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International Journal of Oceanography & Aquaculture

Monitoring of Nitrification Process Occurrence in Aquatic Systems Linked to Water Quality Index

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Research Article

Volume 2 Issue 1

Received Date: December 19, 2017

Published Date: January 24, 2018

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 during winter season and low demand period of Nile River. In this study; raw water samples which were collected seasonally over one year (2013-2014) from selected sampling sites on the seven main canals in Kafr El-Sheikh Governorate were tested and studied for monitoring nitrification process occurrence and studying its relationship with water quality index of these resources. The main results of the study indicated that the activity of ammonia oxidizing bacteria increased mainly during winter season reflecting the effect of low demand period of Nile River and the increase of pollutants during this period. This study also discussed nitrification as a biological process for ammonia removal proposing an infield solution of ammonia increase in water; monitored seasonal variations of AOB communities in raw water resources and proved that AOB activity increased in winter season due to elevated concentrations of ammonia in water.

Keywords: Water resources; ammonia; AOB and water quality index

Introduction

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 [1]. AOB and NOB are collectively known as nitrifying bacteria or nitrifiers and distinguished by their ability for ammonia degradation [2], however 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 [3].

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 [4].

Since then, numerous reports have demonstrated unequivocally that nitrite/nitrate production is not restricted to autotrophic ammonia oxidizers (e.g. Nitrosomonas Sp.) or nitrite oxidizers (e.g. Nitrobacter Sp.) but is a widespread phenomenon among different genera of fungi and heterotrophic bacteria [5]. Furthermore, there is no selective enrichment or isolation method for heterotrophic nitrifying microorganisms [6].

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 (CO2). No chemolithotroph is known that can carry out the complete oxidation of ammonia to nitrate [7]. In a nitrification process, ammonia is first oxidized into nitrite (NO2-) by several genera of autotrophic bacteria; the most important being is Nitrosomonas SP [8].

Originally, the lithoautotrophic nitrifying bacteria were collected together within one family, named Nitrobacteracea, where ammonia oxidizers are characterized by the prefix Nitroso- and the nitrite oxidizers are characterized by the prefix Nitro- [1]. However, phylogenetic investigations have made it evident that ammonia and nitrite oxidizers are not closely related [9-11]. Several genera are recognized on the basis of both morphology and gene-sequence analyses. So far, five genera of ammonia oxidizers, including Nitrosomonas, Nitrosospira, Nitrosovibrio, Nitrosolobus, and Nitrosococcus, and four genera of nitrite oxidizers, including Nitrobacter, Nitrospira, Nitrospina, and Nitrococcus, have been described. According to comparative 16S rDNA sequence analyses, all recognized ammonia oxidizers are members of two lineages within the β- and γ-subclasses of the Proteobacteria [12]. The marine species of the genus Nitrosococcus cluster together in the γ-subclass of Proteobacteria [10]. The four other genera of ammonia oxidizers, Nitrosomonas (including Nitrosococcus mobilis, which belongs phylogenetically to Nitrosomonas), Nitrosospira, Nitrosovibrio, and Nitrosolobus, form a monophyletic assemblage within the β-subclass of Proteobacteria [9].

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 (CO2) 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 0.6-4.0 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 heterotrophy to accomplish. Most of their energy production (80%) is devoted to fixing CO2 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 [13].

In addition to lithotrophic nitrification, various heterotrophic bacteria, fungi, and algae are capable of oxidizing ammonia to nitrate in the presence of O_2 [14]. 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 ecological significant [7]. 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

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a fungus [4]. 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 [5].

Ammonia in surface waters can be of organic origin, the product of decomposition of plant and animal matter, or of inorganic origin, formed due to chemical or biochemical reduction of nitrate and nitrite. Ammonia is an indicator of pollution originating from soil (the excessive use of ammonia rich fertilizers), atmosphere and sewage [15]. Ammonia NH3 is very unstable compound and easily undergoes nitrification. Ammonia is present in all natural waters. Ground water and clean surface waters contain about 0.1 mg/dm3; marine water contains few mg/dm3 and the concentration increases with depth. The highest concentration of ammonia is observed in waters near crude oil beds, and can be as high as 100mg/dm3 [16]. Ammonia is toxic for aquatic organisms. 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 [15].

