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Biosorption of some heavy metals from wastewater *by Pseudoanabaena mucicola*

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THIS STUDY targeted the concentrations of heavy metals (iron, manganese, copper, and zinc) in wastewater sources in Kafr El-Sheikh Governorate, Egypt, and proved their presence in different concentrations heavy metals (Fe, Mn, Cu, and Zn) concentrations in wastewater at Kafr El-Sheikh Governorate, Egypt, and proved their existence with different concentrations. Numerous microalgae were found to significantly remediate different heavy metals from wastewater. Within the study's conclusions were the isolation and identification of *Pseudoanabaena mucicola* under accession OR143298 for the remarkable capability of, chemical oxygen demand (COD), total suspended solid (TSS), total dissolved solid (TDS), biological oxygen demand (BOD) and heavy metals (Fe, Mn, Cu, and Zn) removal from wastewater at 28°C and different pH (7, 8 and 9) for 1-4 weeks. The decreasing capacity by *P. mucicola* were 90.03% for chemical oxygen demand (COD), 42.95% total dissolved solid (TDS), 89.88% biological oxygen demand (BOD), and increasing the TSS by 125.83% after 4 weeks. *P. mucicola* absorbs 100% Fe from wastewater after 3 weeks, Mn from wastewater after 2 weeks, Cu, and Zn from wastewater after 1 week, respectively. The results that were presented showed how promising *P. mucicola* is for biosorbing heavy metals (Fe, Mn, Cu, and Zn) from wastewater.

Keywords: Isolation; *P. mucicola;* heavy metals; removal; DNA barcode analysis.

1. Introduction

Because of their toxicity, persistence, and bioaccumulation, heavy metals are among the most dangerous pollutants and may pose a risk to the ecosystem (Nasr et al. 2006). The following criteria form the foundation of the idea of the thorough mapping of heavy metals soil pollution: (1) Determining the classes of heavy metal pollution in soil in order to estimate the potential for heavy metal pollution (2) Evaluation of the gradient and aspects of soil pollution caused by heavy metals (Abd-El-Hady and Abdelaty 2022). There are three classes of heavy metals are distinguished: *first*) transition elements, which include Ti, Zr, Hf, Rf, V, Nb, Ta, Cr, Mo, W, Mn, Tc, Re, Fe, Ru, Os, and Zn, among other minor amphoteric oxides; *second)* components of rare earth like La and Ac; and *thrid)* components of the p-group that are led by Ga, In, Tl, Sn, Pb, Sb, Bi, and Po (Ameri *et al.* 2020). The limits of heavy metals in wastewater according WHO (World Health Organization) (2004): Cu <1 mg/L, Fe < 0.4 mg/L, Zn < 0.2 mg/L, and Mn< 0.05 mg/L. Microalgae are considered one of the most abundant groups in nature. They are found in fresh and salt water, soil and air. They are used in many fields such as improvement of different crops (Ghazal *et al*. 2018b; Yanni *et al.* 2020; El-Habet and Elsadany, 2020; Geries and Elsadany , 2021. Deyab *et al.* 2021; El-Sheekh *et al.* 2022; El-Adl *et al.* 2022; Elsharkawy *et al.* 2022), and accumulators of heavy metals (Fekry *et al.* 2018a). *Anabaena variabilis* and *Tolypthrix ceytonica* can be used as metals

