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Seasonal Variation of Ammonia Oxidizing Bacterial Community of Raw Water Resources and Physicochemical Relationships

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Abstract: Raw water resources in Kafr El-Sheikh Governorate which are intended to be used as drinking water resources, are affected by many contaminants due to municipal, industrial wastewater and agricultural drains; particularly in winter season, during low demand period of Nile River; water level in the river and main canals is decreased, organic matter is increased which cause decreasing dissolved oxygen, increasing ammonia, nitrite and high bacterial load in water; causing many problems especially in drinking water purification plants located on Nile river or their intakes are located on Rosetta branch and main canals. In this study, physicochemical and microbiological characteristics of raw water samples which were taken seasonally from selected sampling sites of the seven main canals in Kafr El-Sheikh Governorate were tested and studied for monitoring nitrification process occurrence and studying its effect on physicochemical and microbiological characteristics of water. The main results of the study included conductivity and turbidity which both increased during winter, also ammonia, nitrite and nitrate which markedly increased in winter, on the contrary; pH level increased in summer. Heavy metals levels showed that Fe increased in winter and Al increased in spring while microbiological parameters showed that total bacterial count, total coliforms, fecal coliforms and total algal count increased in winter season, also ammonia oxidizing bacteria increased markedly in winter season. This study discussed nitrification as a two-step biological process in which ammonia is oxidized to nitrite via ammonia oxidizing bacteria (AOB) and nitrite is oxidized to nitrate via nitrite oxidizing bacteria (NOB) and proved that this process naturally occurred in raw water resources in Kafr El-Sheikh Governorate affecting water physicochemical characteristics in different ways. Ammonia is oxidized to nitrite in the first step of nitrification by *Nitrosomonas eurapaea* which is isolated from raw water resources and identified through molecular identification. Nitrification in environments which provide unfavorable conditions for autotrophic nitrifying bacteria may result from the activity of heterotrophic microorganisms; *Bacillus amyloliquefaciens* which is isolated and identified to be involved.

Key words: Nitrification · *Nitrosomonas eurapaea* · *Bacillus amyloliquefaciens* · Ammonia Oxidizing Bacteria · Algal Count

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 characters. 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. (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

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development programs [1]. The total amount of wastewater discharged into the main stream of River Nile has been estimated to be 2628 million cubic meters per year, of which the industrial wastewater constitutes 15%. On the other hand, the annual discharge of the river amounts to 55 milliard cubic meters per year, therefore, the high dilution rate of the river amounting to be neglected. Another source of pollution in 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 the potential negative effects of oxygen depletion, increasing poisonous ammonia, increasing the proportion of total dissolved solids, chemical contamination resulting from the hormonal treatment of fish and nutritive materials and remnants of fish output. In addition, using serious kinds of fish food such as blood, meat, fish and poultry remnants, dry sludge and sewage that lead to low water quality, transport these dangerous wastes into potable water then to humans, causing several health problems [2, 3]. Drinking water, according to hygienic rules, should not contain ammonia of organic origin. In case of ammonia of inorganic origin maximum acceptable concentration in drinking water is equal to 0.5 mg/dm^3 according to WHO Guidelines for drinking water and Egyptian standards [4, 5]. Ammonia contamination of water bodies is a widespread environmental problem causes a 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 ammonia [6]. 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. But biological remediation method needs a continuous monitoring, such as pH control, addition of a carbon source, temperature maintenance and also requires the removal of byproducts such as nitrite [7]. 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). Chemolithotrophic nitrifying bacteria obtain energy from these oxidation processes and cellular carbon from carbon dioxide via the Calvin cycle. Nitrification has an important role in the global nitrogen cycle and nitrifiers are widely distributed in terrestrial and aquatic habitats [8]. AOB and NOB are collectively known as nitrifying bacteria or nitrifiers [9]. Biological nitrification and denitrification are slow processes and require large treatment vessels. Therefore, most of the available treatment processes are not particularly effective. Some of the advances made in membrane processes may solve this problem but these technologies were costly when it used with huge amount of drinking water industry [10]. 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 [11]. 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 [12]. Furthermore, there is no selective enrichment or isolation method for heterotrophic nitrifying microorganisms [13]. With the advancement of research, more and more heterotrophic nitrification strains were isolated and characterized such as Bacillus sp. strains which exhibited efficient heterotrophic nitrifying ability with ammonia and Nitrite [14].