Materials and Methods

Sampling

Raw water samples were collected seasonally in winter (December 2013 and February 2014), spring (April 2014), summer (June and August 2014) and autumn (October 2014) for one year (December 2013 to August 2014) from selected sampling sites located on seven main canals of Kafr El-Sheikh Governorate used as raw water resources by water purification plants according to study plan of sampling. 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. Storage and preservation were exactly followed and performed according to standard methods of water and wastewater [17]. Sampling sites were chosen to cover raw water resources in Kafr El-Sheikh Governorate represented by seven main canals fed by Nile River. Table (1) showed the most important main canals in the Kafr EL-Sheikh governorate, their length in kilometers and their source of feed.

Canal	Length (Km)	Source of feed
Al Bahr Al Seidi canal	22	Nile River (Rosetta branch)
Al Qudaba canal	41.2	Nile River – AlRayah Almenofy-Al Baguriah Canal
Met Yazid canal	28.3	Nile River-AlRayah Almenofy- Bahr Shebein canal
Bahr Tirra canal	66.2	Nile River- Bahr Shebein canal
Rewina canal	20.1	Nile River-AlRayah Almenofy- Al Kassed canal
Bahr Nashart canal	7	Nile River-AlRayah Almenofy- Al Khaleg Al Abassy canal
Al Rashidia canal	29.45	Nile River (Rosetta branch)

Table 1: Main canals in Kafr El-Sheikh Governorate

Ammonia

Nitrogen forms as free ammonia (mg/l) by (Nesslerization method) according to standard methods

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.

Water quality index

WQI is a 100 point scale that was used to summarize results from different physico-chemical measurements using computer program created by the National Sanitation Foundation, USA Ali, et al. [18].

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 showed by Ali, et al. [18].

Results and Discussion

Ammonia

During the whole period of study, it was noticed that 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

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during summer season (June 2014). It was also noticed that Al Rashidia canal recorded the highest value of ammonia during all seasons with a remarkable increase during winter and autumn seasons which indicated the effect of low demand period of Nile River on the raw water. Also it was noticed that the ratio of ammonia concentration differences between these canals through seasons didn't record significant disturbances which gave an idea about the seasonal circumstances such as low demand period which resulted in increasing ammonia concentration. Met Yazid canal and Bahr Nashart canal recorded the lowest values of ammonia which reflected the fact that they are the lowest polluted canals; as shown in Tables 2 and 3 and Figure 1.

Ammonia is a form of the nitrogenous compounds present in nature and is essential for the growth and reproduction of plants and animals. The ammonia ion is either released from proteinaceous organic matter and urea or is synthesized by industrial processes [19]. Ammonia serves as a substrate for Nitrosomonas Sp. and is considered one of the most important factors affecting nitrification according to Bitton [20]. Throughout this study, NH3 concentrations showed significant variations both regionally and seasonally. The highest values of ammonia were recorded during winter season while the lowest values were recorded during 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 [21-23]. This finding agrees with Abdel-Satar [24] and Ahmed, et al. [25]. Kahlown, et al. [26] stated that ammonia is an indicator of pollution originating from soil (the excessive use of ammonia rich fertilizers), atmosphere and sewage. Also, the increase of ammonia concentration may be attributed to the activity of denitrifying bacteria which are much higher under anaerobic conditions as mentioned by Ghallab [27]. 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 the ammonia concentrations was related to the decrease in biological activities of aquatic organisms and nitrification [25].

SITE Parameters	Month/ season	Al Bahr Al Seidi canal	Al Qudaba canal	Met Yazid canal	Bahr Tirra canal	Rewina canal	Bahr Nashart canal	Al Rashidia canal
NH_3 (mg/l)	13-Dec	3.81	3.41	0.8	0.89	1.8	0.71	4.95
	14-Feb	2.53	2.91	0.6	0.71	1.48	0.68	4.07
	14-Apr	2.01	2.43	0.44	0.6	0.8	0.52	3.82
	14-Jun	1.34	1.82	0.32	0.49	0.57	0.44	3.26
	14-Aug	0.93	1.41	0.33	0.4	0.42	0.34	2.62
	14-Oct	2.48	2.85	0.75	0.82	0.93	0.63	3.93

* Max.: maximum value, Min.: Minimum value and STD: Standard Deviation.