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accumulators of heavy metal. The maximum removals of Fe^{+2}, Zn^{+2}, Pb^{+2} and Cu^{+2} compared with control (El Bestawy, 2019). Shariful *et al*. (2021) discovered that *Chlorella vulgaris* and its iron-coated counterpart absorb arsenic more efficiently than other types of algae and their iron coatings. Within a day, arsenic contaminated water is absorbed by 23% and 67% of *Chlorella vulgaris* and Fe-coated *Chlorella vulgaris*, respectively. Blanco-Vieites *et al*. (2022) tested that *Tetradesmus obliquus*, *Chlorella* spp., and *Arthrospira* spp. exposure to wastewater contain iron under increasing concentrations of residue. Results showed a reduction 97.9% of iron by these microalgae. Goh *et al.* (2023) started that bioremediation is being pushed more and more as a substitute method for cleaning up a variety of contaminants. In particular, bioremediation which uses microalgae to remove pesticides or change them into less dangerous or harmless compounds is growing in popularity. Fast-growing aquatic plants known as microalgae are found naturally in a wide range of environments across the entire ocean, including freshwater and saltwater ecosystems. Because they are the first stage of trophic transfer and are found at the base of the food chain, microalgae are essential to preserving the balance of the aquatic ecosystem. In recent years, microalgae play role in the recovery of crucial nutrients from secondary effluents, including nitrogen and phosphorus. This method lessens the likelihood of eutrophication and the long-term pollution issues brought on by certain persistent organic micropollutants (Abdelfattah *et al*. 2022; Nguyen *et al*. 2021). In addition to providing tertiary treatment, microalgae culture in wastewater also produces biomass that is commercially attractive and useful for a variety of industrial uses. Furthermore, the biomass obtained from the cultivation of microalgae can be utilized as feedstocks for a wide variety of products with multiple industrial applications as well as for the commercial production of bioenergy, pesticides, dyes, heavy metals, and medications that come from a variety of industrial sectors including home wastewater, agricultural runoff, and pharmaceuticals have all been treated with microalgae. A broad range of organic and inorganic pollutants can be broken down and detoxified by microalgae through the processes of bio-adsorption, bioaccumulation, and biodegradation (Salama *et al.* 2017; Bhatt *et al.* 2022; Arutselvan, *et al.* 2022). A short nucleotide sequence from a standardized gene or gene spacer that has been entered into a major sequence database and is associated with a voucher specimen, the origins and current statuses of which are documented, is the DNA barcode (Hebert and Gregory, 2005). In order to shed light on the phylogenetic connections between various species, the plastid rbcL marker has been extensively employed in research on taxonomic positions of unknown species (Alshehri *et al*. 2019). The rbcL gene's consistent exon structure and strong amino acid sequence similarity made it a dependable marker for these investigations (Kazi *et al*. 2013). This study aims to remove some heavy metals from wastewater using cyanobacteria isolated from seawater.

2. Material and Methods

2.1. Sampling and sampling sites of wastewater

Four wastewater samples were collected during October 2022 from the wastewater in Kafr El-Sheikh Governorate, Egypt. **Table (1)** show the chemical analysis of wastewater in Kafr El-Sheikh Governorate. Samples were taken manually 30 cm under the surface of the wastewater and transferred to the laboratory in icebox. Before the results of the chemical analysis were determined, the samples were thoroughly mixed, filtered through a Millipore filtration system (Millipore Comp. 0.22 µm), and then stored at 4^{o}C in the dark. Chemical oxygen demand (COD), total dissolved solid (TSS), total dissolved solid and biological oxygen demand (BOD) were determined according to standard methods according to **(**Baird and Bridgewater, 2017**)**.

Table 1. The chemical analysis of Kafr El-Sheikh wastewater.

Chemicals	Concentration (mg L^{-1})
Total suspended solid (TSS)	215
Chemical oxygen demand (COD)	510
Electrical Conductivity	945
Biological oxygen demand (BOD)	32.13
Total Dissolved Solid (TDS)	500
Fe	0.70
Mn	0.3 0
Zn	0.0407
Cu	0.0015

Heavy metals were determined by atomic absorption spectrometry (Varian, AAS240FS) according to standard methods. Samples for heavy metals were collected in 250 ml polyethylene bottles containing 2 drops of 10% from HCl. After that, samples were drained for AAS analysis, to measure the concentration of heavy metals, add 10 mL of 1:1 HNO_3, mix the slurry, and cover the mixture with a watch glass or vapor recovery device. Samples should be heated to 95°C ± 5°C and refluxed without boiling for 10 to 15 minutes. Following the incubation periods (1,2,3 and 4 weeks), the measured samples underwent identical testing to determine the removal and biosorption percentages (AMWW, 2023).

2.2. Isolation of *Pseudoanabaena mucicola*

The samples of marine cyanobacteria were taken in the summer of 2022 from various habitats close to Elnakheel beach in the Alexandria Governorate. The samples were collected and brought straight to the laboratory in sterile polyethylene bags. The streaking plate method and serial dilution approach were used to isolate cyanobacterial species (Rippka, 1988). Sterilized BG_{11} media in test tubes was used to create the dilution. One milliliter sample was diluted ten times. Each dilution involved a thorough mixing of test tubes. In several instances, isolating a single species required more dilutions. Dilution tubes were kept in a compartment with continuous light (5000 Lux) and temperature at 28±2 ^{o}C. As soon as cyanobacteria in uni-algal culture was acquired.