The aim of this work was to study the seasonal and spatial variations of physicochemical and microbiological characteristics of raw water resources in Kafr El-Sheikh Governorate regarding Nitrification process and the bio-destruction ability of ammonia oxidizing bacteria to oxidize ammonia in fresh water.

MATERIALS 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 study plan; sampling sites involved: 1- Inlet of Abd El-Rahman water purification plant on Al-Bahr Al-Seidi canal, Sidi-salem city, Kafr El-Sheikh, Egypt, 2- Inlet of Mahlat Abo-Ali water purification plant on Al-Qudaba canal, Dessouk city, Kafr El-Sheikh, Egypt, 3- Inlet of Kafr El-Sheikh water purification plant No. (I) on Met Yazid canal, Kafr El-Sheikh city, Kafr El-Sheikh, Egypt, 4- Inlet of Abo-Araffa water purification plant on Bahr Tirra canal, Al-Hamoul city, Kafr El-Sheikh, Egypt, 5- Inlet of Met Al-Dieba water purification plant on Rewina canal, Qalien city, Kafr El-Sheikh, Egypt, 6- Inlet of Qalien water purification plant on Bahr Nashart canal, Qalien city, Kafr El-Sheikh, Egypt and 7- Inlet of Menyet Al-Morshed water purification plant on Al-Rashidia canal, Muttobies city, Kafr El-Sheikh, Egypt. Samples were collected manually at about 30 cm under water surface in appropriate, clean, free of analyst of interest and free of contaminants containers. Suitable amounts of raw water were collected according to each test requirements. Each lab conditions and instructions for sample collection, storage and preservation were exactly followed and performed according to standard methods of water and wastewater. The temperature of the sample was maintained at ambient conditions prior to and during testing. The collected water was stored and refrigerated (At 4°C) for subsequent testing. The temperature of the sample was adjusted to room temperature before initiating any test. On the other hand, turbidity and pH were measured in field [15].

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 were 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 Argentometeric 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 duplicates and determined by the procedures recommended in the standard methods for the examination of water and wastewater [15].

Microbiological Analysis of Water Samples: Total bacterial count (The heterotrophic plate count method), total coliforms, fecal coliforms and fecal streptococci (Membrane filter technique) were measured [15].

Ammonia Oxidizing Bacteria (AOB): Detection of nitrifying bacteria (9245 the multiple-tube method) and identification of involved bacteria (using conventional PCR and BIOLOG GEN III Microplate system) were carried out [15].

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 summer season followed by autumn season and began to decrease during winter and spring seasons. The highest value of pH was recorded in Al-Qudaba canal during summer season (August 2014) while the lowest pH value was recorded in Rewina canal during winter season (December 2013) (Fig. 1). The maximum conductivity values were recorded during winter season and began to decrease through spring season; the lowest conductivity values were recorded during summer season and began to increase again through autumn season. The highest value of Conductivity was recorded in Al-Rashidia canal during winter season (December 2013) while the lowest Conductivity value was recorded in Rewina canal during summer season (June 2014) (Fig. 1). The maximum turbidity values were recorded during winter and spring seasons; the lowest turbidity values were recorded during summer season and began to increase again through autumn season. The highest value of Turbidity was recorded in Al-Rashidia canal during winter season (December 2013) while the lowest Turbidity value was recorded in Bahr Nashart canal during summer season (June 2014) (Fig. 1). The maximum values of chlorides were recorded during winter season and began to decrease through 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 winter season (December 2013) while the lowest chlorides value was recorded in Bahr Tirra canal and Al-Qudaba canal during 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 winter season (December 2013) while the lowest ammonia value was recorded in Met Yazid canal during summer season (June 2014) (Fig. 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 winter season (December 2013) while the lowest nitrite value was recorded in Bahr Nashart canal during summer season (June 2014) (Fig. 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 winter season (December 2013) while the lowest nitrate value was recorded in Met Yazid canal during summer season (June 2014) (Fig. 2).

