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Antioxidant activity and phycoremediation ability of four cyanobacterial isolates obtained from a stressed aquatic system

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ABSTRACT

Cyanobacteria are natural enormous sources of various biologically active compounds with great contributions in different industries. This study aimed to explore and characterize novel cyanobacterial isolates with antioxidant activity and potent phycoremediation ability from Egyptian wastewater canals. The *in vitro* biological activity of these isolates and their potential ability to take up nutrients and heavy metals from wastewater were examined. The obtained isolates were sequenced and deposited in database under accession numbers, KY250420.1, KY321359.1, KY296359.1 and KU373076.1 for *Nostoc calcicola, Leptolyngbya* sp., *Nostoc* sp., *and Nostoc* sp., respectively. *Leptolyngbya* sp. (KY321359.1) showed the lowest identity (90%) with the nearest deposited sequence in database. While the isolate *Nostoc* sp. (KU373076.1) showed the highest total phenolic content as well as the highest levels of caffeic, ferulic and gallic acids. Consequently, it presented the highest antioxidant scavenging activity. All studied isolates revealed potent ability in chelating nutrients and removing heavy metals from wastewater. In conclusion, this study provides a taxonomic, biochemical and molecular evidence of four novel cyanobacterial isolates with antioxidant activity and potential phycoremediation ability.

1. Introduction

The continuous increase in populations, industrialization and excessive generation of wastewater are major environmental issues threat sustainability of developing countries. The release of polluted water without appropriate treatment into freshwater resources has led to maximizing the problem of water pollution, besides making the water unfit for drinking, irrigation and aquatic life (Egun, 2010). Therefore, there is an urgent need to develop eco-friendly and economic technologies for wastewater treatment, as a sustainable remediation solution for environmental protection, which would require simple infrastructure and less inputs with potential acceptance at the commercial level (Sood et al., 2015; Sunday et al., 2018).

The ecological and economic importance of cyanobacteria is growing rapidly worldwide due to the great diversity of the products that can be developed from cyanobacterial biomass (Hamed, 2016; Badr et al., 2018). The wide range of cyanobacteria biochemical products and the potential use of these compounds in the pharmaceutical, nutraceutical, cosmetic and research industries have led to more concern of cyanobacteria (Pulz and Gross, 2004; Griffiths et al., 2016; Amaro et al., 2018). Commercially, cyanobacteria are used to produce many relatively high-value products such as carotenoids, β-carotene, astaxanthin and long-chain polyunsaturated fatty acids to be used as human nutritional supplements (Borowitzka, 1995, 2013; Assaye et al., 2018).

Phycoremediation is broadly defined as the utilization of microalgae and cyanobacteria (blue-green algae), for the removal of contaminants from wastewater. It is a promising technology offer an inexpensive alternative to conventional forms of tertiary wastewater treatments (Sood et al., 2015; Sunday et al., 2018). The use of cyanobacteria in wastewater treatment is an eco-friendly process with no secondary pollution as long as the produced biomass allows efficient nutrient recycling (Munoz and Guieysse, 2006). Phycoremediation based cyanobacteria is also cost effective when compared with other physical and chemical remediation methods (Han et al., 2007; Sunday et al., 2018). The high requirement of nitrogen and phosphorus for the growth of cyanobacteria strengthens their ability to use the nutrient- rich wastewater as a medium for multiplication. At the same time, assimilated nitrogen and phosphorus can be recycled into algal biomass as biofertilizer (Pittman et al., 2011).

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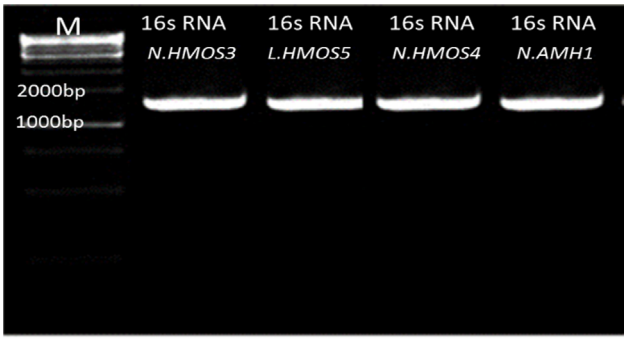

Fig. 1. PCR products of *16S rRNA* gene were almost 1.5 Kb of *Nostoc calcicola* (KY250420.1), *Leptolyngbya* sp. (KY321359.1), *Nostoc* sp (KY296359.1), *Nostoc* sp (KU373076.1) with 1Kp DNA ladder.

Many beneficial cyanobacterial strains belong to the order Nostocales. The importance of these strains refers to their capacity to improve quality, fertility of soils and retain water. They have the ability to release phosphate, nitrogen, trace elements and decrease chemical nitrogen demands. Also, they produce substances with antiviral and antibacterial activities (Hamed, 2016). Additionally, Nostocales have the ability to take up nutrients from water offer the feasibility to recycle the nutrients into algae biomass and thus can be used in wastewater treatment (Sood et al., 2015).

For these findings, cyanobacteria have become one of the most serious tracks for emerging problems that are encountered nowadays (Stephens et al., 2013; Hamed, 2016; Badr et al., 2018). Despite their many advantages, of the 50000-existent species, only a few thousand are now kept in collections and are investigated for their chemical content, and even fewer are cultivated in industrial scales. Therefore, cyanobacteria are still not a well-studied from the biotechnological point of view (Mishra et al., 2015; Hamed, 2016).

Therefore, the present study aimed to identify and characterize novel cyanobacterial isolates obtained from Egyptian irrigated drainage canals' water. Molecular characterization based on 16s rDNA, morphological and biochemical characteristics were determined for the obtained isolates. Moreover, some biotechnological applications of these isolates have been introduced through investigation biological activity, besides *in vitro* potential ability to uptake nutrients and heavy metals from wastewater.

2. Materials and methods

2.1. Samples collection

Water samples were collected from irrigated canals, Qalyubia governorate. Sterile syringes were used to transfer the collected samples to sterile plastic bottles. The bottles were delivered to the laboratory and analyzed within 4 h under sterilized conditions.

2.2. Isolation and selection of cyanobacterial isolates

Serial dilutions method was used to isolate and purify the desired cyanobacterial isolates according to Lee et al. (2014). Cyanobacterial isolates were cultivated on BG-11 and modified BG-11 as a nutrient selective media optimized for the growth of cyanobacterial species. The modified BG-11 medium was prepared from the BG-11 recipe by removing all nitrogen forms to support only heterocystous cyanobacteria (Allen, 1968; Rippka et al., 1979).