Table 2: Concentrations of Ammonia of seven canals samples from December 2013 to October 2014

Par.	Stat. Analy.	Al Bahr Al Seidi canal	Al Qudaba canal	Met Yazid canal	Bahr Tirra canal	Rewina canal	Bahr Nashart canal	Al Rashidia canal
NH_3 (mg/l)	Min.	0.93	1.41	0.32	0.4	0.42	0.34	2.62
	Max.	3.81	3.41	0.8	0.89	1.8	0.71	4.95
	Mean	2.2	2.5	0.54	0.7	1	0.6	3.8
	STD	1.02	0.7	0.21	0.2	0.54	0.15	0.8

Table 3: Data analysis for ammonia concentrations of seven canals samples

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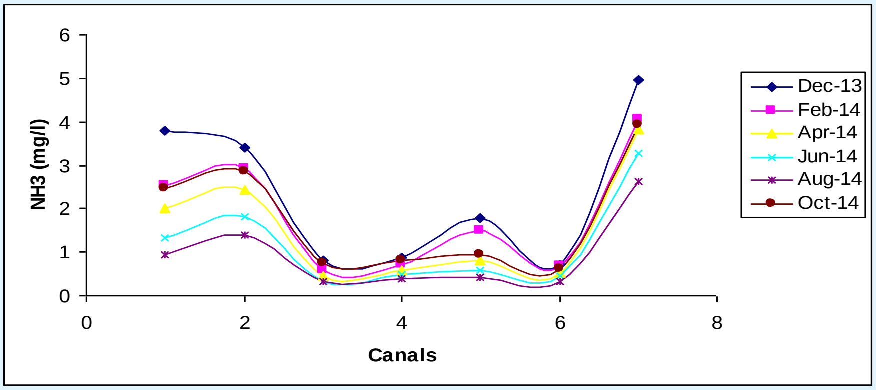

Figure 1: Ammonia concentrations of seven points on the seven major canals from December 2013 to October 2014.

Ammonia Oxidizing Bacteria (AOB)

During the whole period of study, it was noticed that the highest value of AOB was recorded in Al Rashidia canal during winter season (December 2013) while the lowest AOB value was recorded in Rewina canal and al Qudaba canal as well during summer season (August 2014) and Autumn season (October 2014) respectively. It was also noticed that Al Rashidia canal showed the highest AOB count during all season which indicated the occurrence and efficacy of nitrification process in this canal while Rewina canal showed the lowest AOB count which indicated less activity of nitrifiers in Rewina canal. It was also noticed that Al Qudaba canal was in the second rank in winter season then began to decrease gradually in the following seasons to reach the lowest count in autumn season which indicated the fact that the efficacy of nitrification process and the activity of nitrifiers is a process affected by many factors like pH, dissolved oxygen, temperature and existence of ammonia; as shown in Tables 4 and 5 and Figure 2.

The highest count of AOB was recorded during 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. It was found that (AOB) existence, activity and viability were positively correlated to Ammonia concentration which increased remarkably during winter season then started to decrease. It was observed that when raw water is highly polluted during winter season and low demand period, ammonia concentration is remarkably elevated in raw water and microbiological and chemical parameters are markedly influenced. Similar results and correlations are discussed by Hossain, et al. [28]. AOB count showed a significant positive correlations with ammonia concentration ($r = 0.604$) and strong negative correlation with WQI ($r = -0.778$).

High concentration of ammonia in raw water might influence the physicochemical characteristics and other aesthetic characters of water like pH, turbidity, colour, TDS, EC, total alkalinity and odour etc. [28]. 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 contained high concentration of ammonia especially in winter season, so chemical consumption will be increased for the raw water treatment as well as the cost of the water treatment will be raised remarkably. The addition of chlorine (Cl_2) to raw water containing high ammonia (NH_3) during the treatment is formed chloramines [29]; chloramines are known as combined chlorine and is less active than hypochlorous acid (residual chlorine), i.e., one twentieth the power of hypochorous acid [30]. Combined chlorine is not so active against the algae proliferation and ultimately huge amount of algae would be found in the clarified water as well as would be settled on the different parts of the clarifier and filtration systems. To remove ammonia from raw surface water, nitrification process would be used accorfing to [28]. Biological nitrification is more environmentally friendly and cost effective than any other chemical methods of ammonia removal according to Janda and Rudovsky [31]. 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 and Vriends [32].