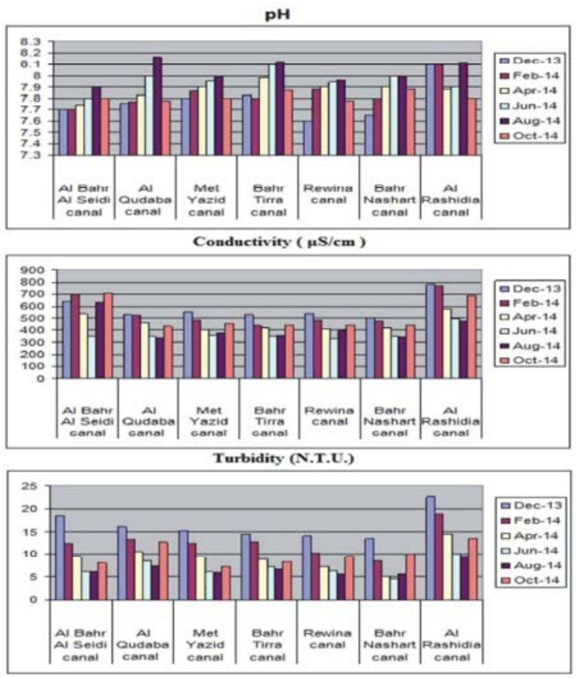

Fig. 1: Represents pH, Conductivity and Turbidity values in different seasons in seven main canals of Kafr El-Sheikh Governorate

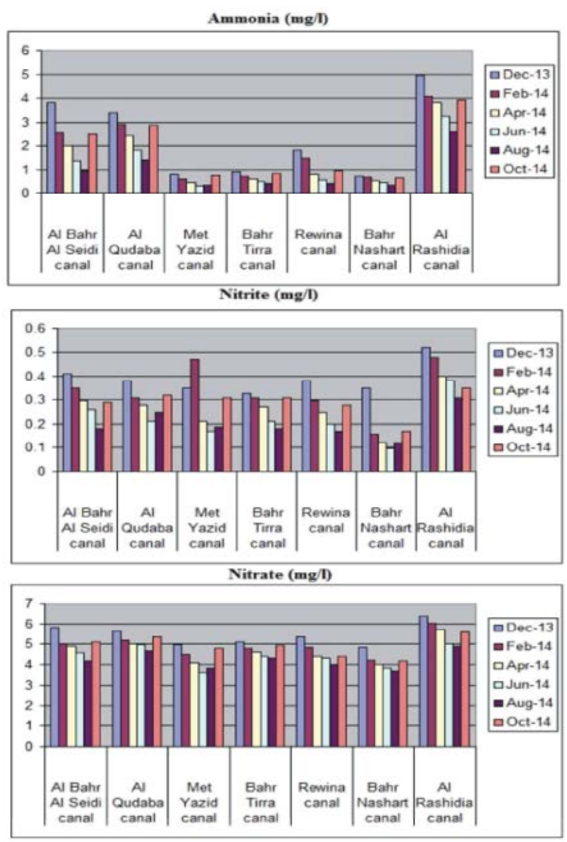

Fig. 2: Represents Ammonia, Nitrite and Nitrate values in different seasons in seven main canals of Kafr El-Sheikh Governorate

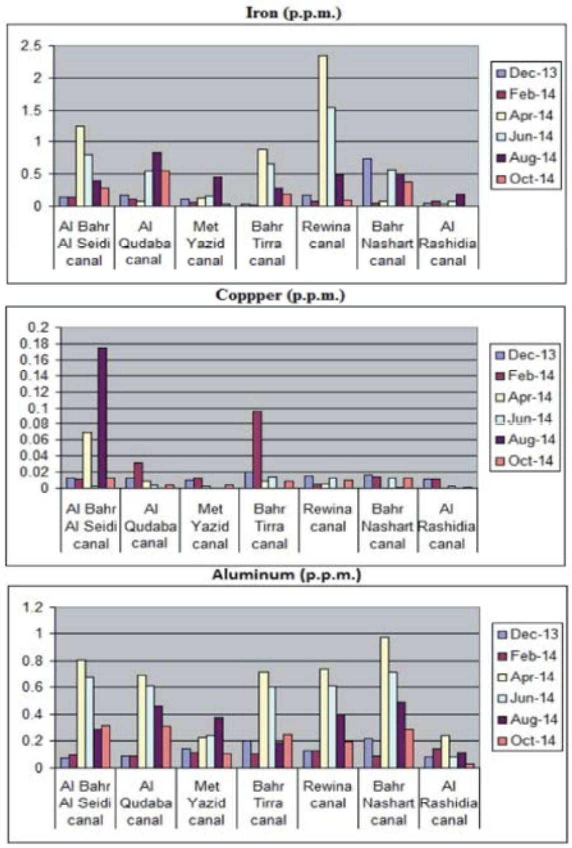

Fig. 3: Represents Iron, Copper and Aluminum concentrations in different seasons in seven main canals of Kafr El-Sheikh Governorate