2.3 Identification of *Pseudoanabaena* sp. by DNA barcode analysis

Identification and molecular characterization of strains *pseudoanabaena* sp. were carried out using a target sequences for chlorophyta was rbcL gene. The sequences rbcL have been submitted to NCBI respiratory and have been assigned to gene bank accession numbers (OR143298). The similarity percentage was maximum for rbcL (97.61%) compared to the database of the same species. The DNA barcode is a short nucleotide sequence from a standardized gene or gene spacer, deposited in a major sequence database, linked to a voucher specimen whose origins and current status are recorded (Hebert and Gregory, 2005). The plastid rbcL marker has been widely used for studying unknown species taxonomic position to clarify phylogenetic relationships between different species (Alshehri, *et al*., 2019). The stable exon structure of rbcL gene and high amino acid sequence similarity supported it as a reliable marker for these studies (Kazi *et al*. 2013).

2.4. DNA barcoding

2.4.1. DNA extraction and purification

Pseudoanabaena sp. was collected from sea water and use a sterile mortar and pestle to grind them into a fine powder while submerged in liquid nitrogen. DNA extraction and purification were carried out using the DNeasy Plant Kit (Qiagen, Germany). The concentration and purity of the extracted DNA were estimated by NanoDrop™ 2000/2000c Spectrophotometers.

2.4.2. PCR and gene sequencing

The PCR reaction was performed as mentioned by Ibrahim ***et al.*** (2019) in a total volume of 50 µL containing 25 µL (2X PCR Master Mix), 2 µL of each primer (10pcmol/µL), 3 µL genomic DNA (10ng/µL), and 18 µL dH2O. DNA barcoding analysis was performed with the chloroplast DNA (cpDNA) gene rbcL. For PCR amplification and sequencing of rbcL, the primer pairs rbcL-F (5'-ATGTCACCACAAACAGAGACTAAAGC-3'), rbcL-R (5'-TCGCATGTACCTGCAGTAGC-3') was used for the PCR. Average amplicon size: 600 bp A Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems, USA) was used to conduct the PCR. It was set up to complete 40 cycles following a 5-minute initial denaturation cycle at 94ºC. The steps in each cycle were as follows: 30 s of denaturation at 94ºC, 30 s of annealing at 50ºC, and 30 s of elongation at 72ºC. In the last cycle, the primer extension phase was prolonged to 7 minutes at 72 degrees Celsius. Electrophoresis was used to separate the amplification products on a 1.5% agarose gel with 0.5 ug/mL of ethidium bromide in 1X TBE buffer at 95 volts. To determine the PCR product size, a 100-bp DNA ladder (Promega, USA) was utilised as a molecular size standard. A UV transilluminator was used to see the gel images, and a Gel Documentation System (BIO-RAD 2000, USA) was used to take pictures. Using a QIAquick PCR Purification Kit (Qiagen, USA), PCR products were purified. Using a BigDye Terminator version 3.1 Cycle Sequencing RR-100 Kit (Applied Biosystems, USA) and a DNA sequencer (ABI 3730XL, Applied Biosystems) (Microgen, Korea), the sequencing of the PCR product was done via the dideoxynucleotide chain termination method in accordance with the manufacturer's instructions. To assign species DNA barcoding for *Pseudoanabaena* sp., the National Centre of Biotechnology Information (NCBI) website's Basic Local Alignment Tool (BLAST) was utilized. The sequence was deposited into the US GenBank.

Effect of pH on remediation of chemicals in wastewater by *Pseudoanabaena mucicola*

One hundred ml of wastewater were used to determine the effect of *P. mucicola* inoculation on removal of chemical oxygen demand (COD), total soluble solid (TSS), total dissolved salts, biological oxygen demand (BOD) and heavy metals (Fe, Mn, Cu, and Zn) from wastewater at 28± °C and different pH (7, 8 and 9) for 1-4 weeks with continuous light (5000 Lux) under shaking twice daily by hand. The

wastewater was inoculated by 10 ml of *P. mucicola* culture (10^8 CFU per ml). The experiments were carried out at pH 7, 8 and 9 and the culture was incubated at 28±2 ºC for 1-4 weeks and three replicates were used. The removal of chemical oxygen demand (COD), total soluble solid (TSS), total dissolved salts, biological oxygen demand (BOD) and heavy metals (Fe, Mn, Cu, and Zn) with *P. mucicola* was as described above **(Eaton *et al.* 2005)**.

