ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Diazotrophic Bacteria Improve the Water Quality of Qarun Lake and El-Bats Drain.

Sayeda M Ali*

Microbiology Department, National Institute of Oceanography and Fisheries, Aswan Research Station, Egypt

ABSTRACT

Qarun Lake is considered an important source of economic fish in El-Fayoum province. Although, Qarun Lake was considered as nature reserve, it received various types of pollution which leads to negative effects on fish and other living organisms. *Azospirillum brasilense* and *Azotobacter chroococcum* were added to water obtained from Qarun lake and El-Bats drain to examine its ability to improve water quality and generate value-added products. Chemical properties for water, net primary productivity (NPP) and chlorophyll *a* were analysed in all treatments. Results were recorded positively increasing at Qarun lake in NPP (134-206%) and chlorophyll *a* (1870-1274%) for *Azospirillum* and *Azotobacter* treatments, respectively. Similarly, in El-Bats drain chlorophyll *a* was increased to 1644% when inoculate with *Azotobacter*. Generally, inoculation improved the chemical properties. particularly, salinity was decreased from 24.6 to 18.8 gl^{-1} in Qarun lake water. Nitrite, nitrate and ammonia were elevated in both Qarun lake and El-Bats drain water. Dissolved oxygen showed slight change in Qarun lake water. The present study indicates the ability of plant growth-promoting bacteria as biotechnological approach, biofertilizers and bioremediators, either in Qarun lake or El-Bats drain water.

Keywords: Diazotrophs, biofertilizers, bioremediators, Qarun lake, El-Bats drain

*Corresponding author
Email: malisayeda@yahoo.com

ISSN: 0975-8585

INTRODUCTION

Qarun Lake is located in El-Fayoum province, at about 80-km southwest of Cairo, between Latitudes 29° 24\ & 29° 33\ N and Longitudes 30° 25\& 30° 50\ E. Qarun Lake was considered as nature reserve back in 1989, where rare kinds of ducks, eagles, falcons, hornbills, macaws and swans have been found. In addition, many wild plants, fish and mammals have been found [1]. Unfortunately, Qarun Lake was received sewage and agricultural drainage water (338 x 10^6 m^3/year), which flows to the eastern and middle basin of the lake via El-Bats (50-km length) and El-Wadi (48.5 km) drains, respectively. Moreover, the lake receives sewage drainage water and fish farms drainage from El- Fayoum Province through a system of twelve drains [2]. Actually, Qarun Lake is a saline basin and the salinity of water is gradually increasing with time because the evaporation rate is higher than the precipitation [3]. This factor led to many adverse effects on flora and fauna of the lake. In addition, the agriculture drainage water of El-Fayoum province influences the environmental characteristics and phytoplankton biomass (chlorophyll *a*) of Qarun Lake [4].

The plant growth-promoting bacteria, PGPB (Diazotrophs) lives as free-living bacteria in soil, fresh water, marine environments and have been used as biofertilizers, detritus processors, fish food organisms and could be supplemented as bioremediators, bioamelioators and biofilters [5]**.** Furthermore, several studies showed success in using PGPB to increase the plant resistance against salinity and reduce the undesirable effects of salinity [6, 7, 8, 9]. For example, [6] recorded that the damaging effects of NaCl on wheat seedlings could be reduced by inoculation with *A. brasilense*. Similarly, *Azospirillum*-inoculated lettuce seeds showed better germination and vegetative growth than non-inoculated controls after being exposed to NaCl [8]. Moreover, diazotrophs has been used in biotreatment and recycled of industrial wastewater. Effluent of the baker's yeast industry [10, 11] and effluent of olive oil industry [12] were used as a culture medium for the growth and biomass production of N_2-fixing bacteria. Furthermore, PGPB have been successfully used as biofertilizers in fish aquaculture. It recorded enhancement in plankton production, net primary productivity and fish biomass [5, 13, 14]. Furthermore, using *Azospirillum* and *Azotobacter* in aquarium wastewater have improved *Chlorella vulgaris* biomass production and water quality [15].

The present study was designed to study possibility of using *Azotobacter* or *Azospirillum* as biofertilizers and bioremediators in water of the Qarun Lake and El-Bats drain.

