was recorded in Bahr Nashart canal during the summer season (June 2014) (Figure 2). The maximum values of nitrate were recorded during winter and autumn seasons; while the lowest values were recorded during summer and spring seasons. The highest value of nitrate was recorded in Al-Rashidia canal during the winter season (December 2013) while the lowest nitrate value was recorded in Met Yazid canal during the summer season (June 2014) (Figure 2).

Concerning heavy metals, it was observed that iron concentration was increased in the winter season (December 2013) then began to decrease through February 2014 and April 2014 except for Rewina canal and Al-Bahr Al-Seidi canal during April 2014 which recorded the highest concentrations. Then iron concentration began to increase again in the summer season in June and August 2014 eventually tending to decrease again during the autumn season in October 2014. The highest concentration of iron was recorded in

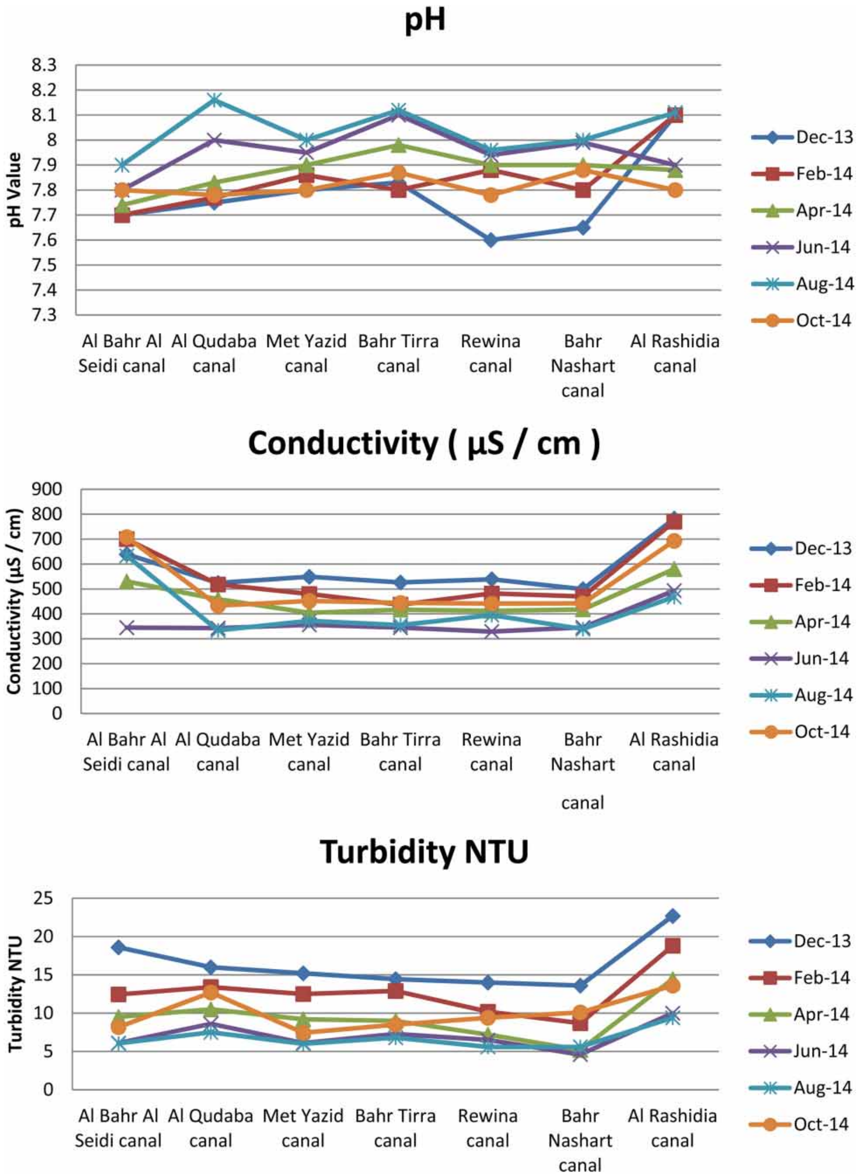


Figure 1 | Values of pH, conductivity and turbidity in different seasons in seven main canals of Kafr El-Sheikh Governorate.

Rewina canal during the spring season (April 2014) while the lowest concentration was recorded in Al-Rashidia canal during the autumn season (October 2014) (Figure 3). Copper concentration showed a similar pattern in most locations which could all be neglected as concentration ranged from non-detected to very small values except for Al-Bahr Al-Seidi canal during spring and summer seasons and Bahr Tirra canal during the winter season (Figure 3). Aluminum concentration was elevated in the spring season (April 2014) in most locations then tended to decrease in summer and autumn seasons; while the lowest concentrations were recorded during the winter season (Figure 3).

Microbiological analysis of raw water samples indicated that total bacterial count showed a slight decrease from winter season to autumn season through spring and summer. The highest total bacterial count was recorded in Al-Rashidia canal during the winter season (December 2013) while the lowest total bacterial count was recorded in Bahr Tirra canal during the summer season (August

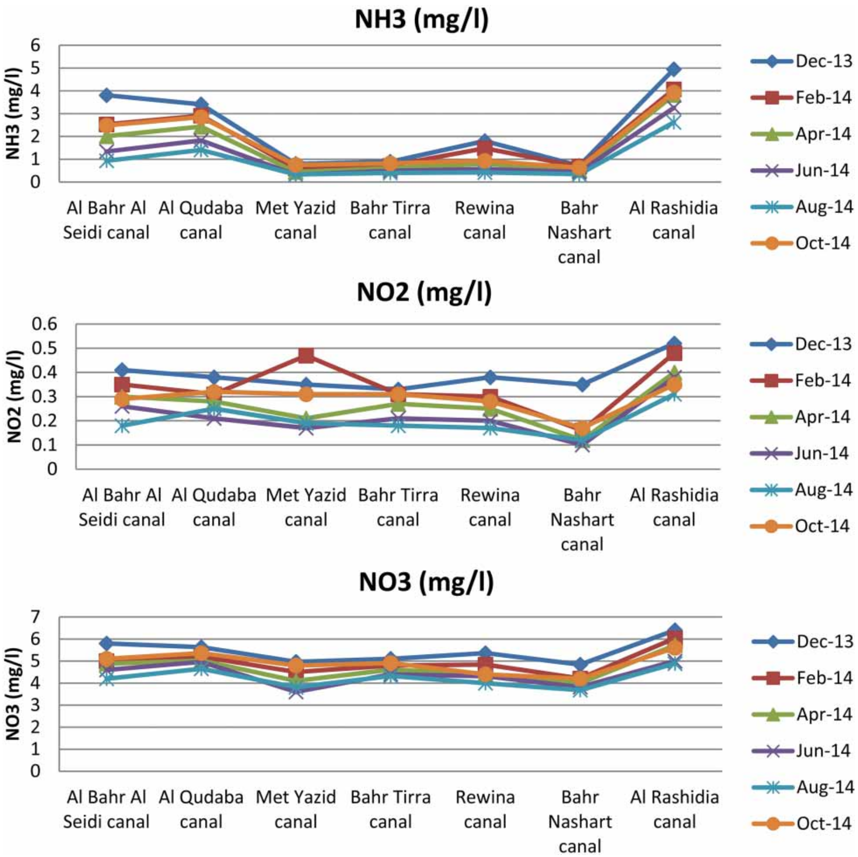


Figure 2 | Ammonia, nitrite and nitrate values in different seasons in seven main canals of Kafr El-Sheikh Governorate.

2014) (Figure 4). Total coliform count showed a remarkable increase during winter and spring seasons and slightly decreased through summer and autumn seasons. The highest value of total coliform count was recorded in Al-Rashidia canal during the winter season (December 2013) while the lowest total coliform count was recorded in Met Yazid canal during the summer season (August 2014) (Figure 4). Fecal coliform count showed an observed decrease from total coliform count in the range of 30–50%, the highest fecal coliform count was recorded during winter and spring seasons, while the lowest count was recorded during summer and autumn seasons. The highest count of fecal coliform was recorded in Al-Rashidia canal during the winter season (December 2013) while the lowest fecal coliform count was recorded in Met Yazid canal, Rewina canal and Bahr Nashart canal during the summer season. Concerning biological characteristics of raw water, the total algal count showed approximate similarity between sampling points through different seasons with a little decreasing through the summer season. The highest total algal count was recorded in Al-Rashidia canal during the winter season (December 2013) while the lowest total algal count was recorded in Rewina canal during the summer season (August 2014) (Figure 4).