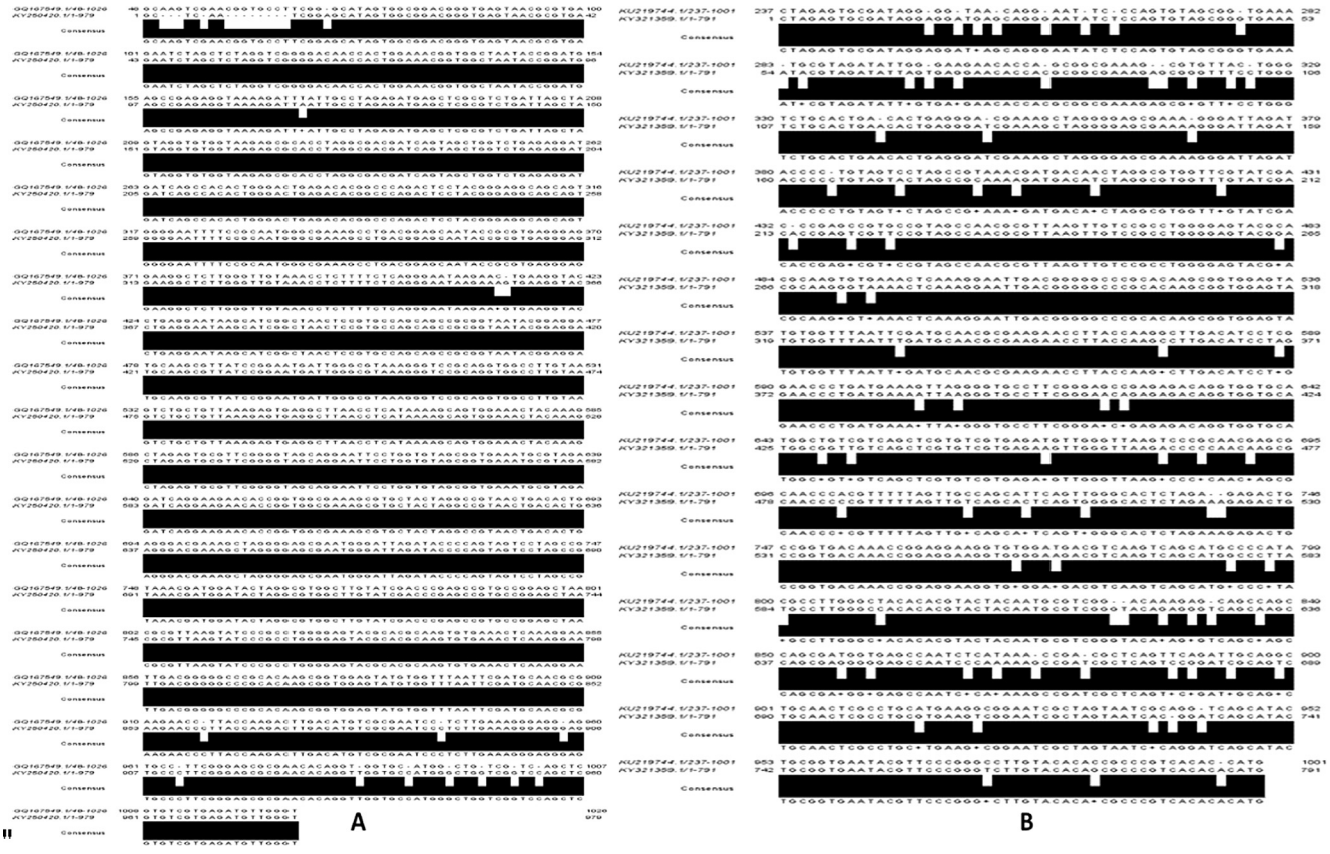

Fig. 2. A. Image showed many SNPs between *Nostoc calcicola* (KY250420.1) and the nearest one *Nostoc calcicola* (*GQ167549.1*) deposited in GenBank Database. B. Image showed many SNPs between our obtained sequence of *Leptolyngbya* sp (KY321359.1) and the nearest one *Leptolyngbya* sp. (KU219744.1) deposited in GenBank Database.

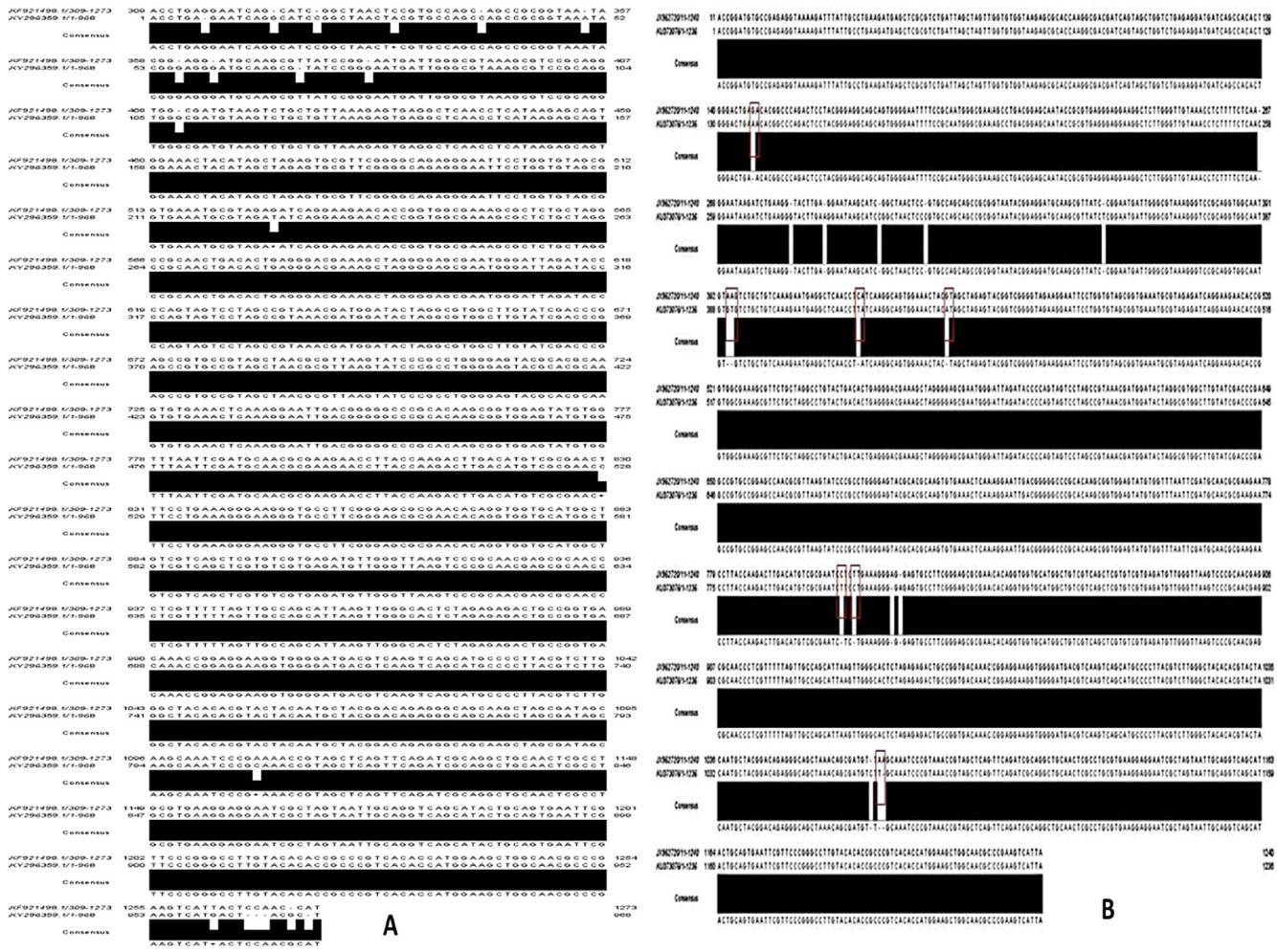

Fig. 3. A. Image showed many SNPs between *Nostoc* sp. (KY296359.1) and the nearest one *Nostoc* sp. *PAN-549* (KF921498.1) deposited in GenBank Database. B. Image showed many SNPs between our obtained sequence of *Nostoc* sp. (KU373076.1) and the nearest one *Nostoc* sp. *UIC 10,279* (JX962720.1) deposited in GenBank Database.