MATERIALS AND METHODS

The nitrogen-fixing bacteria (diazotrophs)

Two strains of diazotrophs were used *Azospirillum brasilense* (Azos. R7) which isolated from *Ricinus communis* and *Azotobacter chroococcum* (Azt) which isolated from *Hordeum vulgare* [16]. They were grown on N-deficient combined carbon sources medium, CCM [17] at 32°C in a rotary shaker for 3 days for *Azospirillum brasilense* and 5 days for *Azotobacter*

chroococcum. Bacterial cells were harvested by centrifugation at 7000 rpm for 15 min and washed twice with sterile solution (0.85% NaCl).

Experimental design

Two sources of waters were separately used (Qarun lake and El-Bats drain).Water samples were collected from the surface water, (ca. <1 m ashore), in five-liter plastic bottles. Each water specimen was inoculated with *A. brasilense* or *A. chroococcum* in three concentrations (5,10, and 20 %v/v). Control groups were carried without bacteria (0% v/v).

Experimental conditions

Two hundred ml of water samples were distributed in 500 ml sterilized Erlenmeyer flasks. The prepared flasks were inoculated with the individual diazotroph strains (10^7cfu ml^{-1}) in four concentrations (0,5,10, 20 %v/v), before incubated in a locally made controlled incubator at temperature of 25±1^{o}C and light intensity of 4000 lux under a day/night program of 14 h light followed by 10 h darkness for 7 days. Samples for analysis were taken after 7 days of incubation.

Determinations

*Chlorophyll (*a)

Chlorophyll (*a) was* estimated by using spectrophotometer at different wavelengths, and its concentration was calculated by the equation of [18].

Net primary productivity (NPP)

Net primary productivity (NPP) measured after three-hour incubation using the modified Winkler method [19].

Chemical analyses

Electrical conductivity (EC), pH, salinity and total dissolved solids (TDS) were measured by using pH meter model JENWAY (4330). Dissolved oxygen (DO) was measured using the modified Winkler method [19]. Chemical oxygen demand (COD) was carried out using the potassium permanganate method [20]. Colorimetric methods were used to determine ammonia and nitrite [19] and nitrate [21].

Bacteriological analyses

The pour plate technique and the total bacteria count agar [19] were used for the enumeration of total bacterial counts at 32˚C incubation temperatures. Total diazotrophs were

counted using the surface-inoculated plate method and the N-deficient combined carbon sources medium, CCM [17].

Statistical analysis

Data were statistically analyzed using analysis of variance, two ways ANOVA using the STATISTICA 6.0 software (Stat soft, Tulsa, USA).

RESULTS

Qarun lake water

Changes in microbial load, chlorophyll *a*, NPP and chemical properties of Qarun lake water after inoculated with diazotrophs were illustrated in Figure (1). Generally, the various concentrations of diazotrophs increased microbial load of Qarun lake water. For example, Total bacterial counts ranged from 863 to 1976 cfu $x10^3$ ml^{-1} and 674 to 6544 cfu $x10^3$ ml^{-1} for *A. brasilense* and *A. chroococcum* treatments respectively, compared to 5 cfu $x10^3$ ml^{-1} in the control. Also, total diazotrophic bacterial counts ranged from 1118 to 2800 cfu $x10^3$ ml^{-1} and 19 to 42 cfu $x10^3$ ml^{-1} for *A. brasilense* and *A. chroococcum* treatments respectively, compared to 1 cfu $x10^3$ ml^{-1} in the control. The highest significant ($p<0.05$) microbial loads were reported with 5% inocula in both microbial treatment. *A.chroococcum* showed a significantly higher ($p<0.05$) in total bacterial count than *A. brasilense* and vice versa in total diazotrophic bacteria count

Generally, net primary productivity (NPP) was positively affected by diazotrophs treatment ($p<0.05$). The increasing in NPP ranged from 97 to 134% and 123 to 206% with *A. brasilense* and *A. chroococcum* treatments respectively. NPP increased with increasing bacterial concentrations, where the highest value was recorded in 20% *A. brasilense* or *A. chroococcum* treatments.