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Increased heterotrophic bacteria are always found in association with nitrifying bacteria when nitrification processes occur [33-35]. Nitrifiers can increase heterotrophic growth by producing soluble organic carbon [36]. Heterotrophs can be beneficial to nitrification by producing stimulating organics for nitrifiers [37] and protecting nitrifiers from detachment [38,39,36]. In other cases, heterotrophs can be detrimental to nitrifiers since they compete for surfaces, dissolved oxygen, and ammonia [38]. The heterotrophic bacteria increased growth could be a result due to organic carbon released from nitrifying bacteria [34]. Many heterotrophic bacteria have also been found to contribute to nitrification [1], 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 [40]. 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 from autotrophic nitrification.

SITE		Al Bahr Al Seidi canal	Al Qudaba canal	Met Yazid canal	Bahr Tirra canal	Rewina canal	Bahr Nashart canal	Al Rashidia canal
Parameters	**Month / season**							
AOB (MPN index-100 ml)	13-Dec	170	280	130	170	60	170	1600
	14-Feb	80	220	90	130	34	70	900
	14-Apr	60	33	50	70	27	50	350
	14-Jun	22	21	27	40	14	27	50
	14-Aug	33	13	14	17	12	17	33
	14-Oct	40	12	17	17	22	26	70

Table 4: Results of ammonia oxidizing bacteria (AOB) of seven canals samples from December 2013 to October 2014.

Par.	Stat. Analy.	Al Bahr Al Seidi canal	Al Qudaba canal	Met Yazid canal	Bahr Tirra canal	Rewina canal	Bahr Nashart canal	Al Rashidia canal
AOB (MPN-100 ml)	Min	22	12	14	17	12	17	33
	Max	170	280	130	170	60	170	1600
	Mean	67.5	96.5	54.7	74	28.2	60	500.5
	STD	54.3	120.64	46.44	63.28	17.6	57.26	632.14

* Max.: maximum value, Min.: Minimum value and STD: Standard Deviation.
Table 5: Data analysis of ammonia oxidizing bacteria (AOB) of seven canals samples

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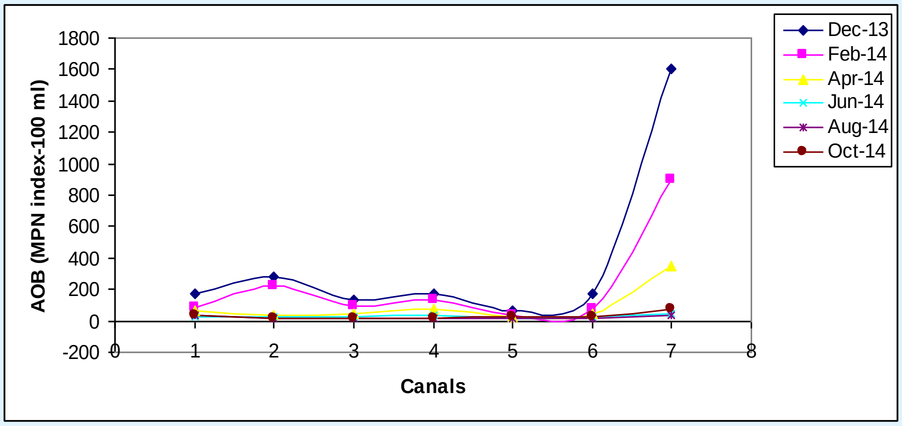

Figure 2: Ammonia oxidizing bacteria (AOB) analysis of seven points on the seven major canals from December 2013 to October 2014.