AOB estimation revealed a progressive increase in count during the winter season particularly in December 2013, the count tended to decrease during the spring season and reached the lowest ratio during summer and autumn. Al-Rashidia canal showed a huge growth of AOB during the winter season (December 2013 and February 2014). The highest count of AOB was recorded in Al-Rashidia canal during the winter season (December 2013) while the lowest AOB count was recorded in Rewina canal and Al-Qudaba canal during the summer season (August 2014) and autumn season (October 2014) respectively (Figure 5). Bacterial strains isolated from AOB broth medium and

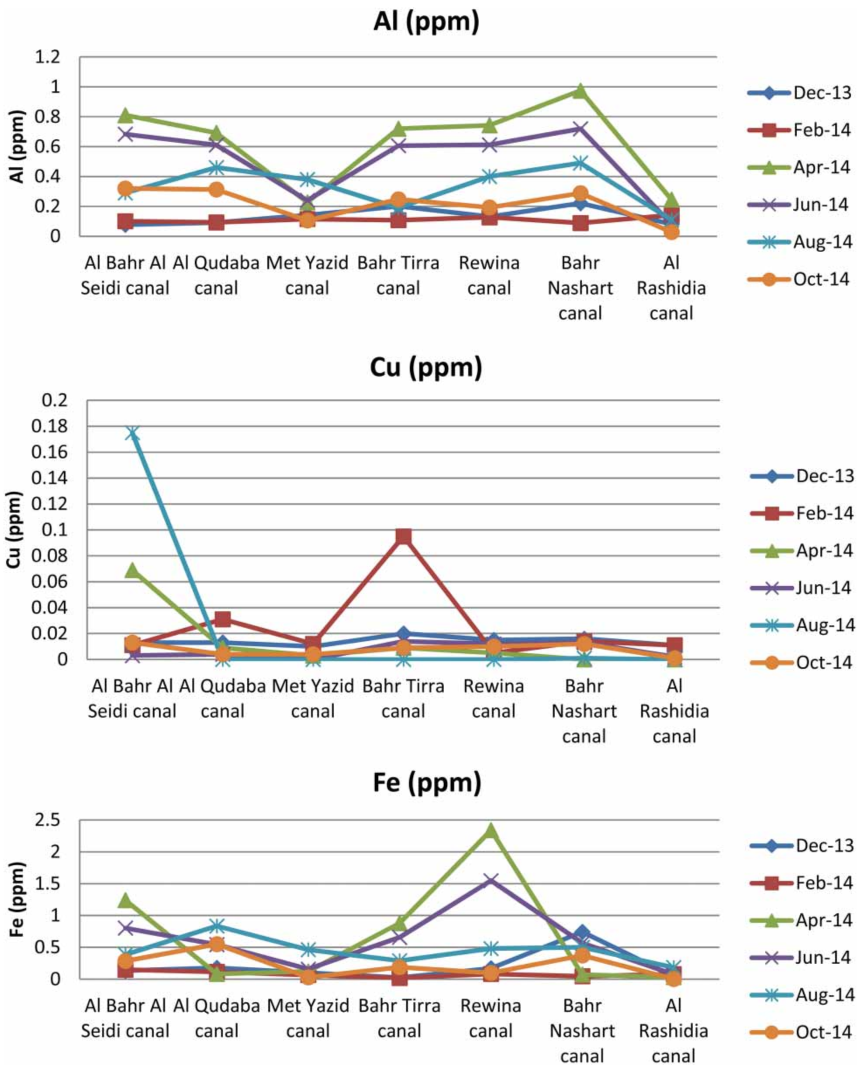


Figure 3 | Iron, copper and aluminum concentrations in different seasons in seven main canals of Kafr El-Sheikh Governorate.

purified on tryptic soy agar (TSA) medium were identified as *Bacillus amyloliquefaciens* using the BIOLOG GEN III Microplate system (Figure 5).

Water quality index

WQI values ranged from 34.36 to 43.52 indicating bad water quality conditions especially during the winter season. The lowest WQI value recorded was 34.36 for Al Rashidia canal during the winter season 2013 while the highest WQI value recorded was 43.52 for Met Yazid canal during the summer season (June 2014). Figure 5 illustrates the results of the WQI testing during the whole period of study.

Statistical analyses

Correlation (predictive statistics) was carried out using the SPSS analytical program. The correlation coefficients are considered significant at the 95% confidence level ($p \leq 0.05$). Table 1 and Figure 6 illustrate the correlation coefficients between physical, chemical and microbiological parameters.

There is a significance between temperature and pH and the relationship is positive which means that increases or decreases in temperature do significantly relate to increases or decreases in pH ($r = 0.519$). There is a statistically significant correlation between ammonia and temperature but the

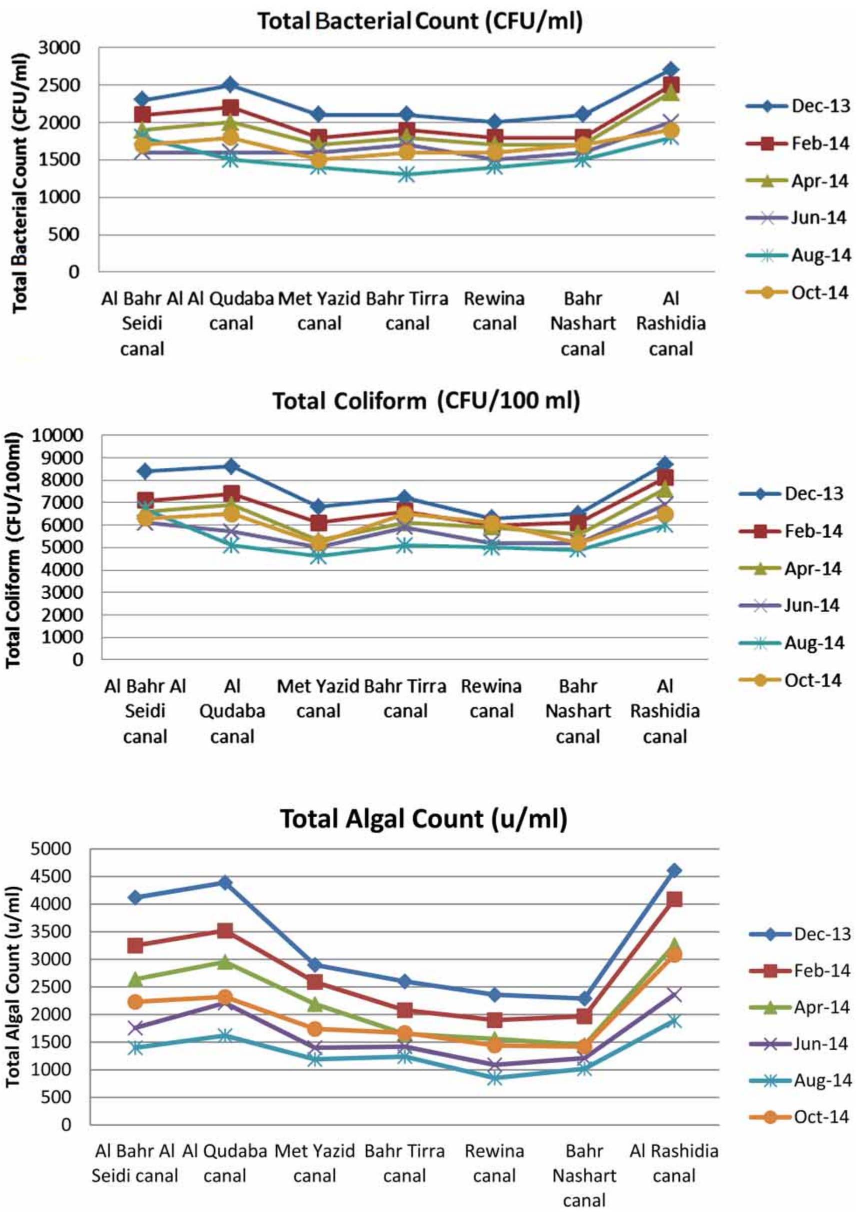


Figure 4 | Total bacterial count, total coliform count and total algal count in different seasons in seven main canals of Kafr El-Sheikh Governorate.

relationship is negative ($r = -0.316$), ammonia and pH have no statistically significant correlation and the relationship is weak negative ($r = -0.100$). Ammonia has strong positive relationships with statistically significant correlations with conductivity, TDS, turbidity, alkalinity, total hardness, Ca hardness, Mg hardness, chlorides, nitrite, nitrate, phosphate, sulfate and total organic carbon (TOC). Ammonia has weak negative relationships with most recorded values of heavy metals except for two weak positive relationships with manganese and cadmium, as shown by the correlation coefficients in Table 1 and Figure 6. Ammonia and all microbiological parameters have strong positive significant relationships, while ammonia and WQI show a strong negative relationship with a statistically significant correlation between both of them as shown in Table 1 and Figure 6. Ammonia and pH showed weak positive relationship with