Chlorophyll *a* values recorded significantly increasing ($p<0.05$), in *A. brasilense* (85-187 μgl^{-1}) and in *A. chroococcum* (81-130 μgl^{-1}) compared to control (9.5 μgl^{-1}). Moreover, 5% *A. brasilense* treatment recorded higher value ($p<0.05$) than *A. chroococcum.*

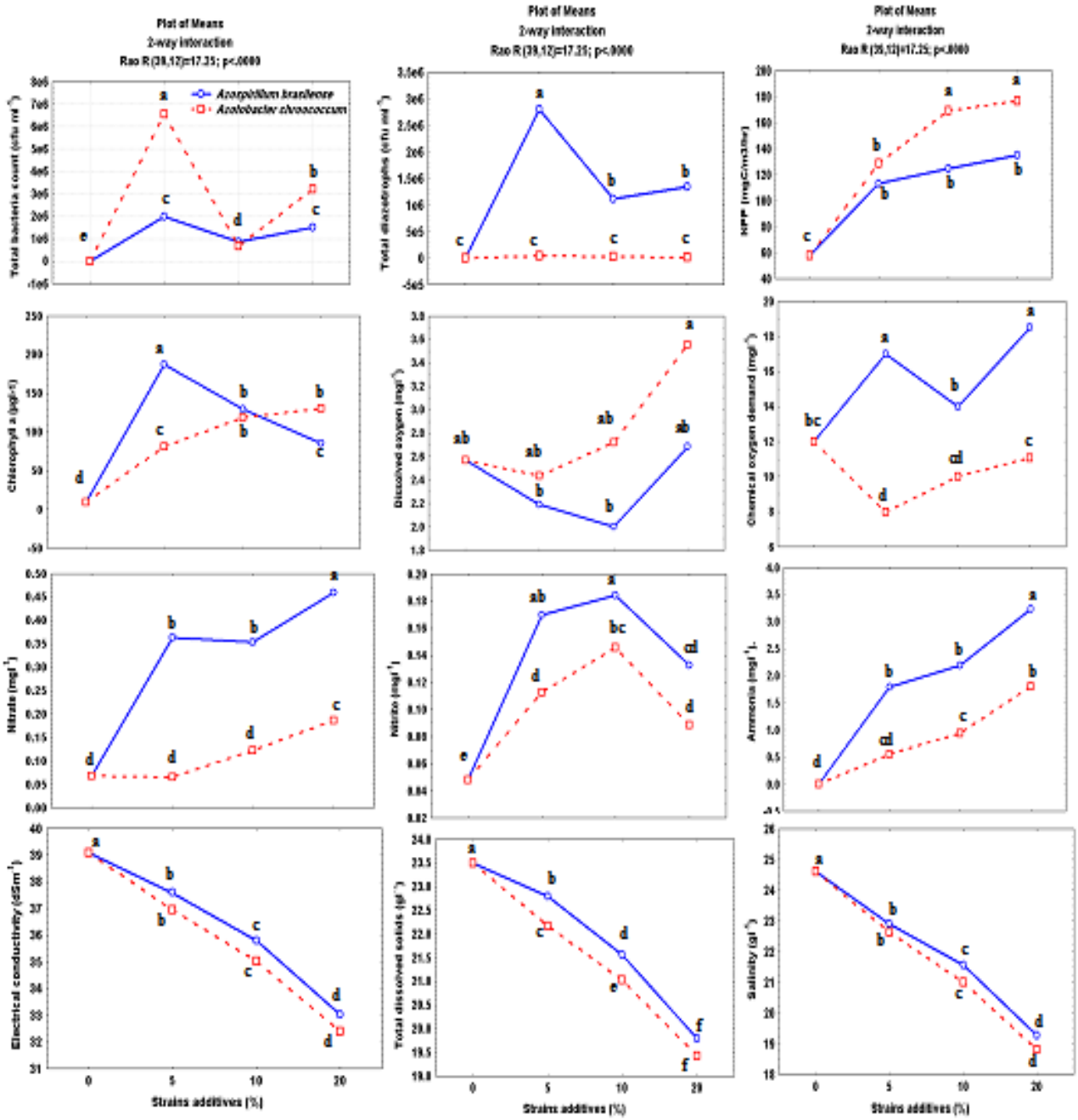
Although, there were changes in DO between treatments, but there was no significant difference ($p>0.05$). The highest DO values were recorded in 20% of *A. chroococcum* (3.55 mgl^{-1}). COD recorded significant increased ($p<0.05$) in *A. brasilense* and significant decreased in *A. chroococcum*, as compared to control.