2.3. DNA extraction and PCR amplification

For PCR amplification, cyanobacteria genomic DNA was extracted from the obtained pure isolates following Fawley and Fawley (2004) methodology. PCR amplification was performed by amplifying the partial sequence of *16S rRNA* gene. The universal primers of *16S rRNA* gene 27F:5́. AGAGTTTGGATCMTGGCTCAG.3′and 1492R:5́CGGTTACC TTGTTACGACTT.3′ were used. The primers were tested by *in silico* PCR tool (http://insilico.ehu.eus/PCR/). The expected PCR amplicon was almost 1.5 kb. The PCR mixture was 50 µL containing 0.4 µM of each primer with concentration of 10 pM, 400 µM of dNTPs mix, 5 µL of 10x PCR reaction buffer, 2 µM $MgCl_2$, 2.5 units of TAKARA Taq DNA polymerase (Cat. #: R001AM), 1 µL of template DNA and the final volume was adjusted with sterilized double distilled water. PCR thermocycler was used under the following conditions: 95 °C for 3 min followed by 35 cycles at 95 °C for 50 secs, 55 °C as annealing temperature for 1 min with an extension of 72 °C for 1 min followed by final extension at 72 °C for 10 min. Amplified PCR products were stored at −20 °C for further purification and downstream application, then 5 µL of PCR amplified product was loaded on 1.2% agarose gel electrophoresis stained with Ethidium bromide using GeneRuler™ 1 kb DNA ladder (Cat. #: SM0313), then visualized under UV Transilluminator (Bio Rad).

2.4. Cloning and sequencing

The expected DNA band, 1500 bp, was eluted from agarose gel and purified according to the manufacturer's QIAquick Gel Extraction Kit (Cat. #: 28704). The purified PCR fragment was ligated into pGEM®-T Easy Vector Systems (Cat. #: A1360) following the manufacturer. Inoue et al. (1990) methodology was used to prepare and transform the competent cells of *E. coli* top 10 strain. The white colonies, transformed, were picked up from LB/Amp/Xgal plates and inoculated on LB/Amp broth media, then incubated overnight at 33 °C with shaking to stabilize the plasmid inside the transformed cells. The reconstruct plasmid was isolated from the transformed *E. coli* cells using the alkaline method of Birnboim and Doly (1979). The purified plasmids were visualized on 1.2% agarose gel stained with Ethidium bromide using electrophoresis. GeneRuler™ 1 kb DNA Ladder was used to confirm the recombinant plasmids which were sequenced by Macrogen Company, South Korea.

The obtained sequences for *16S rRNA* gene were examined for vector contamination using the VecScreen tool (http:// www.ncbi.nlm.nih.gov/tools/vecscreen). While, NEbcuter V2.0 was used to create a restriction map and to identify the GC content of the obtained sequences (Vincze et al., 2003, http://nc2.neb.com/NEBcutter2/). Jalview software (Waterhouse et al., 2009) was applied to show single nucleotide polymorphisms (SNPs) and consensus resulted from the alignment of our obtained sequences with the nearest strains in NCBI database (http://www.jalview.org/). Construction of phylogenetic trees was done using Clustal Omega (https://www.ebi.ac.uk/Tools/

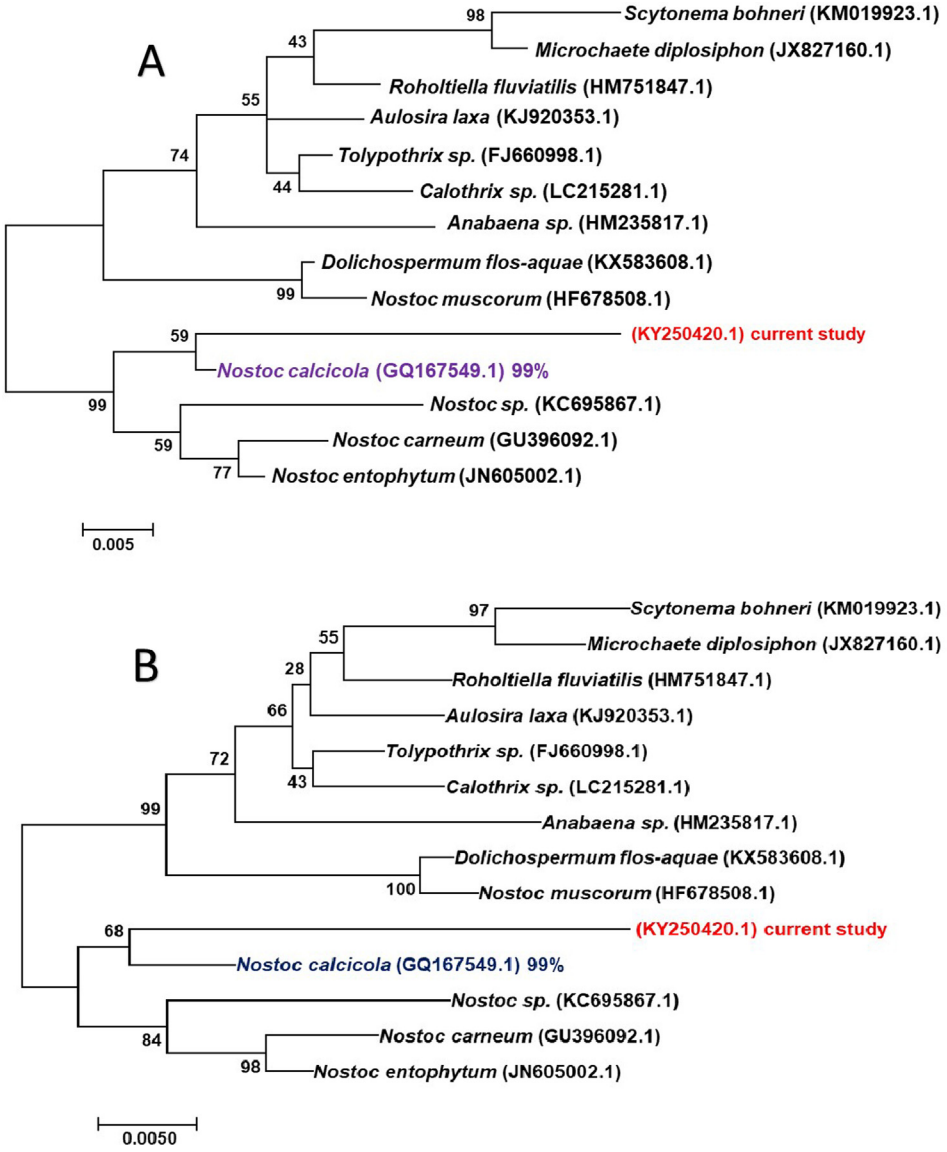

Fig. 4. The phylogenetic tree of the obtained sequence *Nostoc calcicola* (KY250420.1) with the nearest one *Nostoc calcicola* (*GQ167549.1*) recovered by maximum likelihood method (A) and neighbor-joining method (B). Average Bootstrap values, of compared algorithms, were indicated at the branch roots. The bar was represented 0.005 changes per nucleotide. Accession numbers of database extracted sequences were in black, Purple and red colors for the nearest and the obtained sequences, respectively.