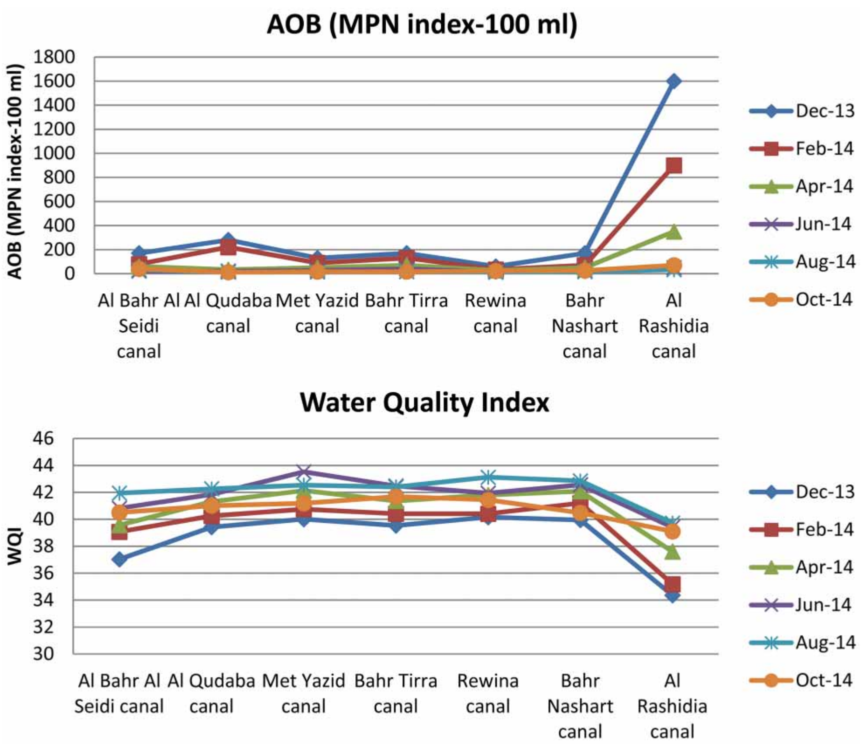


Figure 5 | AOB count and WQI in different seasons in seven main canals of Kafr El-Sheikh Governorate.

no significant correlation. AOB and all chemical and microbiological parameters showed strong positive and significant correlations between each other. AOB and WQI showed a significant and strong negative relationship with correlation coefficient $r = -0.873$, as shown in Table 1.

Identification of heterotrophic bacteria involved in nitrification

One bacterial strain isolated from AOB broth medium and purified on TSA medium was identified as *Bacillus amyloliquefaciens* using the BIOLOG GEN III MicroPlate system by National Research Centre (NRC), El Buhouth, Dokki, Cairo, Egypt, as shown in Figure 7.

DISCUSSION

In this study, many findings were observed by monitoring seasonal variations of physical, chemical and microbiological characteristics of raw water of seven main canals used by water purification plants in Kafr El-Sheikh Governorate; those findings elucidated the quality of these resources and how they are affected by pollutants especially during the winter season and low demand period of the Nile River. This study discussed nitrification process occurrence and how this process and those characteristics would affect each other.

The western borders of Kafr El-Sheikh Governorate are located along 85 km of the Nile River (Rosetta branch). So the Rosetta branch is considered the most important water resource in Kafr El-Sheikh Governorate for drinking water purification, agricultural land irrigation and fisheries. The Rosetta branch part which lies within Kafr El-Sheikh borders is free of industrial wastewater sources, but in the winter season and during the low demand period (January to March), the water level in the river decreases and given that agricultural drains in Al-Munofiya Governorate which discharge into the Rosetta branch (Al-Rahawy drain, Talla drain and Sebbel drain) are heavily polluted with untreated wastewater and sewage, the organic matter in the river increases during this period which leads to decreasing

Table 1 | Correlation coefficient between the different physicochemical and microbiological parameters

	pH	Conductivity	Turbidity	Ammonia	Nitrite	Nitrate	Cu	Fe	Al	Total bacterial count	AOB	Total coliform	Total algal count	WQI
pH	1	−0.281	−0.271	−0.1	−0.266	−0.227	−0.068	0.132	0.233	−0.278	0.235	−0.318*	−0.289	0.145
Conductivity	−0.281	1	0.733**	0.731**	0.700**	0.738**	0.175	−0.382*	−0.504**	0.753**	0.609**	0.759**	0.755**	−0.824**
Turbidity	−0.271	0.733**	1	0.731**	0.866**	0.871**	−0.108	−0.381*	−0.575**	0.899**	0.714**	0.855**	0.905**	−0.890**
Ammonia	−0.1	0.731**	0.731**	1	0.717**	0.882**	−0.126	−0.284	−0.371*	0.764**	0.604**	0.791**	0.839**	−0.829**
Nitrite	−0.266	0.700**	0.866**	0.717**	1	0.868**	−0.144	−0.295	−0.551**	0.770**	0.596**	0.781**	0.812**	−0.837**
Nitrate	−0.227	0.738**	0.871**	0.882**	0.868**	1	−0.139	−0.277	−0.432**	0.813**	0.626**	0.858**	0.873**	−0.877**
Cu	−0.068	0.175	−0.108	−0.126	−0.144	−0.139	1	0.009	0.001	0.035	−0.04	0.121	−0.107	0.072
Fe	0.132	−0.382*	−0.381*	−0.284	−0.295	−0.277	0.009	1	0.637**	−0.325*	−0.217	−0.305*	−0.368*	0.305*
Al	0.233	−0.504**	−0.575**	−0.371*	−0.551**	−0.432**	0.001	0.637**	1	−0.403**	−0.275	−0.394**	−0.450**	0.453**
TBC	−0.278	0.753**	0.899**	0.764**	0.770**	0.813**	0.035	−0.325*	−0.403**	1	0.708**	0.935**	0.920**	−0.873**
AOB	0.235	0.609**	0.714**	0.604**	0.596**	0.626**	−0.04	−0.217	−0.275	0.708**	1	0.630**	0.659**	−0.778**
TC	−0.318*	0.759**	0.855**	0.791**	0.781**	0.858**	0.121	−0.305*	−0.394**	0.935**	0.630**	1	0.909**	−0.843**
TAC	−0.289	0.755**	0.905**	0.839**	0.812**	0.873**	−0.107	−0.368*	−0.450**	0.920**	0.659**	0.909**	1	−0.858**
WQI	0.145	−0.824**	−0.890**	−0.829**	−0.837**	−0.877**	0.072	0.305*	0.453**	−0.873**	−0.778**	−0.843**	−0.858**	1

TBC, total bacterial count; TC, total coliform; TAC, total algal count.
*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

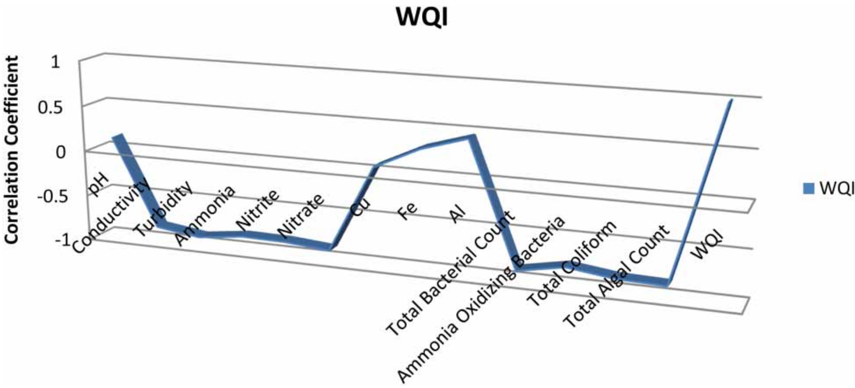


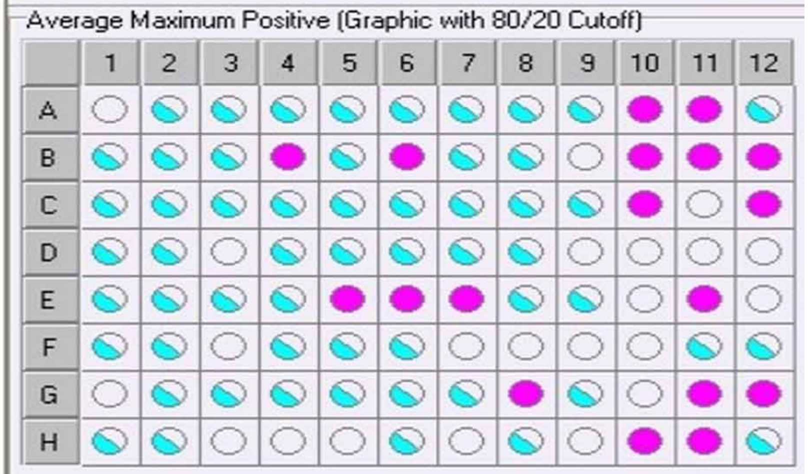
Figure 6 | Positive and negative correlations between WQI and other measured parameters.

dissolved oxygen, increasing ammonia, nitrite, and bacterial load and other seriously dangerous problems in the water. This causes many problems especially for Drinking Water Purification Plants located on the Nile River, or their intakes located on the Rosetta branch and main canals, as ammonia and nitrite rather than other pollutants increase and it is a challenge for purification plants to remove them and produce safe and clean water (Kafr El-Sheikh Environmental Affairs Administration (KFSEAA) 2012). Ammonia in raw water can be removed or decomposed by biological nitrification; however the biological remediation method needs continuous monitoring, such as pH control, addition of a carbon source, and temperature maintenance, and also requires the removal of byproducts such as nitrite (Li *et al.* 2009). In the first step of nitrification; ammonia is oxidized to nitrite by AOB, and then nitrite is further oxidized to nitrate by NOB. AOB and NOB are collectively known as nitrifying bacteria or nitrifiers (Li *et al.* 2009).