Concerning heavy metals, it was observed that Iron concentration was increased in 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 summer season in June and August 2014 then eventually tend to decrease again during autumn season in October 2014. The highest concentration of Iron was recorded in Rewina canal during spring season (April 2014) while the lowest concentration was recorded in Al-Rashidia canal during autumn season (October 2014) (Fig. 3). Copper concentration showed a similar pattern in most locations which are all could be neglected as concentration ranging from non-detected to very small values except for Al-Bahr Al-Seidi canal during spring and summer seasons and Bahr Tirra canal during winter season (Fig. 3). Aluminum concentration was elevated in spring season (April 2014) in most locations then tended to decrease in summer and autumn seasons; while the lowest concentrations were recorded during winter season (Fig. 3).

Microbiological analysis of raw water samples indicated that, total bacterial count showed a slight decreasing from winter season to autumn season through spring and summer. The highest total bacterial count was recorded in Al-Rashidia canal during winter season (December 2013) while the lowest total bacterial count was recorded in Bahr Tirra canal during summer season (August 2014) (Fig. 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 winter season (December 2013) while the lowest total coliform count was recorded in Met Yazid canal during summer season (August 2014) (Fig. 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 winter season (December 2013) while the lowest fecal coliform count was recorded in Met Yazid canal, Rewina

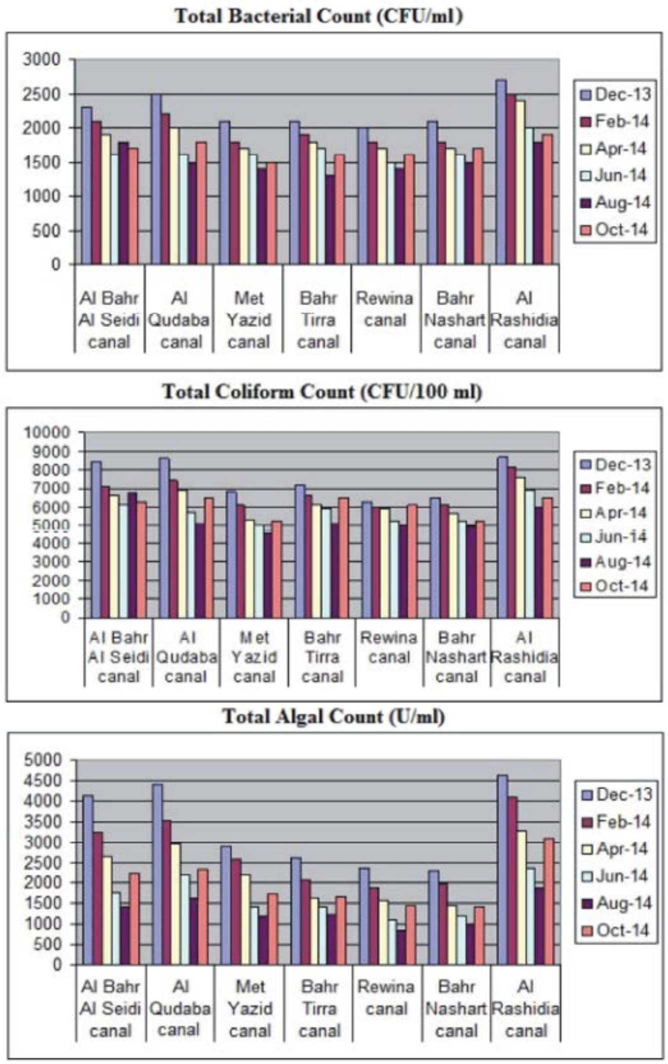

Fig. 4: Represents Total Bacterial Count, Total Coliform Count and Total Algal Count in different seasons in seven main canals of Kafr El-Sheikh Governorate

canal and Bahr Nashart canal during 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 summer season. The highest total algal count was recorded in Al-Rashidia canal during winter season (December 2013) while the lowest total algal count was recorded in Rewina canal during summer season (August 2014) (Fig. 4).