2.5. Statistical analyses

The statistical analysis of the collected data was conducted using the methodology outlined by **Gomes and Gomes (1984)**. **Duncan (1995)** developed the multiple test (MRT), wherein means were compared at $p < 0.05$.

3. Results

3.1. Isolation and identification of the bioremediation agent

Pseudoanabaena sp. was isolated and purified. *Pseudoanabaena* sp. was isolated from the sea water. Using the morphological traits of microalgae strains described in literature as shown in **Fig. 1**. Trichomes actively moving by gliding movement, cylindrical, straight withpolar gas-vacuoles; sheath fine, thin, diffluent, occasionally present under settling of the samples in solid media; cell length > breadth 1.5-1.8 µ width, 3.9- 4.6 µ length. According to **El-Gamal (1995)**, cells 3.86- 470 µ with a mean value of 4.4 µ length, 1.5-167 µ with a mean value of 1.59 µ width.

3.1.1. Morphological characterstics

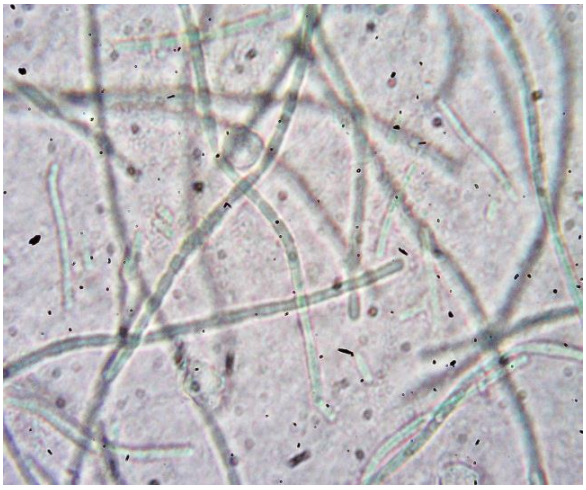

Fig. 1. The micromorphology of *Pseudoanabaena mucicola.*

***3.1.2.* Identification of *pseudoanabaena* sp. by DNA barcode analysis**

DNA barcoding was used to identify and classify the uncommon endemic *pseudoanabaena* sp. microalgae in order to conserve them. The results of BLAST matching and phytogenic tree analysis of *pseudoanabaena* sp. are shown in Table (2) and Fig. 2.

Table 2. DNA barcode of 1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) downloaded from GenBank database including species with similarity percentage of more than 84.48%.

Species	Accession no.	E value	Query coverage (%)	Similarity (%)
Pseudanabaena mucicola	KP698064.1	0.0	100%	97.61%
Pseudanabaena mucicola	KP698061.1	0.0	100%	97.44%
Pseudanabaena mucicola	CP097329.1	0.0	100%	97.09%
Pseudanabaena mucicola	KP698062.1	0.0	100%	97.09%
Pseudanabaena sp.	CP101416.1	0.0	99%	92.64%
Pseudanabaena sp.	AP017560.1	0.0	99%	92.47%
cyanobacterium clone	JX570973.1	0.0	96%	92.57%
Pseudanabaena galeata	CP112874.1	0.0	99%	90.24%
Pseudanabaena galeata	AB505118.1	0.0	99%	90.24%
Pseudanabaena sp.	AB075920.1	0.0	96%	84.49%

