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Microbial Remediation of some Heavy Metals in Wastewaters of Lake Manzala, Egypt

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ABSTRACT

Lake Manzala is Egypt's largest lake and one of the most important fishing grounds in the country. Unfortunately, the lake is confronted with numerous environmental challenges that have an impact on water quality. Thus, the purpose of this study was to evaluate some aspects of lake water. Seasonal water samples were collected from three stations in El-Kaboty area in Lake Manzala during the year 2020 (autumn, winter, spring and summer). Water samples were collected and physically and chemically analyzed. The values of temperature (17.5°C in spring -32.6°C in autumn), pH (6.5 – 7.2), EC (1.0-3.8), Iron (0.212-0.072mg L^{-1}), Manganese (0.017-0.066mg L^{-1}), Copper (0.005-0.009mg L^{-1}), Lead (0.005-0.009mg L^{-1}), Cd (0.003-0.009mg L^{-1}) and Zn (0.018- 0.043mg L^{-1}) were measured. It is noteworthy that the water of Manzala Lake has a percentage of pollution, but it did not exceed the permissible limits of the Egyptian legislation in the national law 48/1982. Reducing the pollution load is recommended to avoid exceeding the permissible percentage that reaches the Mediterranean Sea.The possibility of using isolates to remove iron, manganese, copper, lead, cadmium, and zinc from industrial effluents has been determined. The most effective bioremediation was *Aspergillus niger* . Both minerals were bioprocessed at high rates (Fe 48.1%, Mn50.9%, Cu55.5%, Pb63.4%, Cd 95.5%, Zn 36%). The study on the efficacy of *Aspergillus niger* in reducing heavy metal concentrations is consistent. Thus, it proved its ability to treat other metals with high percentages (Mn100%, Cu88.8%, Pb63.4%, Cd95.5%, Zinc 36%).

INTRODUCTION

Water pollution is a worldwide problem **(Ki *et al*., 2019)**. Pollution from heavy metals is considered particularly pernicious since these contaminants do not break down into less harmful forms and are poisonous to many different kinds of life **(Ismail & Hettiarachchi, 2017)**. A major hazard to the environment and public health is posed by the accumulation of heavy metals in food systems **(Mandour, 2021)**.

Heavy metals, such as Fe, Mn, Cu, Pb, Cd, and Zn are required by biota at trace levels and are recommended as daily dietary supplements. Manzala Lake is the Nile delta's largest shallow lake, located at latitudes (31°10'' to 31°40'' N) and longitudes (31°50'' to

32°25" E) **(Ismail & Hettiarachchi, 2017)**. Manzala Lake has a surface area of approximately 1,471.92 km2. The lake is shallow, with depths ranging from 0.7 to 1.5 m. It is connected to six drains that run along the Southern and Western shores **(Donia & Hussein, 2004)**. Shown in Egypt's North-East region near the Suez Canal **(Ki *et al*., 2019; Mandour, 2021)** (Fig. 1).

The opening of Bogaz, which has three outlets, connects the lake with the Mediterranean: Embassy, El Gemayel, El Gemila El Jadida **(El-Badry & Khalifa, 2017)**. They are responsible for the exchange of water between the lake and the Mediterranean Sea, as well as the revitalization of the lake's ecosystems. The lake is also connected to the Suez Canal through a very narrow canal known as the Kabbati Canal. The El-Anania and El-Routa canals are lateral arteries feeding the lake with the fresh water of the Nile from the Damietta branch **(Hossen & Negm, 2016)**.

Thousands of cubic metres of untreated domestic, industrial, and agricultural drainage water are dumped into the lake each year. The Lake receives a high level of pollution from industrial, domestic, and agricultural sources. Large amounts of water flow through numerous inlets on the western and southern coasts. A substantial amount of wastewater was discharged into the lake including particulate matter, nutrients, bacteria, heavy metals, and other toxic organic contaminants. Every year, the lake receives approximately 7500 million cubic meters of untreated industrial, domestic, and agricultural drainage water, which was discharged via six main drains (Table 1): Bahr El-Baqer (domestic and industrial sewage), Hadous, Ramsis, El-Serw, Matariya and Faraskour drains (agricultural wastewater). This amount of wastewater was reduced to about 4000 million cubic meters after the construction of ElSalam Canal **(El-Ghazalim *et al*., 2015)**.These drains affect the size and quality of the lake, endangering human health and causing a serious pollution problem **(Mageed, 2007)**. As a result of these findings, pollutants such as nutrients and heavy metals were assessed. In the sediments of the lake, heavy metals such as iron, manganese, copper, lead, cadmium and zinc accumulate. Heavy metals were detected in Lake Manzala in the study of **Elewa *et al*. (2007)**.