Both bacterial treatment showed significantly increased ($p<0.05$) in ammonia, nitrite and nitrate compared to control. Irrespective of treatment concentrations, the highest increasing in ammonia, nitrite and nitrate were recorded in *A. brasilense* treatment. Interestingly, Increasing in ammonia value was accompanying with increasing in *A. brasilense* and *A. chroococcum* concentration. All treatment showed a significant difference with control ($p<0.05$). Furthermore, the salinity, EC, and TDS decreased significantly ($p< 0.05$) by increasing

ISSN: 0975-8585

the concentration of diazotroph treatment as compared to control. Where salinity decreased from 24.6 in control to 19.3 and 18.8 gl^{-1} in *A. brasilense* and *A.chroococcum*, respectively. Similarly, TDS and EC decreased from23.5 $gl^{-1}$and 39.1dSm^{-1}in control to 19.8 gl^{-1} and 33.0 dSm^{-1}in *A. brasilense*; and 19.4 $gl^{-1}$and 32.4 dSm^{-1}in *A. chroococcum*, respectively. There was no significant difference between two strains of bacteria in salinity, EC, and TDS ($p< 0.05$).

Fig. 1: Influence of different concentration of diazotrophs (*A. brasilense* and *A. chroococcum*) on microbial load, Chlorophyll*a*, NPP, and chemical analyses of Qarun Lake water. Values followed by the same letter are not significantly different ($p>0.05$). NPP, Net primary productivity