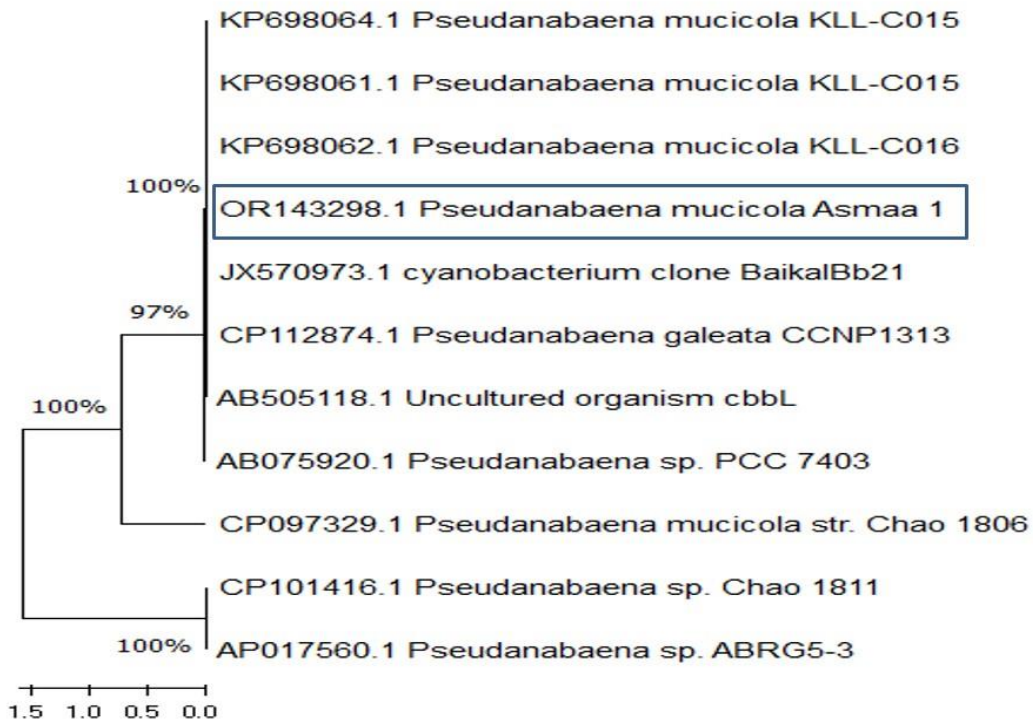

Fig. 2. Phylogenetic tree of sequence using the cpDNA marker rbcL combined ten data sequence alignment from Data base NCBI.

3.2. Effect of pH on Chemical Oxygen Demand removal efficiency from wastewater

The data presented in **Table (3)** show the effect of *Pseudoanabaena mucicola on* COD removal under different pH levels. The removal rate of COD recorded (454.65 mg. L) (90.03%) at pH9 compared with pH7 and 8 while the decreasing of COD % was recorded the maximum values at after four weeks from inoculation.

Table 3. Effect of *Pseudoanabaena mucicola* on wastewater chemical oxygen demand (COD) (mg/L) under different levels of pH.

Treatments	pH	Chemical Oxygen Demand (COD) (mg/L)			
		$Week_1$	$Week_2$	$Week_3$	$Week_4$
Control	7	509.8±0.12 ab	510.2±0.4 a	502.05±0.05 a	502± 1 c
	8	510.5±0.5 a	510.2±0.4 a	506.5 ±0.5 a	506.5 ±0.5 a
	9	509.5±0.5 b	508.7±0.4 b	504±1 a	505± 0 b
P. mucicola	7	282.8 ±0.15 c	169.3±0.2 c	131± 1 c	72.105±0.99 d
	8	277.9 ± 0.05 d	169.3 ±0.2 c	109.7±0.2 5 c	66.2± 0.1 e
	9	265± 1 e	161± 1 d	163.1 ±0.32 b	50.35±0.05 f
Significance -test		**	**	**	**

Control: Wastewater without *P. mucicola*

At the same experimental unit (rows), repeated measured ANOVA used to measure the statistical significance (*: $p < 0.05$; **: $p < 0.01$; ns: non-significant) of the differences between sampling times. ± Standard errors

3.3. Effect of pH on total suspended solid removal efficiency

The results in **Table (4)** show the impact of *P. mucicola* on TSS. TSS increase with increase pH and the maximum value was recorded at pH 9 (488.1) (125.83%) compared pH7 and 8 after three weeks from inoculation and sharp decrease of TSS after four weeks. This due to the wastewater as a source of nutrients such as N, P, and other elements.

Table 4. Effect of *Pseudoanabaena mucicola* on wastewater total suspended solid (TSS) (mg/L) under different levels of pH.

Treatments	pH	Total Suspended Solid (T.S.S) (mg/L)			
		$Week_1$	$Week_2$	$Week_3$	$Week_4$
Control	7	215.3 ±0 f	216.05 ±0.05 e	216.31 ±0.01 d	216.43±0.04 e
	8	215.85±0.05 e	216.2 ±0.01 e	216.325±0.03 5 d	216.5 ±0.01 d
	9	216.25 ±0.25 d	216.75 ±0.05 d	216.14±0.02 d	216.255 ±0.035 f
P. mucicola	7	340.1± 0.1 c	340.945 ±0.025 c	463.8±2.48 c	278.05 ±0.05 a
	8	451.05 ±0.05 b	451.515 ±0.025 b	473.5±0.2 b	219.82 ±0.0 1 c
	9	459.4 ±0.1 a	460.3 ±0.2 a	488.1±0.1 a	259.35±0.05 b
Significance -test		**	**	**	**

Control: Wastewater without *P. mucicola*

At the same experimental unit (rows), repeated measured ANOVA used to measure the statistical significance (*: $p < 0.05$; **: $p < 0.01$; ns: non-significant) of the differences between sampling times ± Standard errors

3.4. Impact of pH on total suspended solid removal efficiency from wastewater

The results in **Table (5)** illustrated the effect of *P. mucicola* on total dissolved solid (TDS). It is clear that the removal of (TDS) increase with time and the highest removal value at four weeks from inoculation (366.5 mg. L.) (42.95%) under pH 9 compared with pH 7, pH 8, and control.