This study aimed to investigate water quality in seven main canals in Kafr El-Sheikh Governorate through examining seasonal and spatial variations of physicochemical parameters, assessment of biological and bacteriological characteristics, including nitrifying bacteria, and monitoring nitrification as a biological process for ammonia removal. pH values were on the alkaline side of the pH scale; values were always higher than 6.5 which is normally expected in raw water due to the presence of carbonates or bicarbonates, as reported by Friedl *et al.* (2004). The increase in pH could be related to photosynthesis and growth of aquatic plants, where photosynthesis consumes CO_2 leading to a rise in the pH values (Yousry *et al.* 2009). The maximum increase in pH values was recorded during the summer season as temperature and pH are positively correlated; this is because the increase in temperature is usually accompanied by hydrolysis of HCO3− and CO32− ions, leading to the appearance of hydroxyl (OH−) ions that increase pH value. A similar relationship was reported by Toufeek & Korium (2009); increase or decrease of pH values was due to the seasonal variation and the previously mentioned factors affecting pH. The optimum pH for nitrification lies between 7.5 and 8.5 (Bitton 2005). Electrical conductivity (EC) is a measure of the ability of water to carry electric current and it is sensitive to variations in dissolved solids (El Shakour & Mostafa 2012); EC values showed a huge seasonal variation and highly spatial differences among the period of study. It was noticed that Al-Rashidia canal gives the highest EC values through all seasons. Values increase more especially in the winter season where organic matter increases in the river during the low demand period. Such results were given by Abd El-Hady & Hussian (2012). Conductivity is a good measure of the total amount of salts in water (e.g. calcium, magnesium, sodium, potassium, carbonate, bicarbonate, sulfate, chloride, nitrate, and others). It is commonly used to determine salinity (Goher 1998). So EC showed a strong positive and high significant relationship with TDS ($r = 0.998$); similar results and correlations were given by Goher (1998) and El Shakour & Mostafa (2012).

Turbidity is the measure of fine suspended matter in water, mostly caused by colloidal particles in addition to suspended organic and inorganic matter (Goher 1998). The turbidity degree of stream water is an approximate measure

Organism Type	GP-Rod-SB
Family	Bacillaceae
Species	Bacillus amyloliquefaciens
Protocol	A



Average Maximum Positive (Percent)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	76	74	79	78	79	75	75	69	100	98	51
B	70	73	51	80	75	80	37	21	0	96	90	87
C	69	70	72	59	32	32	25	29	66	97	18	90
D	78	73	0	23	74	23	36	72	8	15	18	8
E	76	73	77	67	82	82	80	71	69	18	86	8
F	73	39	0	77	39	40	16	8	20	17	29	28
G	3	63	24	79	49	39	73	82	67	18	82	100
H	58	47	15	5	0	78	16	77	19	83	85	38

Figure 7 | Identification of *Bacillus amyloliquefaciens* using BIOLOG GEN III MicroPlate system.

of pollution intensity (Siliem 1984). Turbidity values among the period of study showed high seasonal variation where the highest turbidity values were recorded in the winter season due to the low demand period and the increase of pollutants and colloidal particles at the end of the Nile River. After the low demand period, the turbidity values decrease because of the flow of water. The lowest turbidity values were recorded in the summer season. Turbidity values are negatively correlated with pH (Goher 1998). Turbidity values, TDS and EC revealed positively strong correlations to each other; El Shakour & Mostafa (2012) reported the same correlations. Positive correlations were found between turbidity values and most studied parameters. Turbidity values are negatively correlated with pH ($r = -0.271$); such results were given by El Shakour & Mostafa (2012). Turbidity values revealed a positively strong correlation with TDS ($r = 0.741$) and EC ($r = 0.733$); Toufeek & Korium (2009) reported the same correlations.

Ammonia serves as a substrate for Nitrosomonas spp. and is considered one of the most important factors affecting nitrification according to Bitton (2005). Throughout this study, ammonia concentrations showed remarkable variations both regionally and seasonally. The highest value was observed at Al-Rashidia canal in the winter season and the lowest value was observed at Met Yazid canal in the summer season. Ammonia and nitrogen concentrations of more than 1 mg/l have been given as indicator of organic pollution, such as sewage discharge, industrial effluents and agricultural runoff, and can be toxic to aquatic species if they are higher than 2.5 mg/l (Siliem 1984; Elghobashy *et al.* 2001; Abdel-Satar 2005). Although it is a nutrient required for life, excess ammonia can accumulate in the organism and cause alteration in metabolism or increase body pH (Kahlown *et al.* 2006). On the other hand, the decrease in ammonia concentrations was related to the decrease in biological activities of aquatic organisms and nitrification (Ahmed *et al.* 2011). Statistical analysis showed strong positive and significant correlations of NH_3 with NO_3 ($r = 0.882$), and bacteriological parameters; total bacterial count ($r = 0.764$), AOB ($r = 0.604$), total coliform ($r = 0.791$), fecal coliform ($r = 0.560$), fecal streptococcus ($r = 0.705$) and total algal count ($r = 0.839$), similar findings and correlations were given by El Shakour & Mostafa (2012), while ammonia showed a strong negative significant relationship with WQI ($r = -0.829$). This confirms that the impact of sewage discharge, agricultural runoff and the existence of other pollutants, especially in the winter season with slow water flow rate during the low demand period, had occurred. The recorded mean values violate the permissible limits of law 48/1982 (not to exceed 0.5 mg/l) (El Shakour & Mostafa 2012).

Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate; such oxidation and reduction occurs in waste water treatment plants, water distribution systems, and natural water (Eaton *et al.* 2005). Al-Rashidia canal showed the highest nitrite values among all seasons of the study while Bahr Nashart canal showed the lowest values. It was observed that the highest values of nitrite were measured in the winter season which may be attributed to the decomposition of organic matter during the winter low demand period of the Nile River while pollutants increase due to the very slow flow-rate of water where Nitrosomonas bacteria oxidize ammonia to nitrite by the nitrification process (Tayel 2003; Ahmed *et al.* 2011). The low values of nitrite might be attributed to the fast conversion of NO_2^- by nitrobacteria to NO_3^- (Abdo 2004; Tayel 2007). Nitrite concentrations showed significant positive correlation with ammonia ($r = 0.717$) and AOB ($r = 0.596$). Nitrite concentrations showed a strong positive and significant correlation with nitrate concentration ($r = 0.868$).

Nitrate ion is the final oxidation product of nitrogen compounds in the aquatic environment (Ahmed 2007). The low values of nitrate during the summer season might be attributed to the uptake of nitrate by natural phytoplankton and its reduction by denitrifying bacteria and biological denitrification (Bayomy & Mahmoud 2007; Ahmed *et al.* 2011). On the other hand, the increase of nitrate levels during the winter season might be attributed to sewage wastes and low consumption of phytoplankton as well as the oxidation of ammonia by Nitrosomonas bacteria and biological nitrification (Tayel 2003; Abdo 2010; Ahmed *et al.* 2011).

Concerning heavy metals which were measured in water samples as particulates, increased iron concentrations may be attributed to inorganic fertilizers used in agricultural practice, this was confirmed by the opinions of Khallaf *et al.* (1998) and Arti (2011). Increased copper concentrations may be attributed to the fact that drainage water is the main source of copper; this explanation is in common with El-Baz (2014). Copper is believed to be a key component of the ammonia monooxygenase (AMO) enzyme, which is essential for ammonia oxidation and nitrifier growth (Richardson & Watmough 1999). However, excess copper is known to be toxic to nitrifiers (Braam & Klapwijk 1981). Highest aluminum concentrations may be attributed to several causes depending on the aluminum environmental fate. Aluminum is released to the environment mainly by natural processes. Several factors influence aluminum mobility and subsequent transport within the environment; these include chemical speciation, hydrological flow paths, soil–water interactions, and the composition of the underlying geological materials. Acid environments caused by acid factories' drainage or acid rain can cause an increase in the dissolved aluminum content of the surrounding waters (US Public Health Service 1992; WHO 2006). Aluminum salts are also widely used in water treatment as coagulants to reduce organic matter, color, turbidity, and microorganism levels (Canadian Council of Resource and Environment Ministers (CCREM) 1987).