msa/clustalo/) and MEGA7 software (Kumar et al., 2016). The phylogenetic tree for each cyanobacterial isolate sequence was recovered by maximum likelihood and neighbor-joining methods. Average bootstrap values, of compared algorithms, were indicated at the branch roots. The obtained sequences were registered at NCBI database under accession numbers KY250420.1, KY321359.1, KY296359.1 and KU373076.1 for *Nostoc calcicola, Leptolyngbya* sp., *Nostoc* sp., *and Nostoc* sp., respectively, (http://www.ncbi.nlm.nih.gov).

2.5. In vitro biological activity of cyanobacterial isolates

Determination of flavonoids, vitamin A and E, water-soluble vitamins, β-carotene and zeaxanthin were determined by HPLC (Nogata et al., 1994; Gimeno et al., 2000; Ekinci and Kadakal, 2005; Ahmed et al., 2007). The total phenolic content of the extracts was determined by the Folin–Ciocalteu method (Kaur and Kapoor, 2002). The ability of different extracts to act as hydrogen donors was measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH˙) assay as described by Blois (1958).

2.6. Phycoremediation ability of cyanobacterial isolates

The identified cyanobacterial isolates were cultivated on autoclaved wastewater media obtained from Moshtohor, Toukh, Qalyubia sewage canal under continuous lighting (1000 lx) as a batch culture in 250 ml Erlenmeyer flasks containing 100 ml of BG-11 at 27 ± 2 °C. The nutrients and heavy metals concentration were measured by Perkin EL Mer 3300 Atomic Absorption Spectroscopy before cyanobacteria cultivation and at the end of the experiment. The uptake of heavy metals by the cyanobacteria was calculated by the difference of heavy metals and nutrients as percentage removal; (B-A)/B × 100 where, B; concentration of nutrients and heavy metals in wastewater media before cyanobacteria cultivation (control) and A; concentration of nutrients and heavy metals in wastewater media after cyanobacteria cultivation period. Wastewater medium was filtered through Whatman filter paper (No. 42) before the final measurement. The experiment lasted for 16 days assuming that all heavy metals removed from water were taken up by the grown cyanobacteria.

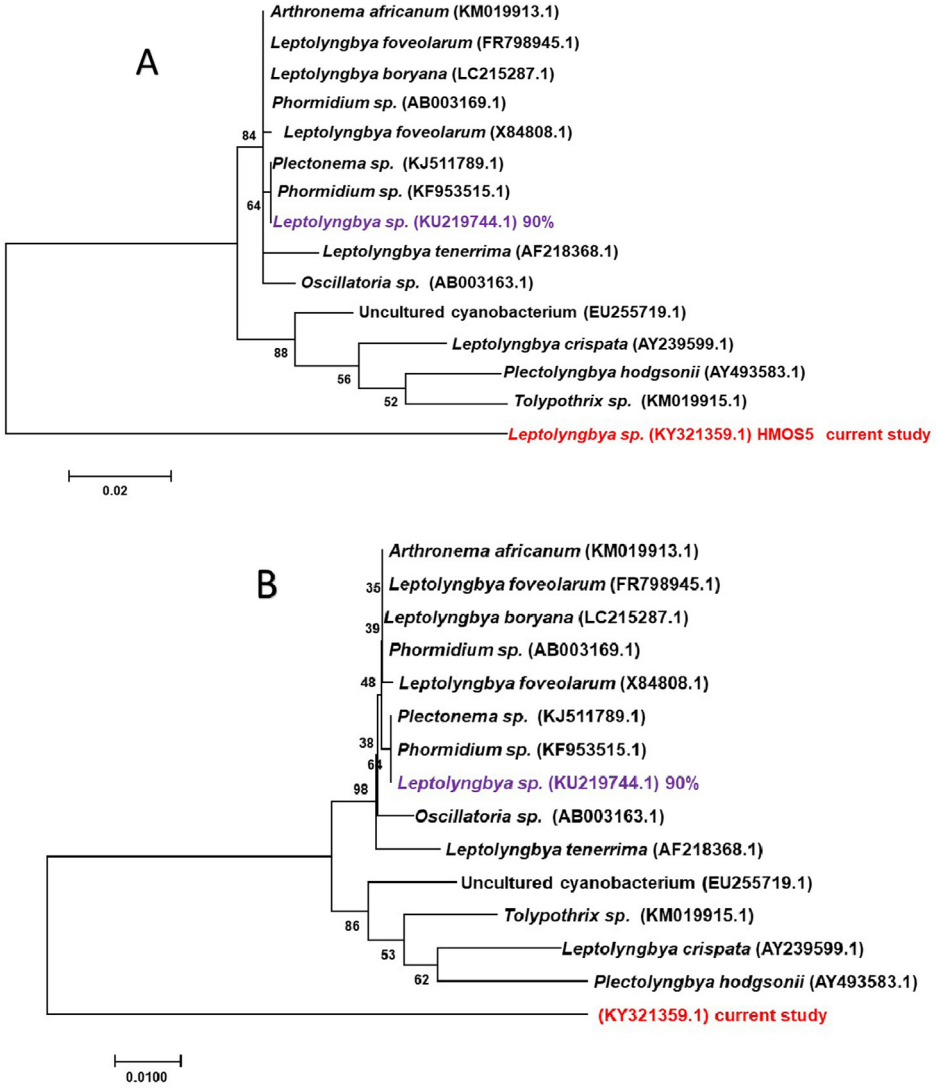

Fig. 5. The phylogenetic tree of the obtained sequence of *Leptolyngbya sp* (KY321359.1) with the nearest deposited sequence *Leptolyngbya* sp. (KU219744.1) recovered by maximum likelihood method (A) and neighbor-joining method (B). Average Bootstrap values, of compared algorithms, were indicated at the branch roots. The bar was represented 0.02 and 0.01 changes per nucleotide. Accession numbers of database extracted sequences were in black, Purple and red colors for the nearest and the obtained sequences, respectively.

2.7. Statistical analysis

The values were expressed as mean ± SE for 5 replicates of different cyanobacterial isolates. Means and standard error were calculated using SAS software for Windows, version 13.0, (SAS, 2004).