Table 5. Effect of *Pseudoanabaena mucicola* on wastewater total dissolved solid (TDS) (mg/L) under different levels of pH.

Treatments	pH	Total Dissolved Solid (TDS) (mg/L)			
		$Week_1$	$Week_2$	$Week_3$	$Week_4$
Control	7	637.4±0.22 ab	598.5±0.98 e	639.04 ±1.01 b	598.6±1.02 b
	8	626.7±0.06 b	617.2 ±0.98 d	608.4±0.98 d	597.6 ±1.01 b
	9	643.2±1 ab	633.6±1.00 c	643.17 ±0.94 a	644.17±1.07 a
P. mucicola	7	662.8±0.06 ab	701.32±1.05 a	642.8±1.03 a	487.6±0.97 c
	8	648.7±1.52 ab	703.3±2.59 a	642.3±1.12 a	452.40±0.62 d
	9	772.5±1.7 a	688.42 ±1.03 b	630.09±1.03 c	366.5±0.86 e
Significance -test		**	**	**	**

Control: Wastewater without *P. mucicola*

At the same experimental unit (rows), repeated measured ANOVA used to measure the statistical significance (*: $p < 0.05$; **: $p < 0.01$; ns: non-significant) of the differences between sampling times ± Standard errors

3.5. Effect of pH on Biochemical Oxygen Demand removal efficiency

The data presented in **Table (6)** shows the effect of *P. mucicola* on BOD removal under different pH levels. BOD is the term for the ability of microorganisms to use molecular oxygen as an oxidizing agent in order to oxidize organic material to CO_2 and water. As a result, BOD can cause receiving water's dissolved oxygen to decrease, resulting in the anaerobic system. High removal percentages of BOD from wastewater using *Pseudoanabaena* was observed. After four weeks of inoculation, the average percentage BOD reduction determined was 89.88%.

Table 6. Effect of *Pseudoanabaena mucicola* on wastewater biochemical oxygen demand (BOD) (mg/L) under different levels of pH.

Treatments	pH	Biochemical Oxygen Demand (BOD) (mg/L)			
		$Week_1$	$Week_2$	$Week_3$	$Week_4$
Control	7	316.1±1.01 b	318.4±2.67 ab	339.9±3.12 b	331.3 ±1 b
	8	329.2±2.23 a	490.6±291.1 a	324.4±0.86 c	334.6±0.87 a
	9	316.5± 0.89 b	325.5±0.75 ab	343.4 ±1 a	323.2±1 c
P. mucicola	7	179.6 ±1.50 c	112.2±1.05 b	82.2 ±1 e	46.8 ±1.00 d
	8	181.6±1.015 c	108.3±1.12 b	73.84±0.7 5 f	41.04 ±0.06 e
	9	173.9±1 d	104.6±1 b	86.8±2.99 d	32.72±0.98 f
Significance -test		**	**	**	**

Control: Wastewater without *P. mucicola*

At the same experimental unit (rows), repeated measured ANOVA[A] used to measure the statistical significance (*: $p < 0.05$; **: $p < 0.01$; ns: non-significant) of the differences between sampling times. ± Standard errors

3.6. Effect of *P. mucicola* inoculation on Fe^{++} removal after three weeks

The Fe removal percentage from wastewater was showed in **Table (7)** under different pH levels. The results obtained by removal efficiency showed that *P. mucicola* was able to remove 68.97% of the total iron content at pH 9 after two weeks from inoculation and removal 100% after three weeks in all treatments.

Table 7. Effect of *P. mucicola* inoculation on Fe^{++} (mg/L) removal.