Total bacteria count increased in the winter season either because more nutrients are carried by the Nile then or because of recently drowned vegetation in the autumn season providing bacteria with organic matter, encouraging their reproduction (Saleh 1976). Such a negative relationship between total bacterial count and transparency in autumn and winter seasons has previously been reported (Saleh 1976; Elewa & Azazy 1986; Rabeh 1999), as suspended matter is very important, due to bacterial adherence to particles. The increased counts during the winter season also might be attributed to the low demand period of the Nile River during the winter season which decreases the water level and increases organic matter existence because of drains polluted by sewage discharged to the Rosetta branch, e.g. El-Rahawy Drain. The lowest total and fecal coliform counts were measured during the summer season while the highest counts were recorded in the winter season. This indicates the high activity of total and fecal coliform bacteria during the winter season because of the low demand period of the River Nile, and increase of pollutants, nutrients and favourable growth conditions. It may also be attributed to human sewage pollution associated with high organic loads favouring the bacterial survivability. These findings agreed with Gad (2005). Total bacterial count showed a significant positive correlation with ammonia concentration ($r = 0.764$) and AOB ($r = 0.708$), while total bacterial count showed a significant strong negative correlation with WQI ($r = -0.873$). Total coliform showed a significant positive correlation with ammonia concentration ($r = 0.781$) and AOB ($r = 0.630$) and a significant strong positive correlation with total bacterial count ($r = 0.935$), while total coliform showed a significant strong negative correlation with WQI ($r = -0.843$).

The word Algae is a general term for small, chlorophyll-containing plants. In this study, the lowest total algal count was recorded in the summer season while the highest total algal count was recorded in the winter season due to the high level of nutrients enabling the algae to grow and reproduce quickly. If algae grow in high density on the surface, this will block sunlight from reaching plants at greater depths which will cause the plants to die. When algae die, the decaying process uses oxygen in the water, and decreasing the amount of dissolved oxygen will cause aquatic organisms to die. The process of aquatic overgrowth, followed by death, decay, and oxygen depletion is called eutrophication (Harrison & De Mora 1996; Goher 1998). Results of this study indicated that: total algal count was positively correlated to ammonia concentration; algae are able to uptake and assimilate ammonia and would enhance nitrification, also they are able to assimilate various types of nitrogen and they could be of great concern when used as a biological treatment; and some algae prefer ammonia as their nitrogen source, however nitrate would be utilized in the absence of ammonia. This agreed with Hii *et al.* (2011) who indicated that some microalgae grew faster with ammonia than nitrate, presumably due to the higher uptake rate and could be used as a potential biological treatment for aquaculture water. Baskaran *et al.* (1992) showed that combined algal/bacterial biofilms grown on surfaces immersed in the lagoons showed potential for greatly increasing the extent of nitrification; photosynthetic activity in the biofilm greatly enhanced nitrification efficiencies at low dissolved oxygen levels. Concerning biological parameters, the total algal count including diatoms, green algae and blue green algae increased markedly in the winter season then started to decrease in spring and reached the lowest count in summer, and then began to increase again in the autumn season. Total algal count showed significant strong positive correlations with ammonia ($r = 0.839$), total bacterial count ($r = 0.920$), AOB ($r = 0.659$), diatoms ($r = 0.977$), green algae ($r = 0.956$) and blue green algae ($r = 0.879$), while total algal count showed a significant and strong negative correlation with WQI ($r = -0.858$).

Nitrification and nitrifying bacteria

Nitrification is the biological oxidation of ammonia with oxygen into nitrite followed by the oxidation of these nitrites into nitrates. Nitrification is an important step in the nitrogen cycle. Under oxic conditions, the most important group of organisms involved in nitrification are aerobic chemolithoautotrophic nitrifying bacteria, the ammonia and nitrite oxidizers. For these organisms, the oxidation of inorganic nitrogen compounds serves as their characteristic energy source. They can derive all cellular carbon from carbon dioxide (CO_2). No chemolithotroph is known that can carry out the complete oxidation of ammonia to nitrate (Werner & Newton 2005). In a nitrification process, ammonia is first oxidized into nitrite (NO_2^-) by several genera of autotrophic bacteria; the most important being *Nitrosomonas* sp. (Chen *et al.* 2006). Nitrifying bacteria are classified as obligate chemolithotrophs. This simply means that they must use inorganic salts as an energy source and generally cannot utilize organic materials. They must oxidize ammonia and nitrites for their energy needs and fix inorganic carbon dioxide (CO_2) to fulfill their carbon requirements. They are largely non-motile and must colonize a surface (gravel, sand, synthetic bio-media, etc.) for optimum growth. They secrete a sticky slime matrix which they use to attach themselves. Species of Nitrosomonas and Nitrobacter are gram negative, mostly rod shaped, microbes ranging between 0.6 and 4.0 microns in length. They are obligate aerobes and cannot multiply or convert ammonia or nitrites in the absence of oxygen. Nitrifying bacteria have long generation times due to the low energy yield from their oxidation reactions. Since little energy is produced from these reactions they have evolved to become extremely efficient at converting ammonia and nitrite. Scientific studies have shown that Nitrosomonas bacterium are so efficient that a single cell can convert ammonia at a rate that would require up to one million heterotrophs to accomplish. Most of their energy production (80%) is devoted to fixing CO_2 via the Calvin cycle and little energy remains for growth and reproduction. As a consequence, they have a very slow reproductive rate. Nitrifying bacteria reproduce by binary division. Under optimal conditions, Nitrosomonas may double every 7 hours. None of the Nitrobacteraceae is able to form spores. They have a complex cytomembrane (cell wall) that is surrounded by a slime matrix. All species have limited tolerance ranges and are individually sensitive to pH, dissolved oxygen levels, salt, temperature, and inhibitory chemicals. Unlike species of heterotrophic bacteria, they cannot survive any drying process without killing the organism. In water, they can survive short periods of adverse conditions by utilizing stored materials within the cell. When these materials are depleted, the bacteria die (Ramadan 2007). In addition to lithotrophic nitrification, various heterotrophic bacteria, fungi, and algae are capable of oxidizing ammonia to nitrate in the presence of O_2 (Fiencke *et al.* 2005). However, in contrast to lithotrophic nitrification, heterotrophic nitrification is not coupled to energy generation. Consequently, the growth of heterotrophic nitrifiers is dependent on the oxidation of organic substrates. During heterotrophic nitrification, either ammonia or reduced nitrogen from organic compounds is co-oxidized. The rate of heterotrophic nitrification is much slower than that accomplished by the chemolithotrophic nitrifying bacteria and may not be ecologically significant (Werner & Newton 2005). Nitrification in environments which provide unfavorable conditions for autotrophic nitrifying bacteria may result from the activity of heterotrophic microorganisms. The phenomenon of heterotrophic nitrification was first described in 1894 for a fungus (Stutzer & Hartleb 1894). Since then, numerous reports have demonstrated unequivocally that nitrite/nitrate production is not restricted to autotrophic ammonia oxidizers (e.g. Nitrosomonas) or nitrite oxidizers (e.g. Nitrobacter) but is a widespread phenomenon among different genera of fungi and heterotrophic bacteria (Robertson *et al.* 1995). Lin *et al.* (2007) initiated a study suggesting that the removal of ammonia nitrogen which was 74.7% was mostly attributed to nitrification by *Bacillus* sp. *LY*. The pure microorganism which could nitrify via a heterotrophic pathway was likely responsible for nitrification in that case.

Bacillus species are important candidates for developing commercial biological agents for nitrogen removal and water quality enhancement (Hong *et al.* 2005). Previously, a few studies reported that some strains of B. subtilis (Rui *et al.* 2009; Chen & Hu 2011), B. lichenformis (Rui *et al.* 2009) and B. cereus (Lalloo *et al.* 2007) exhibited strong nitrite removal ability. Physiological studies on Bacillus spp. also showed that Bacillus spp. could utilize nitrate and nitrite as alternative electron acceptors and nitrogen sources (Xie *et al.* 2013). In the past, B. amyloliquefaciens were applied in enzyme production (Wei *et al.* 2011), plant disease control (Arrebola *et al.* 2010) and food preservation (Junhua 2006). Xie *et al.* (2013) referred to its potential application in improving water quality and suggested that B. amyloliquefaciens might be an important alternative Bacillus species for ammonia and nitrite removal. Therefore, to create a microbial agent which can simultaneously eliminate nitrite and ammonia, B. amyloliquefaciens can be formulated with efficient nitrifying bacteria (Rui *et al.* 2009).