Water quality index

WQI values ranged from 34.36 to 43.52 indicated bad water quality conditions especially during winter season. The lowest WQI value was recorded 34.36 for Al Rashidia canal during winter season December 2013 while the highest WQI value was recorded 43.52 for Met Yazid canal during summer season (June 2014). Tables (6 and 7) and Figure (3) illustrate the results of WQI during the whole period of study.

This index transforms huge amount of data to a single number, which then used to rank water into one of five descriptive categories of water qualities ranged 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 ranged from 34.36 to 43.52 indicating poor conditions of raw water resources according to water quality index scale. The lowest values were recorded during winter season indicating the effect of 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., [18].

SITE		Al Bahr Al Seidi canal	Al Qudaba canal	Met Yazid canal	Bahr Tirra canal	Rewina canal	Bahr Nashart canal	Al Rashidia canal
Parameters	Month / season							
WQI	13-Dec	37.03	39.42	40.01	39.54	40.16	39.95	34.36
	14-Feb	39.07	40.25	40.75	40.41	40.41	41.2	35.18
	14-Apr	39.57	41.3	42.13	41.36	41.8	42.07	37.6
	14-Jun	40.82	41.84	43.52	42.47	41.94	42.56	39.41
	14-Aug	41.94	42.26	42.54	42.4	43.12	42.85	39.72
	14-Oct	40.5	41.01	41.19	41.68	41.44	40.48	39.1

* 0 – 25: Very Bad, 25 – 50: Bad, 50 – 70: Medium, 70 – 90: Good and 90 – 100: Excellent.
Table 6: WQI values* of the seven main canals samples from December 2013 to October 2014.

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Par.	Stat. Analy.	Al Bahr Al Seidi canal	Al Qudaba canal	Met Yazid canal	Bahr Tirra canal	Rewina canal	Bahr Nashart canal	Al Rashidia canal
WQI	Min	37.03	39.42	40.01	39.54	40.16	39.95	34.36
	Max	41.94	42.26	43.52	42.47	43.12	42.85	39.72
	Mean	39.82	41.01	41.69	41.31	41.48	41.52	37.56
	STD	1.7	1.04	1.3	1.15	1.1	1.2	2.3

* Max.: maximum value, Min.: Minimum value and STD: Standard Deviation.
Table 7: Data Analysis for WQI values of seven canals samples.

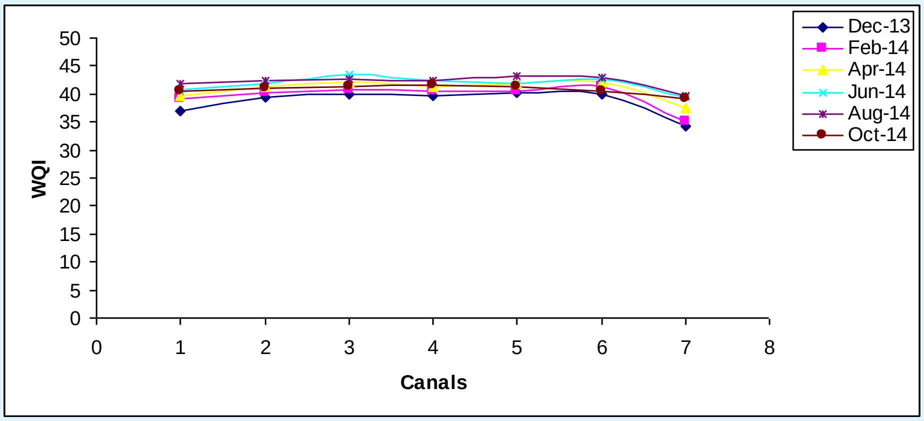

Figure 3: Represents Water Quality Index for the seven main canals from December 2013 to October 2014.

Conclusion

This study monitored the seasonal variations of Ammonia Oxidizing Bacterial communities in raw water resources and proved that AOB activity increased in winter season due to elevated concentrations of ammonia in water during that period. This study discussed nitrification as a biological process for ammonia removal proposing an infield solution of ammonia increase in water. This study revealed that all raw water resources recorded few points on Water Quality Index scale; indicating poor conditions mainly during winter season indicating the effect of low demand period, decreasing of water level and increasing of pollutants during this period resulting in a deteriorated water situation.

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