Treatments	pH	Removal of (Fe^{++}) (mg/L) from wastewater		
		$Week_1$	$Week_2$	$Week_3$
Control	7	0.709±0.002 ^{a}	0.70 ±0.002 ^{a}	0.709±0.002 ^{a}
	8	0.709±0.002 ^{a}	0.70 ±0.002 ^{a}	0.709±0.002 ^{a}
	9	0.709±0.002 ^{a}	0.709±0.053 ^{a}	0.709±0.002 ^{a}
P. mucicola	7	0.514±1.03 ^{c}	0.26±0.01 ^{b}	0±0 ^{b}
	8	0.53±0.003 ^{b}	0.22±0.01 ^{bc}	0±0 ^{b}
	9	0.523±0.01 ^{b}	0.22±0.01 ^{c}	0±0 ^{b}
Significance -test		**	**	**

Control: Wastewater without *P. mucicola*

At the same experimental unit (rows), repeated measured ANOVA used to measure the statistical significance (*: $p < 0.05$; **: $p < 0.01$; ns: non-significant) of the differences between sampling times. ± Standard errors

3.7. Effect of *P. mucicola* inoculation on Mn^{++} removal

The results in **Table (8)** show that the removal percentage of Mn from wastewater reached 100% after one week from inoculation with *P. mucicola* at pH7, 8 and 9 at 28±2 ºC compared with control treatments.

Table 8. Effect of *P. mucicola* inoculation on Mn^{++} (mg/L) removal.

Treatments	pH	Removal of (Mn^{++}) (mg/L) from wastewater	
		$Week_1$	$Week_2$
Control	7	0.3±0.015 a	0.33±0.015 a
	8	0.33 ±0.015 a	0.33±0.015 a
	9	0.3 ±0 b	0.33±0.015 a
P. mucicola	7	0.011c ±0.001	0 ±0 b
	8	0.013 ±0 c	0 ±0 b
	9	0 ±0 c	0 ±0 b
Significance -test		**	**

Control: Wastewater without *P. mucicola*

At the same experimental unit (rows), repeated measured ANOVA[A] used to measure the statistical significance (*: $p < 0.05$; **: $p < 0.01$; ns: non-significant) of the differences between sampling times. ± Standard errors.

3.8. Effect of *P. mucicola* inoculation on Cu^{++} and Zn^{++} removal

The results in **Table (9)** show that the removal percentage of **Cu^{++} and Zn^{++}** from wastewater. **Cu^{++} and Zn^{++}** removal reached 100% after one week from inoculation with *P. mucicola* at pH7, 8 and 9 compared with control treatments.

Table 9. Effect of *P. mucicola* inoculation on Cu^{++} and Zn^{++} (mg/ L) removal.

Treatments	pH	Removal of Cu^{++} and Zn^{++} mg/L from wastewater after one week	
		Cu^{++}	Zn^{++}
Control	7	0.001±4.04 [a]	0.001±4.04 [a]
	8	0.001±4.04 [a]	0.001±4.04 [a]
	9	0.001±4.04 [a]	0.001±4.04 [a]
P. mucicola	7	0±0 [b]	0±0 [b]
	8	0±0 [b]	0±0 [b]
	9	0 ±0 [b]	[A]0 ±0 [b]
Significance -test		**	**

Control: Wastewater without *P. mucicola*

- At the same experimental unit (rows), repeated measured ANOVA[A] used to measure the statistical significance (*: $p < 0.05$; **: $p < 0.01$; ns: non-significant) of the differences between sampling times. ± Standard errors

4. Discussion

Microalgae are the source of O_2 in the aquatic atmosphere and the hyperaccumulator of heavy metals. These species generate a lot of biomass, which can be utilized for innovative products like biodiesel, biomethane, organic fertilizers, nanoparticles, and pharmaceuticals as well as for remediation. Because different strains have distinct physical and metabolic characteristics, it is evident that different heavy metal remediation techniques vary depending on the strain (Chugh *et al*. 2022). The strain's micrographs were analyzed (Rani *et al.* 2022). The strain was found to have close resemblance with *Pseudoanabaena mucicola* The *pseudoanabaena* species of the highest percentages of similarity are represented. Newly generated sequences of the rbcL marker barcode. The alignment of rbcL sequence against GenBank accessions yielded query coverage between 96 to 100%. Sequencing for rbcL regions of *pseudoanabaena* resulted in 585-bp sequences (affected length of the query). Sequence alignment analysis revealed highest similarity percentages 97.61% the genus *Pseudanabaena mucicola.*