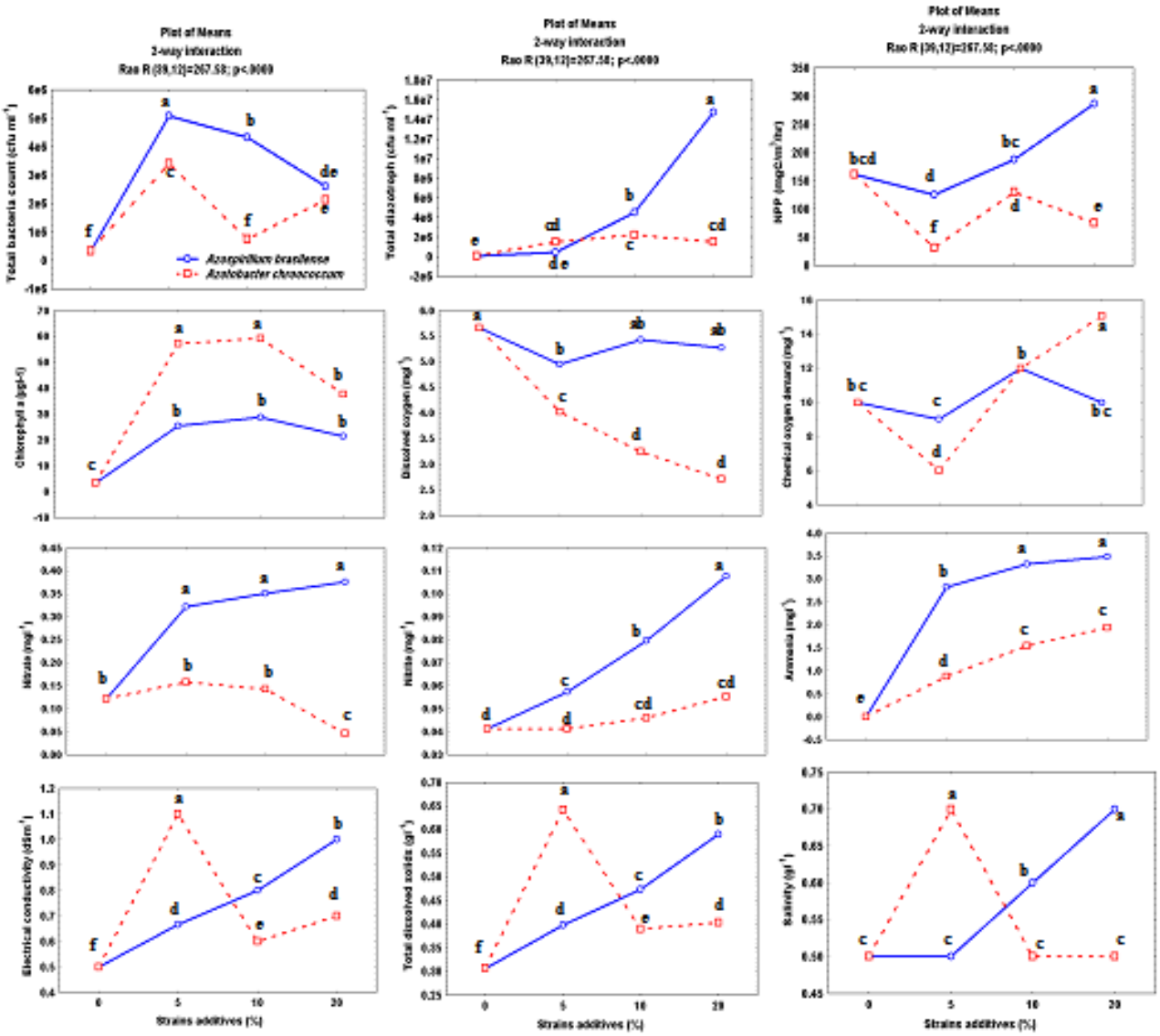


ISSN: 0975-8585

El-Bats drain water

The effects of diazotroph inoculation on microbial load, net primary productivity, chlorophyll *a* and chemical properties of El-Bats drain water were illustrated in Figure (2). The results indicated that, diazotrophs positively increased ($p<0.05$) total bacterial counts and total diazotrophic bacterial counts where it reached5064 $x10^3$cfu $ml^{-1}$and 14720 $x10^3$ cfu ml^{-1} in *A. brasilense* treatments, as well as 3392 $x10^3$cfu ml^{-1} and 2188 $x10^3$cfu ml^{-1} for *A. chroococcum* treatments compared to controls (334 $x10^3$cfu ml^{-1} and 93 $x10^3$cfu ml^{-1}, respectively).

Fig. 2: Influence of different concentration of diazotrophs (A. brasilense and A. chroococcum) on microbial load, Chlorophyll a, NPP, and chemical analysesof El-Bats drain water. Values followed by the same letter are not significantly different (p>0.05). NPP, Net primary productivity



Although, *A. chroococcum* showed decreasing in NPP (31-130 mgC/m^3/hr), ($p<0.05$), but *A. brasilense* recorded increasing especially in 10 and 20% concentrations (188 and 287 mgC/m^3/hr, respectively), as compared to control (161 mgC/m^3/hr).

Both bacterial treatments showed significant increasing in chlorophyll *a* ($p<0.05$) with increasing treatment concentrations as compared to control(3.4 μgl^{-1}). Moreover, *A. chroococcum* treatments recorded remarkable increasing (from 38 to 59 μgl^{-1})than *A. brasilense* treatments (from 21 to 29 μgl^{-1}).

In respect to chemical analyses, the DO was decreased than control where *A. brasilense* treatments decreased from 4 to 13% and from 29 to 52% in *A. chroococcum* treatments. As comparing with control(10 mgl^{-1}), the highest COD values were recorded in 20% and 10% of *A. chroococcum* (12 and 15 mgl^{-1}), respectively, while the highest COD value was only recorded in 10% of *A. brasilense* (12 mgl^{-1}). With exception of 5% and 20% of *A. chroococcum*, all concentration did not showed any significant difference as compared to control ($p>0.05$). All concentrations of *A. brasilense* showed highly significant value in ammonia, nitrite and nitrate ($p< 0.05$) as compared with *A. chroococcum* and control. With exception of nitrate, the highest concentrations recorded the highest values. The increasing in ammonia was distinguished in all diazotroph treatments (*A. brasilense,* 2.8-3.5 mgl^{-1} and *A. chroococcum* 0.9-2.0 mgl^{-1}). EC recorded an increase ($p <0.05$) in *A. brasilense* (0.7-1.0 dSm^{-1}) and *A. chroococcum* (0.6-1.1 dSm^{-1}) compared to control (0.5 dSm^{-1}). Also, TDS was increased in both treatment bacteria (0.6 gl^{-1}), compared to control (0.3 $gl^{-1)}$. The highest salinity values ($p<0.05$) were recorded in 10 and 20% *A. brasilense* (0.6,0.7 gl^{-1} respectively) and 5% *A. chroococcum* (0.7 gl^{-1}).