Ammonia oxidizing bacteria estimation revealed a progressive increase in count during winter season particularly in December 2013, count tend to decrease during spring season and reached the lowest ratio during summer and autumn. Al-Rashidia canal showed a huge and massive growing of AOB during winter season (December 2013 and February 2014 respectively). The highest count of AOB was recorded in Al-Rashidia canal during winter season (December 2013) while the lowest AOB count was recorded in Rewina canal and Al-Qudaba canal as well during summer season (August 2014) and autumn season (October 2014) respectively (Fig. 5). *Nitrosomonas europaea*-like AOB were detected in samples by molecular identification using primer pair NSMR52f and NSMR53r for identification of bacteria responsible for Ammonia oxidation in Freshwater Aquaria (Fig. 5). Another two bacterial strains isolated from AOB broth medium and purified on TSA medium were identified as *Bacillus amyloliquefaciens* using BIOLOG GEN III Microplate system (Fig. 5).

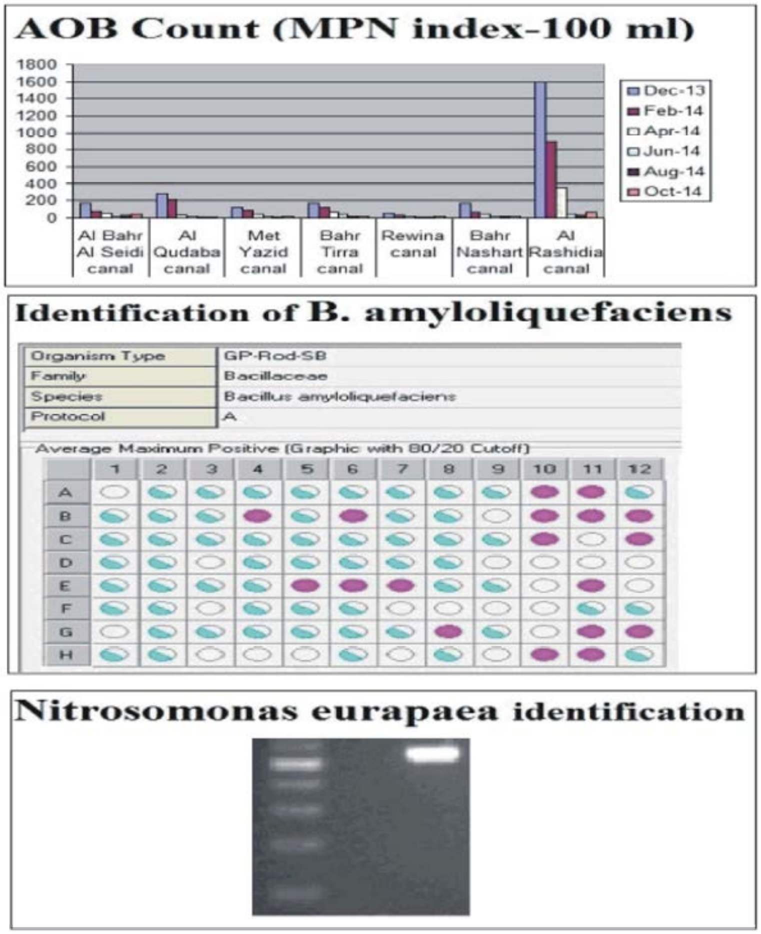

Fig. 5: Represents AOB count in different seasons in seven main canals of Kafr El-Sheikh Governorate and identification of responsible bacteria