3. Results and discussion

Qalyubia irrigated drainage canals characterized with the presence of various wastewater types, human sewage, livestock wastes, agro-industrial wastes, industrial wastes, piggery effluent, food processing waste and other agricultural waste substrates. Many investigations have examined the impact of some environmental stresses on the distribution and species composition of fresh water algal communities (Abdel-Raouf et al., 2012). Hence, there are a potentiality to discover new algal species in such aquatic stressed system. In this study, the first microscopic investigation confirmed that there were many different shapes of diatoms and cyanobacteria. The variation in the obtained isolates may be due to the presence of various wastewater types in Qalyubia irrigated canals. Serial dilutions method resulted in purifying four cyanobacterial cultures. Three isolates were belonged to *Nostocaceae* family and one belonged to the family of *Pseudanabaenaceae*.

3.1. Molecular characterization of the cyanobacterial isolates

The amplified PCR products of *16S rRNA* genes almost 1.5 kb (Fig. 1) from cyanobacterial isolates were sequenced. The assembled sequences were deposited in NCBI database under accession numbers KY250420.1, KY321359.1, KY296359.1 and KU373076.1 for *Nostoc calcicola, Leptolyngbya* sp., *Nostoc* sp., *and Nostoc* sp., respectively. Analysis of the obtained sequences via VecScreen tool showed no contamination with vector sequence. While the BLAST alignment showed that the nearest deposited sequences in database to the obtained sequences were *Nostoc calcicola* 99 (GQ167549.1), *Leptolyngbya* sp. *NIES-504* (LC319755.1), *Nostoc* sp. PAN-549 (KF921498.1) and *Nostoc* sp. *UIC 10,279* (JX962720.1) with identity ratio 99%, 90%, 98% and 99% as shown in Fig. 2A, B and Fig. 3A, B, respectively.

The isolate *Leptolyngbya* sp. showed the lowest identity ratio (90%) with other deposited sequences in database where, the divergence seemed in high number of SNPs (54) and GAPs (26) between it and the nearest sequence. These results supported by Thompson et al. (2013) who reported that strains from different microbial species share less

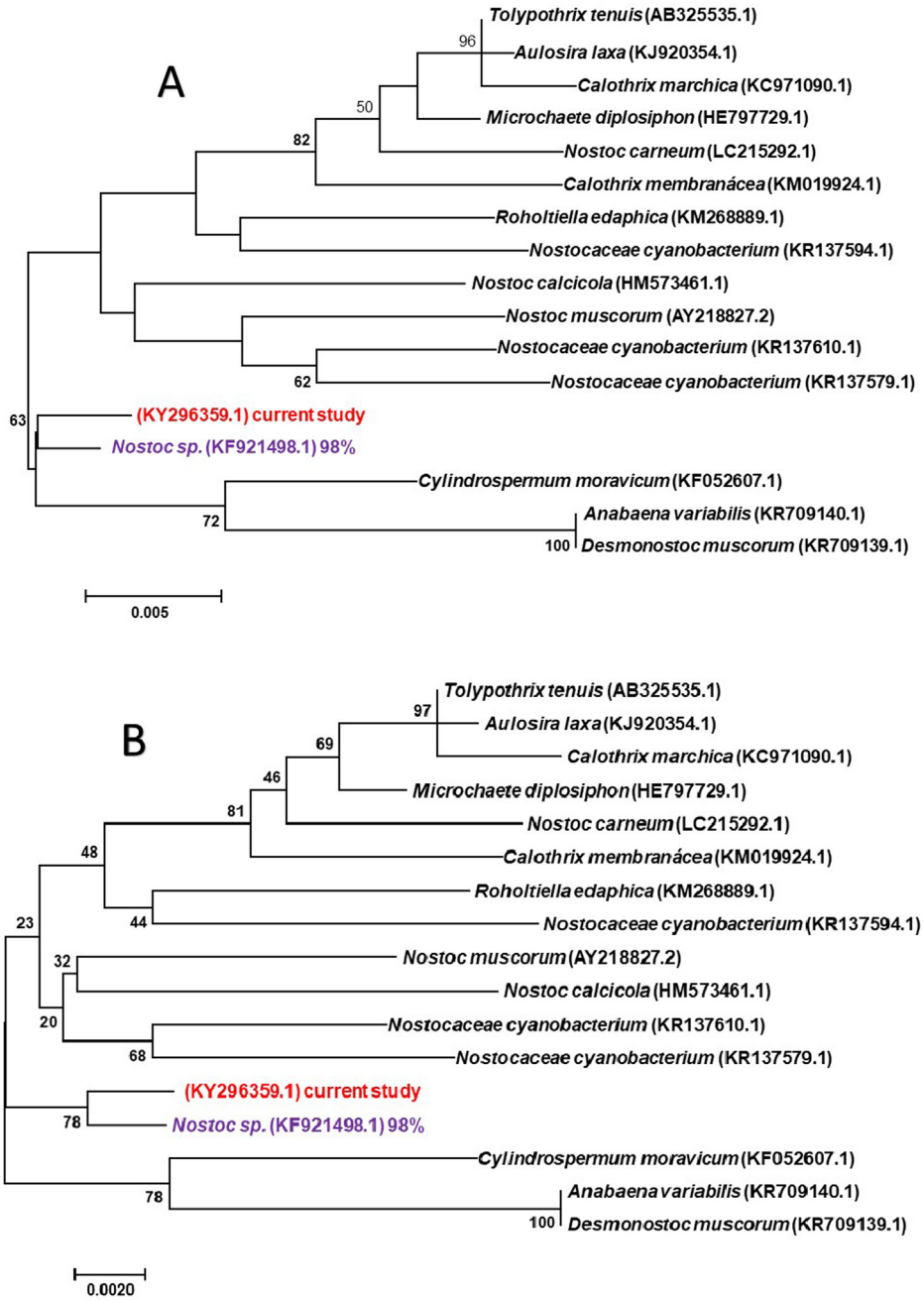

Fig. 6. The phylogenetic tree of the obtained sequence *Nostoc* sp. (KY296359.1) with the nearest one *Nostoc* sp. *PAN-549* (KF921498.1) recovered by maximum likelihood method (A) and neighbor-joining method (B). Average Bootstrap values, of compared algorithms, were indicated at the branch roots. The bar was represented 0.005 and 0.002 changes per nucleotide. Accession numbers of database extracted sequences were in black, Purple and red colors for the nearest and the obtained sequences, respectively.

than 95% Average Nucleotide Identity (ANI). Hence, this isolate could be a new species with novel and unique characteristics and this could be attributed to the area of isolation where high concentration of pollutants is found in the irrigated wastewater cannels. The phylogenetic tree confirmed the same identity ratios on the roots of clades. It clearly diverged from the nearest sequence (*Leptolyngbya* sp. *NIES-504*, LC319755.1,) and found to be developed in a new clade sharing the same ancestor with the family of *Pseudanabaenaceae* (Fig. 5). In contrast, the obtained isolates *Nostoc calcicola, Nostoc* sp. (KY296359.1), and *Nostoc* sp. (KU373076.1) were in the same clades with the nearest sequences *Nostoc calcicola* 99 (GQ167549.1), *Nostoc* sp. PAN-549 (KF921498.1) and *Nostoc* sp. *UIC 10,279* (JX962720.1) in database (Fig. 4, Fig. 6, and Fig. 7). These results reflect close similarity that seemed in the less number of SNPs and GAPs among the obtained sequences and the nearest sequences in database. The expected restriction maps of all obtained sequences (Fig. 8) displayed different endonucleases sites that could be important in building genetic maps and biodiversity studies. These results supported by Badr et al., (2018) study, who genetically identified two novel cyanobacterial isolates, *Sphaerospermopsis aphanizomenoides* and *Cronbergia siamensis*, via bioinformatics analysis of *16S rRNA* gene sequence, where they exhibited identity ratio 94% and 93% with the nearest NCBI deposited sequences.