DISCUSSION

In El-Fayoum province, Qarun Lake is considered an important source of economic fish and commercial salts [22]. Although, Qarun Lake is considered as nature reserve, it was received sewage and agricultural drainage water. So, Qarun Lake needs sustainable wastewater management. The N_2-fixing bacteria play an important role in the environmental, where, it used as biofertilizers (in soil and aquaculture) and bioremediation of wastewater [10,11, 14]. Generally, this study revealed that, using *A. chroococcum* or *A. brasilense* treatment (either to Qarun Lake or El-Bats drain water) has significantly increased chlorophyll *a*, Net primary productivity and total bacterial counts. This could reflects the role of these bacteria as microalgae-growth promoting bacteria (MGPB) [23], where, it have ability to produce various phytohormones and vitamins [24] and production an active metabolites [25]. Similar to our results, the biomass yield and chlorophyll *a* and *b* of the fresh water microalgae *C. vulgaris* were positive affected by the addition of either *A. brasilense* or *A. chrococcum* to aquarium wastewater and synthetic wastewater [15]. Similarly, feeding *Oreochromis niloticus* with *Azotobacter* and *Azospirillum*, led to an increasing in total bacterial count and total diazotrophic bacterial counts and NPP at water aquarium [14]

In Qarun Lake, treatment with *A. chroococcum* recorded the highest significant value for NPP and total bacterial counts compared to *A. brasilense* treatments. This may be attributed to

that *Azotobacter sp.* used a wide range of organic compounds as carbon source [26]. Moreover, the ability of *Azotobacter* to form capsular polysaccharides qualifies these particular organisms to entrap heavy metals [27]. On the other hand, *A. brasilense* treatments recorded the highest significant value of chlorophyll *a* and total diazotrophis compared to *A. chroococcum*. Such an increasing in aquatic microorganisms growth have been reported by [28] who noticed an increasing in microalga *Chlorella vulgaris* growth and nutrient removal after incubated with *A. brasilense* compared to using microalga alone.

Moreover, *A. brasilense* recorded highly significant ($p < 0.05$) increase in all parameters than *A. chroococcum* in El-Bats drain water. As well as, comparing the effect of bacterial treatment revealed a highly positive effect of microbial load, NPP and Chlorophyll *a* in Qarun Lake than El-Bats drain water. This may be attributed to high amount of total organic carbon and low content of soluble nitrogen form in Qarun Lake, such this environment is considered as a good environment for the growth of N_2- fixing bacteria [29]. Also, using *A. brasilense* with *C. vulgaris* recorded the highest value of chlorophyll a and b in both aquarium wastewater and synthetic waste water media compared to using *A. chrococcum* [15].

[29] noticed an accumulation of ammonium in N-free synthetic growth medium after inoculated with *Bacillus pumilus* (PGPB). This is agreement to our result which recorded highly increase in N-forms in Qarun lake and El-Bats drain water after inoculated with either *A. chrococcum* or *A. brasilense*.

The results recorded decreasing in salinity level and total dissolved solid with both bacterial treatment in Qarun Lake. This is due to the important role of diazotrophs in biotechnology to reduce the undesirable effects of salinity and increase the microorganisms' resistance against salinity [8, 30, 31]. Our observations agreed with [32] who found that inoculating wheat by polysaccharide-producing bacteria and polysaccharides prevented plant root from absorb sodium. Moreover, soil inoculation by *Azotobacter* strain decreased the adverse effects of NaCl and proline (an osmoprotectant) concentration compared to control at the same salinity level [30].

This study concluded that diazotrophs could use successfully as biofertilizers and bioremediators in Qarun Lake and El-Bats drain water in order to protect this environment from pollution and generate value-added products.

ACKNOWLEDGMENT

The authoress would like to thank the Environmental Studies and Research Unit (ESRU), Faculty of Agriculture, Cairo University, Prof. Ahmad K. El-hammady, Dr. Elham Awad and Prof. Wafaa T. Abbas for providing the required facilities.

ISSN: 0975-8585

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