DISCUSSION

The western borders of Kafr El-Sheikh Governorate are located along the Nile River (Rosetta Branch) for 85 Km. so Rosetta branch is considered the most important water resources in Kafr El-Sheikh Governorate for drinking water purification, agricultural land irrigation and fisheries, but in winter season and during low demands period (January of every year), water level in the river is decreased and regarding that agricultural drains in Al-Munofiya Governorate which discharge into Rosetta branch (Al-Rahawy drain, Talla drain and Sebbel drain) are heavily polluted with untreated wastewater and sewage, the organic matter increase in the river during this period decreasing dissolved oxygen, increasing ammonia, nitrite, bacterial load and other seriously dangerous problems. Ammonia in raw water can be removed or decomposed by biological nitrification, however biological remediation method needs a continuous monitoring, such as pH control, addition of a carbon source, temperature maintenance and also requires the removal of byproducts such as nitrite [7]. In the first step of nitrification; ammonia is oxidized to nitrite by ammonia-oxidizing bacteria (AOB) and then nitrite is further oxidized to nitrate by nitrite-oxidizing bacteria (NOB), AOB and NOB are collectively known as nitrifying bacteria or nitrifiers [9].

This study is 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 in the alkaline side of the pH scale; values are always higher than 6.5 which are normally expected in raw water due to the presence of carbonates or bicarbonates as reported by Friedl *et al*. [16]. The increase in pH could be related to photosynthesis and growth of aquatic plants, where photosynthesis consumes CO_2 leading to arise in the pH values [17]. The maximum increase in pH values was recorded during summer season as Temperature and pH are positively correlated; this is due to the increase in temperature is usually accompanied by hydrolysis of HCO_3^- and CO_3^{2-} ions, leading to the appearance of hydroxyl (OH^-) ions that increase pH value. Similar relationship was reported by Toufeek and Korium [18]. The optimum pH for Nitrification lies between 7.5 and 8.5 [19]. Electric conductivity (EC) is a measure of the ability of water to carry electric current and it is sensitive to variations in dissolved solids [20], EC values showed a huge seasonal variation and a 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 over especially in winter season where organic matter increases in the river during low demands period. Such results were given by Abd El-Hady and Hussian [21]. Turbidity is the measure of fine suspended matter in water, mostly caused by colloidal particles in addition to suspended organic and inorganic matter [20]. The turbidity degree of stream water is an approximate measure of pollution intensity [22]. Turbidity values among the period of study showed high seasonal variation where the highest turbidity values were recorded in winter season due to low demands period and the increase of pollutants and colloidal particles at the end of the Nile River, After low demands period, the turbidity values decrease because of the flow of water. The lowest turbidity values were recorded in summer season. Turbidity values are negatively correlated with pH [20]. Turbidity values, TDS and EC revealed positively strong correlation to each other, Toufeek and Korium [18] 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 [19]. Throughout this study, ammonia concentrations showed remarkable variations both regionally and seasonally, the highest value was observed at Al-Rashidia canal in winter season and the lowest value was observed at Met Yazid canal in summer season. Ammonia and nitrogen concentrations more than 1 mg/l have been given as indicator of organic pollution such as, sewage discharge, industrial effluents and agriculture-runoff and can be toxic to aquatic species if they are higher than 2.5 mg/l [23-25]. Although it is a nutrient required for life, excess of ammonia can accumulate in the organism and cause alteration in metabolism or increase body pH [26]. On the other hand, the decrease in ammonia concentrations was related to the decrease in biological activities of aquatic organisms and nitrification [27]. 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 occur in waste water treatment plants, water distribution systems and natural water [15]. 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 winter season which may be attributed to the decomposition of organic matter during winter low demand period of Nile River while pollutants are increased due to the very slow flow-rate of water where Nitrosomonas bacteria oxidize ammonia to nitrite by nitrification process [27, 28]. The low values of nitrite might be attributed to the fast conversion of NO_2^- by nitrobacteria to NO_3^- [29, 30]. Nitrate ion is the final oxidation product of nitrogen compounds in the aquatic environment [31]. The low values of nitrate during summer season might be attributed to the uptake of nitrate by natural phytoplankton and its reduction by denitrifying bacteria and biological denitrification [27, 32]. On the other hand, the increase of nitrate levels during 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 [27, 28, 33].

Concerning heavy metals, Increased Iron concentrations may be attributed to inorganic fertilizers used in agriculture practice, this was confirmed by the opinions of Khallaf *et al*. [34] and Arti [35]. 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 [36]. Copper is believed to be a key component of the ammonia monooxygenase (AMO) enzyme, which is essential for ammonia oxidation and nitrifier growth [37]. However, excess copper is known to be toxic to nitrifiers [38]. Highest Aluminum concentrations may be attributed to several reasons depending on its 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 [4, 39]. Aluminum salts are also widely used in water treatment as coagulants to reduce organic matter, colour, turbidity and microorganism levels [40].