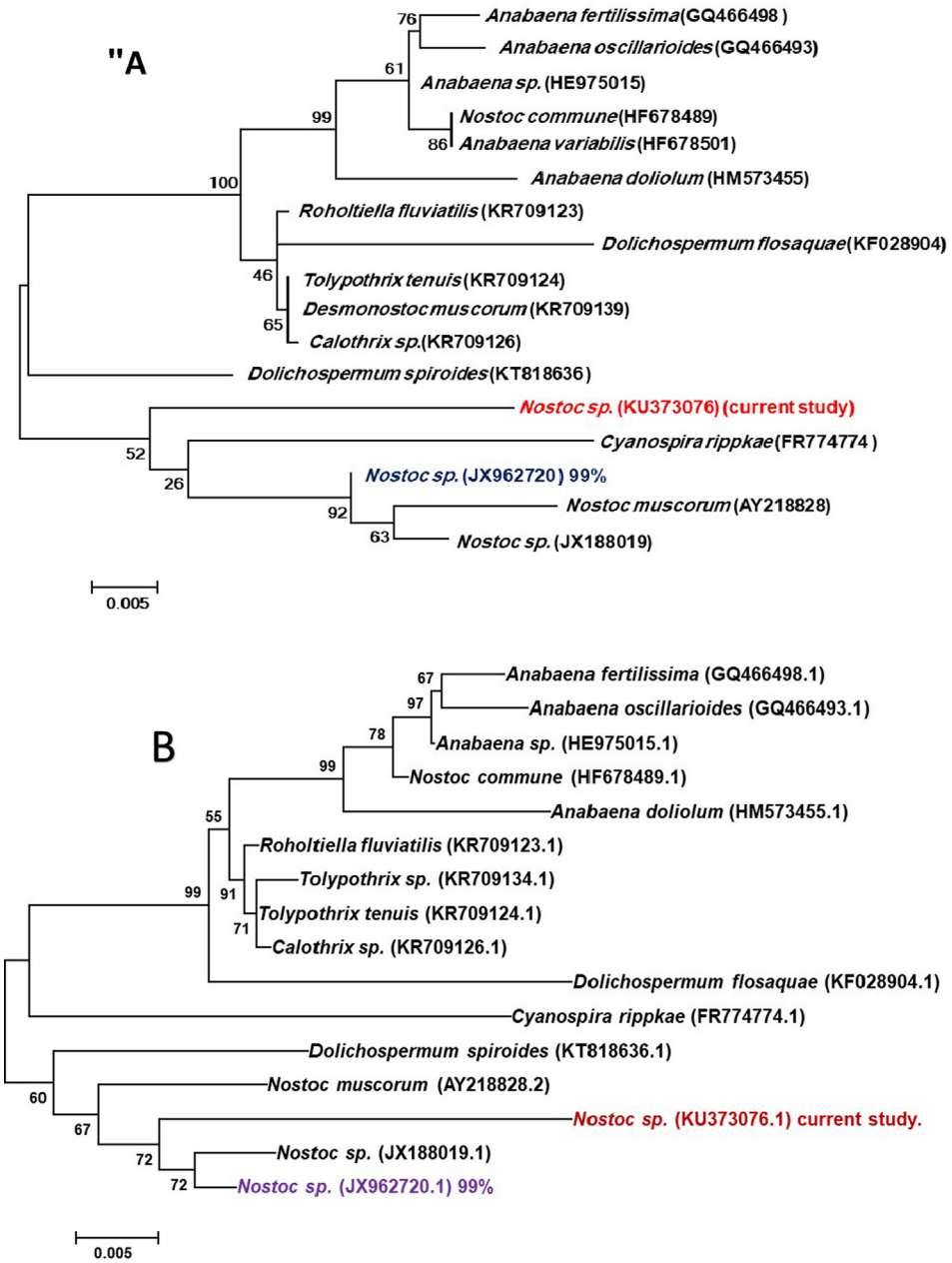

Fig. 7. The phylogenetic tree of the obtained sequence of *Nostoc* sp. (KU373076.1) with the nearest deposited sequence *Nostoc* sp. *UIC 10,279* (JX962720.1) recovered by maximum likelihood method (A) and neighbor-joining method (B). Average Bootstrap values, of compared algorithms, were indicated at the branch roots. The bar was represented 0.005 changes per nucleotide. Accession numbers of database extracted sequences were in black, Purple and red colors for the nearest and the obtained sequences, respectively.

3.2. Biochemical profile of the cyanobacterial isolates

Phenolic compounds have been detected in all the studied cyanobacterial isolates, where the phenolic content varied from 6.25 in *Nostoc calcicola* (KY250420.1) to 13.85 in *Nostoc* sp. (KU373076.1) μg gallic/g dried cyanobacteria (Table 1). From the results of nutritional profile, *Nostoc* sp. (KU373076.1) recorded the highest level of ferulic, coumaric, cinnamic, pyridoxine, gallic, and salicylic acids as well as zeaxanthin, riboflavin and vitamin A (Table 2). These results agree with Singh et al. (2017) who clearly demonstrated that, cyanobacteria contain a wide range of carotenoids, flavonoids, and phenolic compounds with a potential antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities. Phenolic compounds are considered as one of the most important classes of natural antioxidants (Machu et al., 2015). Various investigations reported that microalgae and cyanobacteria are natural sources of phenolic compounds however, few studies have focused on their identification and quantification (Cirulis et al., 2013; Safafar et al., 2015; Jerez-Martel et al., 2017). On contrast, Jerez-Martel et al. (2017) reported that phenolic constituents were not detected in *Nostoc* sp., *Leptolyngbya protospira, Nodularia spumigena, Phormidiochaete* sp., *Arthrospira platensis,* and *Caespitella pascheri* as well as they reported that among the studied cyanobacterial isolates gallic acid was only identified in *Nostoc commune*. However, Klejdus et al. (2009) stated that *Spongiochloris spongiosa, Spirulina platensis, Anabaena doliolum, Nostoc* sp., and *Cylindrospermum* sp. exhibited phenolic compounds at μg levels per gram of biomass. However, the current study results showed that the caffeic acid level was high compared with the results reported by Goiris et al. (2014) who screened flavonoids in different microalgal and cyanobacterial species and reported that none of the algal biomass samples contained neither the flavanone