Ammonia oxidizing bacteria

Concerning AOB, the highest count of AOB was recorded during the winter season while the lowest AOB count was recorded during summer and autumn seasons. Al Rashidia canal showed very high activity of AOB during the entire period of study in comparison with the other six points of the main canals. AOB count showed significant positive correlations with ammonia concentration ($r = 0.604$), nitrite ($r = 0.596$), nitrate ($r = 0.626$), EC ($r = 0.609$), alkalinity ($r = 0.719$) and turbidity ($r = 0.714$), while AOB showed a significant and strong negative correlation with WQI ($r = -0.778$).

Many factors contribute to the viability of nitrifying bacteria and, as a result, nitrification episodes have been observed at pH levels ranging from 6.6 to 9.7 according to Odell *et al.* (1996). pH values in the present study ranged between 7.6 and 8.16 which were optimal for the activity and viability of nitrifying bacteria; AOB showed an insignificant positive correlation with pH ($r = 0.235$). Normally, nitrifying bacteria need phosphate for good functioning (Hossain *et al.* 2007). In the present study, raw water contained a sufficient amount of phosphate (i.e. 0.32 to 1.78 mg/l), which was helpful for the nitrification process; AOB showed a significant strong positive correlation with phosphate ($r = 0.780$). In the nitrification process, ammonia is oxidized and subsequently forms nitrite and the end product is nitrate. Occurrence of nitrification was confirmed by monitoring for nitrate and nitrite; it was observed that the increase of nitrite and nitrate is positively correlated to the increase of ammonia concentration; the increase of nitrite, nitrate and ammonia concentration is positively correlated to the increase of AOB count and activity, as shown in Table 1; and those findings agreed with Blasiola & Vriends (1991) and Eaton *et al.* (2005). In the water treatment processes, the elevated nitrate concentrations do not create any problem with chlorine which is injected into the raw water at the time of treatment. On the other hand, 30 mg/l of nitrate-N is acceptable for drinking water (Jamil *et al.* 2013).

High concentration of ammonia in raw water might influence the physicochemical characteristics and other aesthetic characteristics of water like pH, turbidity, color, TDS, EC, total alkalinity and odor etc. (Hossain *et al.* 2007). Considering the impact of raw water ammonia on the physicochemical parameters of raw water of the seven main canals in Kafr El-Sheikh Governorate used as feed water by WTPs, it was obvious that some critical problems arise when raw water contains a high concentration of ammonia, especially in the winter season. So chemical consumption will be increased for the raw water treatment as well as the cost of the water treatment being raised remarkably. The addition of chlorine (Cl_2) to raw water containing high ammonia (NH_3) during the treatment forms chloramines (Angers 2002). Chloramines are known as combined chlorine which is less active than hypochlorous acid (residual chlorine), having only one twentieth the power of hypochlorous acid (Cairncross & Feachem 1993). Combined chlorine is not so active against the algae proliferation and ultimately a huge amount of algae would be found in the clarified water as well as settled on the different parts of the clarifier and filtration systems. To remove ammonia from raw surface water, the nitrification process would be used according to Hossain *et al.* (2007). Biological nitrification is more environmentally friendly and cost effective than any other chemical methods of ammonia removal according to Janda & Rudovsky (1994). Nitrifying bacteria in the nitrification process detoxify ammonia in two steps. First, *Nitrosomonas* spp. converts toxic ammonia to nitrite, in the second step; nitrite is converted to nitrate by *Nitrobacter* spp., according to Blasiola & Vriends (1991).

Increased heterotrophic bacteria are always found in association with nitrifying bacteria when nitrification processes occur (Wilczak *et al.* 1996; Powell 2004). Nitrifiers can increase heterotrophic growth by producing soluble organic carbon (Rittmann *et al.* 1994). Heterotrophs can be beneficial to nitrification by producing stimulating organics for nitrifiers (Hockenbury *et al.* 1977) and protecting nitrifiers from detachment (Rittmann & Manem 1992; Furumai & Rittmann 1994; Rittmann *et al.* 1994). In other cases, heterotrophs can be detrimental to nitrifiers since they compete for surfaces, dissolved oxygen, and ammonia (Rittmann & Manem 1992). The heterotrophic bacteria increased growth could be a result of organic carbon released from nitrifying bacteria (Wilczak *et al.* 1996). Many heterotrophic bacteria have also been found to contribute to nitrification (Watson *et al.* 1989), although with a slower rate and different mechanisms than autotrophic nitrifiers. Some heterotrophic bacteria are capable of oxidizing inorganic nitrogen compounds, but the rates of heterotrophic nitrification are normally four orders of magnitude lower than those of autotrophic nitrification, according to Atlas (1988). This is of special interest in drinking water systems, because unlike autotrophic nitrification, heterotrophic bacteria do not consume dissolved inorganic carbon, and net changes to water chemistry from heterotrophic nitrification will differ to those from autotrophic nitrification.

Concerning AOB, it was very remarkable that Al-Rashidia canal showed very high activity of AOB during the entire period of the study in comparison with the other six points of the main canals. It was found that AOB existence, activity and viability were positively correlated to ammonia concentration which increased remarkably in the winter season then started to decrease. It was observed that when raw water is highly polluted during the winter season and low demand period, ammonia concentration is remarkably elevated in raw water and as a result, microbiological, physical and chemical parameters of the water are markedly influenced. Similar results and correlations are discussed by Hossain *et al.* (2007). Increased heterotrophic bacteria are always found in association with nitrifying bacteria when nitrification processes occur (Wolfe *et al.* 1990). Nitrifiers can increase heterotrophic growth by producing soluble organic carbon (Rittmann *et al.* 1994). Heterotrophs can be beneficial to nitrification by producing stimulating organics for nitrifiers (Hockenbury *et al.* 1977) and protecting nitrifiers from detachment (Rittmann *et al.* 1994). Many heterotrophic bacteria have also been found to contribute to nitrification, although with a slower rate and different mechanisms than autotrophic nitrifiers (Watson *et al.* 1989).

In the identification of bacterial isolates responsible for bioremediation, bacterial strains isolated from AOB broth medium and purified on TSA medium were identified as *Bacillus amyloliquefaciens* using the BIOLOG GEN III Microplate system. In addition to lithotrophic nitrification, various heterotrophic bacteria, fungi, and algae are capable of oxidizing ammonia to nitrate in the presence of O_2 (Fiencke *et al.* 2005). However, in contrast to lithotrophic nitrification, heterotrophic nitrification is a slow process and is not coupled to energy generation (Werner & Newton 2005). *Bacillus* species are important candidates for developing commercial biological agents for nitrogen removal and water quality enhancement (Hong *et al.* 2005). Xie *et al.* (2013) mentioned the efficiency of *Bacillus* species in ammonia removal, and explained that ammonia removal efficiency of a single Bacillus strain has been reported to reach 90%. Xie *et al.* (2013) indicated that *B. amyloliquefaciens* strain, isolated from the activated sludge, was also a very efficient ammonia-N and nitrite-N cleaner.

Water quality index

The WQI is a 100-point scale that was used to summarize results from different physicochemical measurements using a computer program created by the National Sanitation Foundation, USA (Roy *et al.* 2017). The used parameters are: pH, temperature, PO_4, NO_3, turbidity and fecal coliform count. This index transforms a huge amount of data to a single number, which is then used to rank water into one of five descriptive categories of water qualities ranging from very bad conditions (0–25), to excellent conditions (90–100). It was observed that all sites during the whole period of study recorded few points, ranging from 34.36 to 43.52 indicating poor conditions of raw water resources according to the WQI scale. The lowest values were recorded during the winter season indicating the effect of the low demand period, decreasing of water level and increasing of pollutants during this period resulting in deteriorated water situation. WQI showed high significant negative relationships with most physicochemical and bacteriological parameters: with ammonia ($r = -0.829$), with nitrite ($r = -0.837$), with nitrate ($r = -0.877$), with turbidity ($r = -0.890$), with chloride ($r = -0.828$), with total bacterial count ($r = -0.873$), with AOB ($r = -0.778$) and with total algal count ($r = -0.858$). Similar findings and correlations were given by Ali *et al.* (2014).