Total bacteria count increased in winter season either because more nutrients are carried by the Nile or because of recently drowned vegetation in autumn season, provides bacteria with organic matter, encouraging their reproduction [41]. Such a negative relationship between total bacterial count and transparency in Autumn and winter seasons had previously been reported [41-43] as suspended matter is very important, due to bacterial adherence to particles. The increased counts during winter season also might be attributed to low demand period of Nile River during winter season which decreases water level and increases organic matter existence because of drains polluted by sewage which discharge to Rosetta branch, e.g, El-Rahawy Drain. The lowest total and fecal coliform counts were measured during summer season while the highest counts were recorded in winter season which indicated the high activity of total and fecal coliform bacteria during winter season because of low demand period of River Nile, increase of pollutants, nutrients and favorable growth conditions, this also might be attributed to human sewage pollution associated with high organic loads favoring the bacterial survivability, this findings agreed with Gad [44].

Algae are a general term for small, chlorophyll containing plant. In this study, the lowest total algal count was recorded in summer season while the highest total algal count was recorded in winter season due to high level of nutrients so algae grow and reproduce quickly. If algae grow in high density on the surface, it will block sunlight from reaching plants at greater depths; this will cause the plants to die; and when algae die, the decaying process uses oxygen in the water; 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 [45, 46]. 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, some algae prefer ammonia as its nitrogen source; however nitrate would be utilized in the absence of ammonia, this agreed with Hii *et al.* [47] 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.* [48] 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 ammonia oxidizing bacteria, it was very remarkable that Al-Rashidia canal showed very high activity of ammonia oxidizing bacteria 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 winter season then starts to decrease. It was observed that when raw water is highly polluted during winter season and low demands 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*. [49]. Increased heterotrophic bacteria are always found in association with nitrifying bacteria when nitrification processes occur [50]. Nitrifiers can increase heterotrophic growth by producing soluble organic carbon [51]. Heterotrophs can be beneficial to nitrification by producing stimulating organics for nitrifiers [52] and protecting nitrifiers from detachment [51]. Many heterotrophic bacteria have also been found to contribute to nitrification, although with a slower rate and different mechanisms than autotrophic nitrifiers [8].

Identification of nitrifiers: *Nitrosomonas europaea*-like AOB was detected with primer pair NSMR52f and NSMR53r first used by Burrell *et al*. [53] for identification of bacteria responsible for ammonia oxidation in freshwater aquaria, which proved the existence of *Nitrosomonas europaea*-like AOB in the sample. 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 [54]. In a nitrification process, ammonia is first oxidized into nitrite (NO_2^-) by several genera of autotrophic bacteria; the most important being is Nitrosomonas Sp. [55]. Another two bacterial strains isolated from AOB broth medium and purified on TSA medium were identified as

Bacillus amyloliquefaciens using 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 [56]. However, in contrast to lithotrophic nitrification, heterotrophic nitrification is slow process and is not coupled to energy generation [54]. *Bacillus* species are important candidates for developing commercial biological agents for nitrogen removal and water quality enhancement [57]. Xie *et al.* [58] marked 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.* [58] indicated that *B. amyloliquefaciens* strain, isolated from the activated sludge, was also a very efficient ammonia-N and nitrite-N cleaner.

CONCLUSION

In this study it was concluded that Physicochemical and microbiological characteristics of raw water resources in Kafr El-Sheikh Governorate are affected and changed mainly in winter season; the annual increase of ammonia concentration during low demands period is a continued problem making a vital challenge for water treatment plants to provide a safe and clean drinking water. Nitrification process occurrence in raw water resources helps to get rid of ammonia which is oxidized to nitrite by ammonia oxidizing bacteria; the most important being is *Nitrosomonas eurapaea* which had been isolated and identified in raw water. Various heterotrophic bacteria are capable of oxidizing ammonia to nitrate through heterotrophic nitrification; *Bacillus amyloliquefaciens* is isolated from raw water, identified and proved to be an important Bacillus species for ammonia and nitrite removal.

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