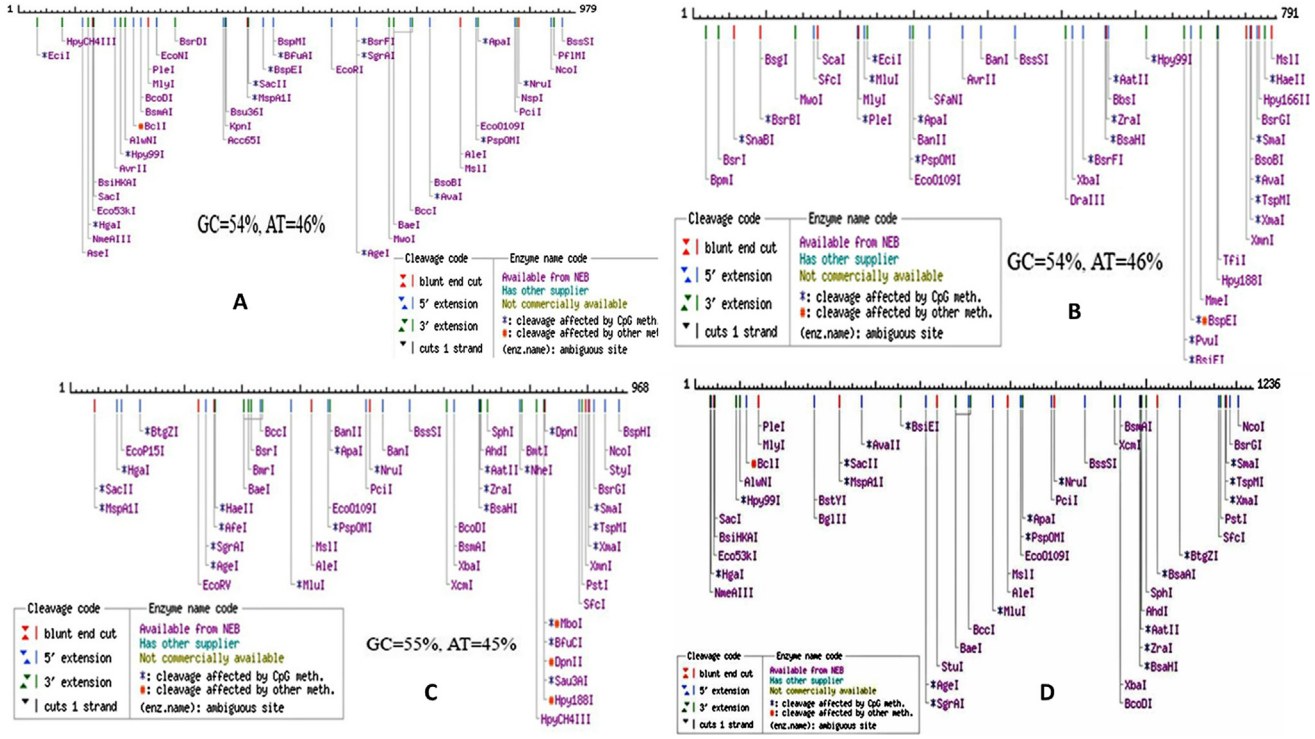

Fig. 8. Restriction map of the partial sequences of 16S rRNA genes for A- *Nostoc calcicola* (KY250420.1) B- *Leptolyngbya* sp. (KY321359.1) C- *Nostoc* sp. (KY296359.1) *D- Nostoc* sp. (KU373076.1) with available commercially restriction enzymes.

Table 1
Total phenolic compounds in the extracts of obtained cyanobacterial isolates.

Cyanobacterial extracts	Total phenolic compound (µg gallic/g dried algae)
Nostoc calcicola (KY250420.1)	6.25 ± 0.12
Nostoc sp. (*KY296359.1*)	8.45 ± 0.28
Leptolyngbya sp. (*KY321359.1*)	10.41 ± 0.21
Nostoc sp. (*KU373076.1*)	13.58 ± 0.17

● Data shown as mean ± SD for 5 replicates for total phenolic compounds of different cyanobacterial isolates.

Table 3
DPPH radical scavenging activity (%) of cyanobacteria.

Cyanobacterial isolate	Concentration µg /ml	Scavenging activity %
Nostoc calcicola (KY250420.1)	50	18.25 ± 0.37
Nostoc sp. (*KY296359.1*)	50	19.16 ± 0.39
Leptolyngbya sp. (*KY321359.1*)	50	22.94 ± 0.47
Nostoc sp. (*KU373076.1*)	50	29.33 ± 0.60

● Data shown as mean ± SD for 5 replicates for scavenging activity of different Cyanobacterial isolates.

eriodictyol nor its precursor caffeic acid. Thus, *Nostoc* sp. (KU373076.1) is considered a potent and promising isolate as a natural antioxidant source.

The DPPH (2,2-diphenyl-1-picrylhydrazyl), free radical scavenging assay, is used widely for evaluating antioxidant activity due to its stability, simplicity and reproducibility (Kuda et al., 2007). The ability of a compound to scavenge DPPH radicals is dependent on their ability to pair with the unpaired electron of a radical; the higher DPPH-scavenging activity, the higher antioxidant potentiality of the tested sample

Table 2
Nutritional profile of cyanobacterial isolates powder.

Contents	*Nostoc* sp. (*KY296359.1*)	*Nostoc calcicola (KY250420.1)*	*Leptolyngbya* sp. (*KY321359.1*)	*Nostoc* sp. (*KU373076.1*)
Thiamine HCL µg/g powder	0.0104 ± 0.0004	0.0089 ± 0.0003	0.0146 ± 0.0006	0.0343 ± 0.0015
Riboflavin µg/g powder	0.0038 ± 0.0001	0.0033 ± 0.0001	0.0054 ± 0.0002	0.0194 ± 0.0008
Nicotinamide µg/g powder	0.0461 ± 0.0020	0.0396 ± 0.0017	0.0649 ± 0.0029	0.1971 ± 0.0088
Pyridoxine µg/g powder	0.0716 ± 0.0032	0.0615 ± 0.0027	0.1008 ± 0.0045	0.1684 ± 0.0075
α- tocophero µg/g powder	0.0138 ± 0.0006	0.0118 ± 0.0005	0.0194 ± 0.0008	0.038 ± 0.0016
Vitamin A IU/ g powder	79.28 ± 3.54	68.12 ± 3.04	111.67 ± 4.99	269.02 ± 12.03
Total carotenoid				
β-carotene µg/g powder	0.0315 ± 0.0014	0.0271 ± 0.0012	0.0443 ± 0.0019	0.1792 ± 0.0080
Zeaxanthin µg/g powder	0.0246 ± 0.0011	0.0211 ± 0.0009	0.0347 ± 0.0015	0.0641 ± 0.0028
Phenolic Compound				
Caffeic acid µg/g powder	0.4154 ± 0.0185	0.3569 ± 0.0159	0.5851 ± 0.0261	1.1547 ± 0.0516
Cinnamic acid µg/g powder	ND	ND	0.6442 ± 0.0288	0.4261 ± 0.0190
Coumaric acid µg/g powder	0.0693 ± 0.0030	0.0595 ± 0.0026	0.0976 ± 0.0043	0.2511 ± 0.0112
Ferulic µg/g powder	ND	0.2793 ± 0.0124	0.4578 ± 0.0204	0.8103 ± 0.0362
Gallic acid µg/g powder	0.8394 ± 0.0375	0.6823 ± 0.0305	1.1822 ± 0.0528	2.5969 ± 0.1161
Salicylic acid µg/g powder	0.0125 ± 0.0005	ND	ND	0.9815 ± 0.0438

● Data shown as mean ± SD for 5 replicates for nutritional profile of different cyanobacterial isolates.