CONCLUSION

In this study it was concluded that most physicochemical characteristics and microbiological parameters of raw water resources in Kafr El-Sheikh Governorate were affected and changed dramatically mainly during the winter season, reflecting the effect of the low demand period of the Nile River and the increase of pollutants during this period. The major sources of pollution of raw water resources in Kafr El-Sheikh Governorate are municipal, industrial wastewater and agricultural drains. This study revealed that all raw water resources recorded few points on WQI scale, indicating poor conditions mainly during the winter season, indicating the effect of the low demand period, decreasing of the water level and increasing of pollutants during this period, resulting in a deteriorated water situation. This study monitored the seasonal variations of ammonia oxidizing bacterial communities in raw water resources and proved that AOB activity increased in the winter season due to elevated concentrations of ammonia in the water during that period. This study discussed nitrification as a biological process for ammonia removal, proposing an infield solution of ammonia increase in water. *Bacillus amyloliquefaciens* was isolated and identified for its capability of bioremediation of ammonia in raw water. This study provided predictive statistics which proved that AOB and most physicochemical and microbiological parameters showed strong positive and high significant correlations between each other. This study provided the following recommendations which could be useful for the application of the ammonia bioremediation process in the future, especially for water purification applications: (1) sanitary wastewater should be treated properly before discharging to drains; (2) agricultural drainage water should be used for irrigation only away from raw water resources intended to be used as drinking water resources; (3) laws governing industrial wastewater discharging on the Nile River should be applied to avoid problems which may arise from the irresponsible discharging of polluted industrial wastewater; and (4) drinking water purification plants which are located on the Nile or on one of the main canals must take precautions during winter season, especially during the low demand period of the Nile River due to low water levels and increasing of pollutants during this period. Further investigations are recommended for the development of the nitrification process, making it possible to be applied in raw water resources, to find an effective and efficient solution for ammonia problems in raw water resources.

REFERENCES

Abd El-Hady, H. H. & Hussian, A.-E. M. 2012 Regional and seasonal variation of phytoplankton assemblages and its biochemical analysis in Ismailia Canal, River Nile, Egypt. *Journal of Applied Sciences Research* **8** (7), 3433–3447.

Abdel-Satar, A. M. 2005 Water quality assessment of river Nile from Idfo to Cairo. *Egyptian Journal of Aquatic Research* **31** (2), 200–223.

Abdo, M. 2004 Seasonal variations of some heavy metals in macrophytes and water of Damietta branch, River Nile, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries* **8**, 195–211.

Abdo, M. 2010 Environmental and water quality evaluation of Damietta branch, River Nile, Egypt. *African Journal of Biological Sciences* **6** (2), 143–158.

Ahmed, N. 2007 *Effect of River Nile Pollution on Clarias gariepinus Located Between El-Kanater El-Khayria and Helwan*. MSc Thesis, Faculty of Agriculture, Zagazig University.

Ahmed, S., Nasr, A. M., El-Sayed, E.-D. A., Ismaiel, T. S. & Mohamed, A. N. 2011 Haematological and histopathological studies on Clarias gariepinus in relation to water quality along Rossetta branch, River Nile, Egypt. *The Egyptian Journal of Experimental Biology (Zoology)* **7** (2), 223–233.

Ali, E., Dessouki, S., Soliman, A. & El Shenawy, A. 2014 Characterization of chemical water quality in the Nile River, Egypt. *International Journal of Pure and Applied Biosciences* **2** (3), 35–53.

Angers, J. 2002 *What Happens When Ammonia Occurs Naturally in a Chlorinated Well.* Opflow. American Water Works Association (AWWA), New York.

Arrebola, E., Jacobs, R. & Korsten, L. 2010 Iturin A is the principal inhibitor in the biocontrol activity of *Bacillus amyloliquefaciens* PPCB004 against postharvest fungal pathogens. *Journal of Applied Microbiology* **108** (2), 386–395.

Arti, S. D. D. 2011 Assessment and treatment of municipal wastewater of Indore city of India. *Archives of Applied Science Research* **3** (1), 450–461.

Atlas, R. M. 1988 *Experimental Microbiology: Fundamentals and Applications*, 2nd edn. Benjamin-Cummings Publishing Company, San Francisco, United States.

Baskaran, K., Scott, P. & Connor, M. 1992 Biofilms as an aid to nitrogen removal in sewage treatment lagoons. *Water Science and Technology* **26** (7–8), 1707–1716.

Bayomy, M. & Mahmoud, S. 2007 Some hematological and histological studies on Clarias gariepinus fish living in different sites of the River Nile in relation to water quality criteria. *Journal-Egyptian German Society of Zoology* **54** (c), 33–47.

Bitton, G. 2005 *Wastewater Microbiology.* John Wiley & Sons, Hoboken, New Jersey, USA.

Blasiola, G. C. & Vriends, M. M. 1991 *The New Saltwater Aquarium Handbook*. Barron, New York, pp. 84–88.

Braam, F. & Klapwijk, A. 1981 Effect of copper on nitrification in activated sludge. *Water Research* **15** (9), 1093–1098.

Cairncross, S. & Feachem, R. 1993 *Environmental Health Engineering in the Tropics: An Introductory Text,* No. Ed. 2. John Wiley & Sons, Inc., Hoboken, New Jersey, USA.

Canadian Council of Resource and Environment Ministers (CCREM) 1987 *Task Force on Water Quality Guidelines of the Canadian Council of Resource and Environment Ministers Canadian Water Quality Guidelines*. Ottawa, Ontario.

Chen, S. & Hu, Y. 2011 Use of Bacillus subtilis in purification of slightly-polluted water. *Acta Scienctiae Circumstantiae* **31** (8), 1594–1601.

Chen, S., Ling, J. & Blancheton, J.-P. 2006 Nitrification kinetics of biofilm as affected by water quality factors. *Aquacultural Engineering* **34** (3), 179–197.

Eaton, A., Clesceri, L. S., Rice, E. W., Greenberg, A. E. & Franson, M. 2005 *APHA: Standard Methods for the Examination of Water and Wastewater*. Centennial Edition. APHA, AWWA, WEF, Washington, DC.

El-Baz, S. M. 2014 Benthic foraminifera as bioindicators of heavy metal pollution in Lake Burullus, Egypt. *Arabian Journal of Geosciences* **8** (8), 1–19.

Elewa, H. H. 2010 Potentialities of water resources pollution of the Nile River Delta, Egypt. *The Open Hydrology Journal* **4** (1), 1–13.

Elewa, A. & Azazy, M. 1986 Comparative studies on microbiological and chemical characteristics of the High Dam Lake within the last ten years. *Bulletin of the National Institute of Oceanography and Fisheries* **12**, 315–322.

Elghobashy, H. A., Zaghloul, K. H. & Metwally, M. 2001 Effect of some water pollutants on the Nile tilapia Oreochromis niloticus collected from the River Nile and some Egyptian Lakes. *Egyptian Journal of Aquatic Biology and Fisheries* **5** (4), 251–279.

El Shakour, E. H. A. & Mostafa, A. 2012 Water quality assessment of river Nile at Rosetta branch: impact of drains discharge. *Middle-East Journal of Scientific Research* **12** (4), 413–423.

Fiencke, C., Spieck, C. & Bock, E. 2005 *Nitrifying Bacteria. Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment*. Springer, Dordrecht, The Netherlands, pp. 255–276.

Friedl, G., Teodoru, C. & Wehrli, B. 2004 Is the Iron Gate I reservoir on the Danube River a sink for dissolved silica? *Biogeochemistry* **68** (1), 21–32.

Furumai, H. & Rittmann, B. 1994 Evaluation of multiple-species biofilm and floc processes using a simplified aggregate model. *Water Science and Technology* **29** (10–11), 439–446.

Gad, N. S. 2005 Impact of environmental pollution in the southern region of lake Manzalah Egypt on some biochemical parameters of Tilapia zillii. *Journal-Egyptian German Society of Zoology* **48** (A), 279.

Goher, M. E. M. 1998 *Factors Affecting on the Precipitation and Dissolution of Some Chemical Elements in River Nile at Damietta Branch*, Doctoral Dissertation, MSc Thesis, Faculty of Science, Menofia University, Egypt.

Harrison, R. M. & De Mora, S. J. 1996 *Introductory Chemistry for the Environmental Sciences*, Vol. 7. Cambridge University Press, Cambridge, UK.

Hii, Y., Soo, C., Chuah, T., Mohd-Azmi, A. & Abol-Munafi, A. 2011 Interactive effect of ammonia and nitrate on the nitrogen uptake by Nannochloropsis sp. *Journal of Sustainability Science and Management* **6** (1), 60–68.

Hockenbury, M. R., Grady, C. & Daigger, G. T. 1977 Factors affecting nitrification. *Journal of the Environmental Engineering Division* **103** (1), 9–19.

Hong, H. A., Duc, L. H. & Cutting, S. M. 2005 The use of bacterial spore formers as probiotics. *FEMS Microbiology Reviews* **29** (4), 813–835.

Hossain, M. A., Fakhruddin, A. & Khan, S. I. 2007 Impact of raw water ammonia on the surface water treatment processes and its removal by nitrification. *Bangladesh Journal of Microbiology* **24** (2), 85–89.