The highest specific growth rate of *Pseudanabaena* sp. was in the pH range from 7 to 9. The higher biomass yield of microalgae and better nutrient removal rate when the pH of secondary effluent was 9.0 and the removal rate of COD was 52.69% (Gao, *et al*. 2018; Wang *et al.* 2023). These findings demonstrated that the high alkaline (pH 9) environment was more favorable for nutrient removal. Low pH affects microalgae's ability to absorb nutrients and also lowers the activity of enzymes involved in photosynthesis, which may be the reason for the inhibition of nutrient removal in an acidic environment (Sari *et al.* 2013). *Pseudoanabaena* sp. prefers alkaline environments for survival, according to experiment results. Rather *et al*. (2023) reported microalgae have proven to be excellent at biosorbing substances and to be very successful at clearing pollutants out of a variety of water environments. During wastewater treatment, *Chlorella vulgaris* exhibited an approximately 85% removal efficiency for total phosphorus (TP) and an approximately 89% removal efficiency for total nitrogen efficiency (1.8 g/L) was attained in organic nutrients, particularly phosphate and nitrogen, which are necessary for their metabolic development.

Dolatabadi, and Hosseini (2016) discovered that TDS had decreased by more than 50%. TDS (2000 mg/L), pH (9.36), light intensity (4,010 lux), and duration of light-dark phase (15 h) were considered to be the ideal conditions for predicting 76% TDS removal efficiency. The maximum biomass efficiency of *S. maxima* (1.8 g/L) was achieved at 2,000 mg/L of TDS and ideal conditions for the remaining parameters. Nutrients, particularly nitrogen and phosphate, are necessary for their metabolic growth (Ghalharia *et al*. 2022). This result shows the *Pseudoanabaena* can absorption and removing of dissolved salts and nutrients from wastewater.

BOD is the term for the ability of microorganisms to use molecular oxygen as an oxidizing agent in order to oxidize organic material to CO_2 and water. As a result, BOD can cause receiving water's dissolved oxygen to decrease, resulting in the anaerobic system. *Pseudoanabaena*, grows when carbon dioxide and light are present. Thus, with the aid of an enzyme known as carbonic anhydrites, microalgae can use bicarbonate ions (HCO_3) or carbon dioxide as a carbon source for photosynthesis. Furthermore, inorganic nutrients are necessary for their metabolic growth, particularly phosphate and nitrogen. (Munoz and Guieysse, 2006). Highest removal efficiency of COD and BOD was 81% and 19% in the mixotrophic

growth with *S. parvus* (Ooi, *et al*. 2023). *P. mucicola* was able to remove 68.97% of the total iron content at PH 9 after two weeks from inoculation and removal 100% after three weeks in all treatments, which are similar to the iron removal ratio obtained by (Serr *et al.* 2020; Blanco-Vieites *et al.* 2022). The manganese, a nutrient that is essential for microalgae, prevents algal growth. Significant amounts of Mn (III/IV) oxides were found both intracellular and extracellular, resulting from Mn(II) oxidation. This suggests that photosynthetic algae may modify the Mn cycle by converting soluble Mn (II) to intracellular bound Mn and subsequently to solid-state Mn (III/IV) oxides. Through indirect oxidation, microalgae may also accelerate Mn (II) oxidation by raising the pH of the solution and producing more dissolved oxygen as they grow and this agreement with (Knauer *et al*. 1999). Microalgae recorded high removal efficiency towards copper, cobalt, lead and manganous from sterilized sewage wastewater after 10 days of incubation at percentages of 33.3, 33.3, 86.2 and 40, respectively (El-Sheekh *et al.* 2005). Up to 81.7% of the copper was eliminated by microalgae, with a final concentration of 7.8 ppb achieved after 10 days (Alison *et al*. 2018). Cu, Mn, and Zn can be adsorbed by microalgae due to binding groups on their cell surfaces (Saavedra *et al*. 2018). After ten days, zinc had decreased by up to 94.1%, to 0.6 ppb (Alison *et al*. 2018).

5. Conclusions

The isolated *pseudoanabaena* from sea water belong to *mucicola* specie. *P. mucicola* able to removal of heavy metals from wastewater and decrease TSS, COD, and BOD. This finding demonstrates the potential of microalgae culture as a cutting-edge method for recovering compounds from waste materials and bringing wastewater in the future closer to a circular economy.

Conflicts of interest: In accordance with our policy on Conflict of interest please ensure that a conflicts of interest statement is included in your manuscript here. Please note that this statement is required for all submitted manuscripts. If no conflicts exist, please state that “There are no conflicts to declare”.

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