Table 4
% of decreased heavy metals by the different cyanobacterial isolates.

Metals percentage	*Nostoc calcicola* (KY250420.1)	*Leptolyngbya sp* (KY321359.1)	*Nostoc sp* (KY296359.1)	*Nostoc sp* (KU373076.1)
P%	89.2	80	93.8	96.7
No3%	73.9	49	65.3	76.85
k%	96.5	95.7	90.2	97.3
Mg + %	97.3	96.5	91.1	94.7
Fe + 2%	34.2	30.1	32.8	23.2
Zn%	100	100	100	100
Cu + 2%	90	100	100	100
Mn + 2%	41	54.8	20	50
Co + 2%	100	100	100	100
Cd + 2%	100	100	100	100
Pb + 2%	100	100	100	100

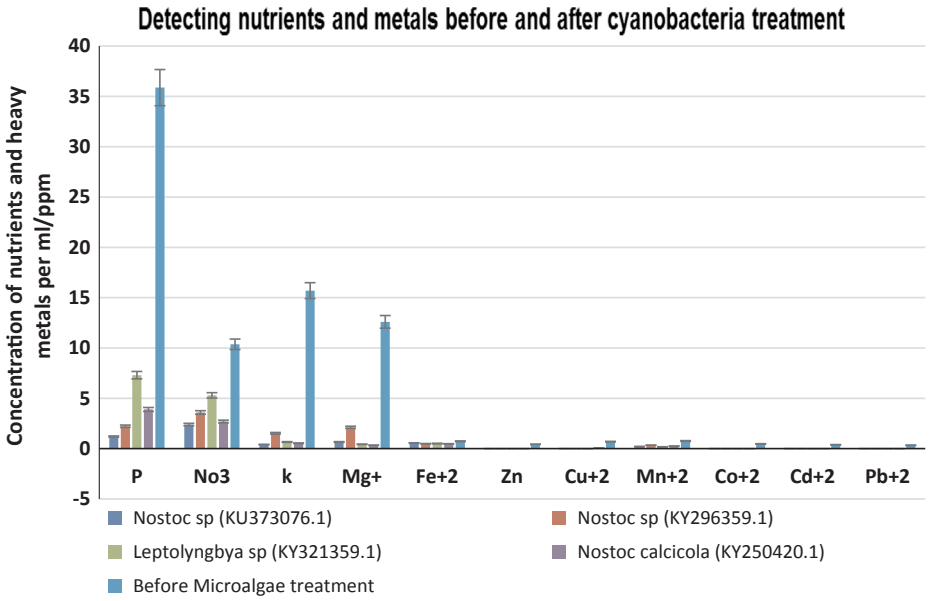

Fig. 9. concentration of nutrients and heavy metals before and after cyanobacteria treatments.

(Park et al., 2004). From the results presented in Table 3, all cyanobacterial isolates exhibited high capacity values to scavenge free DPPH radicals that ranged from 18.25% (*Nostoc calcicola*, KY250420.1) to 29.33% (*Nostoc* sp., KU373076.1) at concentration of 50 µg/ml. In the current study, cyanobacterial isolates displayed stronger relative radical scavenging efficiencies. These findings are relevant with Jerez-Martel et al. (2017) study, who determined the antioxidant activity of *Nostoc* sp., *Leptolyngbya protospira, Nodularia spumigena, and Phormidiochaete* sp., where DPPH radical ranged from 7.65% (*Leptolyngbya protospira*) to 27.89% (*Nostoc* sp.). The DPPH scavenging ability is a significant indicator of a potential antioxidant activity that may be due to the presence of phenolic compounds and flavonoids in the crude extracts of the isolates as confirmed by Hossain et al. (2016).

3.3. Wastewater treatment with the obtained cyanobacterial isolates

The isolate *Nostoc* sp. (KU373076.1) revealed the best phycoremediation ability via reducing phosphorus, nitrate, potassium and magnesium by 96.7%, 76.85%, 97.30% and 94.70%, respectively, (Table 4). Interestingly, all cyanobacterial isolates decreased lead, cadmium, zinc and cobalt by 100% (Fig. 9) and this may be due to that samples collected from Qalyubia irrigated canals have received contaminants from domestic, agricultural and industrial sources.

Cyanobacteria-based phycoremediation technologies have gained much attention recently as alternative bioremediation techniques over traditional methods for eco-friendly clean-up of metal-contaminated wastewater (Rawat et al., 2016; Sunday et al., 2018). Cyanobacterial wastewater treatment is effective and applicable in the removal of nutrients (K, N and P) and heavy metals also, the reduction of chemical and biological oxygen demand, removal of xenobiotic compounds and other contaminants (Olguín, 2003; Rawat et al., 2011; Abdel-Raouf et al., 2012; Cai et al., 2013). Wastewaters production are increasing with the increase in human populations especially in developing countries, where the aquatic environments are suffering from contamination with heavy metals which is one of the most serious problem in Egypt (Zahran et al., 2015). Many previous studies stated the ability of different cyanobacterial species to treat wastewater through removal of nitrogen, phosphors and heavy metals mainly by uptake these elements into algal cells as essential macronutrients for the growth of cyanobacteria (Aslan and Kapdan, 2006; Garcia et al., 2006; Ji et al., 2013). In this manner, the study of Foad et al. (2017) reported that dual bio treatment of *Chlorella vulgaris* and *Micrococcus luteus* achieved the best removal of pollutants from wastewater via reducing phosphorus by 78.71%, nitrate (65.46%), potassium (49.9%) and magnesium (78.8%) within an incubation period of 16 days. Also, Shanab et al. (2012) reported that *Pseudochlorococcum typicum* from aqueous solution showed high ratio of metal bioremoval in the first 30 min of contact by 97% (Hg2+), 86% (Cd2+) and 70% (Pb2+). In the current study, the studied cyanobacterial isolates were obtained from highly polluted sites with high concentration of heavy metals. So, these isolates exhibited high metal resistance with incomparable phycoremediation ability. These results agree with Shanab et al. (2012) and Qari et al. (2014) who stated that algae appearing in polluted sites are either metal-tolerant or metal- resistant species.

4. Conclusion

The four identified cyanobacterial isolates exhibited antioxidant activity and phycoremediation ability. The isolate *Leptolyngbya* sp. (KY321359.1) could be novas species due to the low identity ratio (90%) with the nearest sequences in the database. On the other hand, Nostoc sp. (KU373076.1) revealed the highest DPPH radical scavenging activity which could be attributed to its high content of the phenolic constituents like caffeic and ferulic acids. All cyanobacterial isolates revealed incomparable phycoremediation ability since they have been collected from stressed aquatic systems with different types of contaminates. Therefore, these cyanobacteria can be utilized either as agents for providing raw martials for cosmeceuticals and pharmaceutical or as potent and promising cyanobacterial species for wastewater treatment. This study shed the light on to what extent the environmental stresses could affect and stimulate cyanobacteria for the induction of different important secondary metabolites in such aquatic systems.

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