ICARDA 2011 *Water and Agriculture in Egypt, Technical Paper Based on the Egypt-Australia-International Center for Agricultural Research in the dry Areas Workshop on On-Farm Water-use Efficiency*. International Center for Agricultural Research in the Dry Areas, Cairo, Egypt.

Jamil, T. S., Dijkstra, I. & Sayed, S. 2013 Usage of permeate water for treated domestic wastewater by direct capillary nanofiltration membrane in agriculture reuse. *Desalination and Water Treatment* **51** (13–15), 2584–2591.

Janda, V. & Rudovsky, J. 1994 Removal of ammonia from drinking water by biological nitrification. *Aqua-Journal of Water Supply: Research and Technology* **43** (3), 120–125.

Junhua, W. 2006 Antifungal characterization of *Bacillus amyloliquefaciens* Q-12. *Food and Fermentation Industries* **32** (6), 47.

Kahlown, M., Tahir, M., Rasheed, H. & Bhatti, K. 2006 *Water Quality Status, National Water Quality Monitoring Programme*. Fourth Technical Report. Pakistan Council of Research in Water Resources.

KFSEAA 2012 The environmental characteristics of Kafr El-Sheikh Governorate. *Environmental Affairs Administration, Kafr El-Sheikh Governorate* **1**, 56–59.

Khallaf, E., Galal, M. & Authman, M. 1998 Assessment of heavy metals pollution and their effects on Oreochromis niloticus in aquatic drainage water. *Journal of the Egyptian German Society of Zoology* **26** (B), 39–74.

Lalloo, R., Ramchuran, S., Ramduth, D., Görgens, J. & Gardiner, N. 2007 Isolation and selection of Bacillus spp. as potential biological agents for enhancement of water quality in culture of ornamental fish. *Journal of Applied Microbiology* **103** (5), 1471–1479.

Li, M., Feng, C., Zhang, Z., Zhao, R., Lei, X., Chen, R. & Sugiura, N. 2009 Application of an electrochemical-ion exchange reactor for ammonia removal. *Electrochimica Acta* **55** (1), 159–164.

Lin, Y., Kong, H., He, Y., Liu, B., Inamori, Y. & Yan, L. 2007 Isolation and characterization of a new heterotrophic nitrifying Bacillus sp. *strain. Biomedical and Environmental Sciences* **20** (6), 450.

Mandowara, A. & Bhattacharya, P. K. 2011 Simulation studies of ammonia removal from water in a membrane contactor under liquid–liquid extraction mode. *Journal of Environmental Management* **92** (1), 121–130.

Odell, L. H., Kirmeyer, G. J., Wilczak, A. & Jacangelo, J. G. 1996 Controlling nitrification in chloraminated systems. *American Water Works Association Journal* **88** (7), 86.

Powell, R. 2004 Implementation of chloramination by a Florida utility: The good, the bad, and the ugly. In: *Proceedings of Water Quality Technology Conference*, San Antonio, Texas.

Rabeh, S. 1999 Monitoring of faecal pollution in Wadi El-Raiyan Lakes, Fayoum, Egypt. In: *The Second Scientific Conference on the Role of Science in the Development of Egyptian Society and Environment*, pp. 23–24.

Ramadan, A. E. K. 2007 Removal of ammonia and phenol from industrial wastewater. In: *11th International Water Technology Conference (IWTC) Sharm El-Sheikh, Egypt*.

Richardson, D. J. & Watmough, N. J. 1999 Inorganic nitrogen metabolism in bacteria. *Current Opinion in Chemical Biology* **3** (2), 207–219.

Rittmann, B. E. & Manem, J. A. 1992 Development and experimental evaluation of a steady-state, multispecies biofilm model. *Biotechnology and Bioengineering* **39** (9), 914–922.

Rittmann, B. E., Regan, J. M. & Stahl, D. A. 1994 Nitrification as a source of soluble organic substrate in biological treatment. *Water Science and Technology* **30** (6), 1–8.

Robertson, L. A., Dalsgaard, T., Revsbech, N. P. & Kuenen, J. G. 1995 Confirmation of 'aerobic denitrification' in batch cultures, using gas chromatography and 15N mass spectrometry. *FEMS Microbiology Ecology* **18** (2), 113–120.

Roy, R., Majumder, M. & Barman, R. N. 2017 Assessment of water quality by RSM and ANP – a case study in Tripura, India. *Asian Journal of Water, Environment and Pollution* **14** (1), 51–58.

Rui, M., LianSheng, H., BeiDou, X., Xiang, H. & YueYan, L. 2009 Experimental study on purifying aquaculture wastewater between Bacillus and nitrifying bacteria. *Environmental Science & Technology (China)* **32** (11), 28–31.

Saleh, A. 1976 *A Study on the Phenomenon of Taste and Odour in Lake Nasser*. Report to the Academy of Scientific Research and Technology (ASRT) Cairo, Egypt.

Siliem, T. 1984 *Chemical Studies on Pollution in the Damietta Nile Branch Between the Faraskour Dam and Ras El-Bar Outlet*. PhD Thesis, submitted to Fac. Sci. Alex. Univ. Egypt.

Stutzer, H. & Hartleb, R. 1894 About nitrification. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Zweite Naturwissenschaftliche Abteilung: Allgemeine, Landwirtschaftliche und Technische Mikrobiologie*. Reihe A, **22**, 701.

Tayel, S. 2003 *Histopathological, Biochemical and Hematological Studies on Tilapia zillii and Clarifies gariepinus in Relation to Water Quality Criteria at Different Localities in Delta Barrage*. PhD Thesis, Fac. Sci., Benha Branch, Zagazig Univ.

Tayel, S. 2007 Histological and biochemical seasonal changes of Oreochromis niloticus muscles in relation to water quality at Zefta and El-Mansoura Cities, Damietta branch River Nile, Egypt. *Journal of Egyptian Academic Society for Environmental Development* **8** (2), 81–92.

Toufeek, M. A. & Korium, M. A. 2009 Physicochemical characteristics of water quality in Lake Naser water. *Global Journal of Environmental Research* **3** (3), 141–148.

US Public Health Service 1992 *Toxicological Profile for Aluminum and Compounds*. Clement International Corp. (Contact No. 205-88-0608).

Watson, S., Bock, E., Harms, H., Koops, H. & Hooper, A. 1989 Nitrifying bacteria. *Bergey's Manual of Systematic Bacteriology* **3**, 1808–1834.

Wei, X., Luo, M., Xu, L., Zhang, Y., Lin, X., Kong, P. & Liu, H. 2011 Production of fibrinolytic enzyme from *Bacillus amyloliquefaciens* by fermentation of chickpeas, with the evaluation of the anticoagulant and antioxidant properties of chickpeas. *Journal of Agricultural and Food Chemistry* **59** (8), 3957–3963.

Werner, D. & Newton, W. E. (eds) 2005 *Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment*, Vol. 4. Springer Science & Business Media B.V., Dordrecht, The Netherlands.

WHO 2006 *Guidelines for the Safe Use of Wastewater, Excreta and Greywater: Policy and Regulatory Aspects*. World Health Organization, Geneva.

Wilczak, A., Jacangelo, J. G., Marcinko, J. P. & Odell, L. H. 1996 Occurrence of nitrification in chloraminated distribution systems. *American Water Works Association Journal* **88** (7), 74–85.

Wolfe, R. L., Lieu, N. I., Izaguirre, G. & Means, E. G. 1990 Ammonia-oxidizing bacteria in a chloraminated distribution system: seasonal occurrence, distribution and disinfection resistance. *Applied and Environmental Microbiology* **56** (2), 451–462.

Xie, F., Zhu, T., Zhang, F., Zhou, K., Zhao, Y. & Li, Z. 2013 Using *Bacillus amyloliquefaciens* for remediation of aquaculture water. *SpringerPlus* **2** (1), 119.

Yang, X.-P., Wang, S.-M., Zhang, D.-W. & Zhou, L.-X. 2011 Isolation and nitrogen removal characteristics of an aerobic heterotrophic nitrifying–denitrifying bacterium, Bacillus subtilis A1. *Bioresource Technology* **102** (2), 854–862.

Yousry, M., El-Sherbini, A., Heikal, M. & Salem, T. 2009 Suitability of water quality status of Rosetta Branch for West Delta water conservation and irrigation rehabilitation project. *Water Science* **46**, 47–60.

Zaki, M. S., Authman, M. M., Hammam, A. M. M. & Shalaby, S. I. 2014 Aquatic environmental pollution in the Egyptian countryside and its effect on fish production (review). *Life Science Journal* **11** (9), 1024–1029.

First received 12 March 2017; accepted in revised form 5 July 2017. Available online 23 August 2017