MATERIALS AND METHODS

2.1 Sampling and sites

Water samples were collected from 3 sites (L1, L2 and L3) from the El Kabbati region (31°25'06.0000"N latitude and 32°25'60.0000"E) (Fig. 1) during all seasons (winter, spring, summer and autumn) of 2020. Water samples were collected from a depth of 10-15cm below water surface in all study locations, using a clean sterile glass and preserved with 5ml of 70% conc. nitric acid that was added to 250ml of each sample **(Soad , 2018)**.

Fig. 1. Lake Manzala geographical location

2.2. Analytical procedures

2.2.1. Physico-chemical analysis of water

Temperature of the water, pH value, and electric conductivity (EC) were immediately measured in the field, using pH meter model HI 8314 and digital conductivity meter HI2300 Hanna Ins. Romania, respectively.

2.2.2. Heavy metals analyses

The concentrations of heavy metals were determined using atomic emission spectrophotometry with inductive coupling plasma (ICP-OES) on a PERKIN ELMER Optima 2000 DV device, Canada.

2.2.3. Microbial remediation procedure

Psudomonas.aeruginosa and *A.Niger* were isolated from lake's water and used in this process in full compliance with the remediation method recommended by the American Public Health Association **(APHA, 2012)**. Pure cultures of *Psudomonas.aeruginosa and A.Niger* were selected to study the activity of some microorganisms during the removal of heavy metals reported in the wastewater of the Manzala Lake. The synthetic wastewater contained mixed concentrations of heavy metals presented in the lake wastewater. The heavy metals were Fe (0.1225 mgl^{-1}), Mn (0.0346mgl^{-1}), Cu (0.007mgl^{-1}), Pb (0.0075mgl^{-1} &Cd (0.006 mgl^{-1}), and Zn (0.032 mgl^{-1}). The concentrations of heavy metals are more or less equal to those recoded in lake water. For *Psudomonas.aeruginosa* procedure, 3 of 250ml conical flask with each containing 100ml of prepared synthetic wastewater was sterilized.

Flasks were inoculated by 20ml inoculum medium uniform bacteria cell density of approximatly 3.8×10^3cfu mL^{-1} and 3.8×10^3cfu mL^{-1} for fungal strain efficacy, respectively. For 14 days, samples were incubated at 30°C. An atomic emission spectrophotometry was used with inductive coupling plasma (ICP-OES) on a PERKIN ELMER Optima 2000 DV device to measure the residual heavy metal.

2.3. Statistical analysis

The one-way ANOVA and Duncan multiple range tests were used to determine whether there was a significant difference in concentrations between the study sites. A probability of 0.05 or less was considered significant **(Bailey, 1982)**. In addition, the standard deviations were calculated.

RESULTS

3.1 Physico-chemical parameters of water in Lake Manzala

Temperature, salinity, pH, conductivity, and total dissolved solid (TDS) of Lake Manzala water were seasonally measured at all sampling locations during the study period, and the results are summarized in Table (2).

Table (2) shows that there's no large discrepancy in water temperatures between the three sampling locations during the same season. Autumn temperature ranged from 31.2 to 33.4°C, winter temperature ranged from 18.5 to 20.5°C, spring temperature ranged from 17.3 to 17.8°C, and that of summer ranged from 22.9 to 22.1°C. No discernible temperature difference was detected between the investigated locations (Fig. 3A). Temperature has an impact on the chemistry and biological activities of organisms in water. Thus, it was assumed to have an effect on other variables such as pH and conductivity.

Furthermore, no statistically significant differences in water pH were observed between the two sampling locations or seasons. The pH ranged from 7.1-7.5 in autumn to 7.1-7.3 in winter, 6.9-7.1 in spring to 6.2-6.9 in summer (Fig. 3B). The high pH value at some locations is due to the lake's direct connection to the Mediterranean Sea, which allows water to flow into Lake Manzala. Organic matter fermentation, as well as the release of hydrogen sulphide and methane gases and raw sewage freshwater drains could account for the lower pH value of 6.5 observed in sites. When organic matter in drain water degrades, it consumes oxygen and emits CO2, causing the lake to become acidic. This finding is consistent with the findings of **Fathi and Abdelzahar (2003)** and **Ahmed *et al*. (2013)** who reported that, the changing values of the water pH in EL-Kaboty was always between 6.5 and 7.2.

Salinity (electrical conductivity) values of water samples collected from the study sites (Fig. 3 C, D) iw represented in accordance with the time period.The salinity of the water is not high; it ranged between 2.6 and 6.3. The high salinity rate is due to the proximity of the location to the sea/lake connection. The water's salinity, on the other hand, is one-quarter of that of the sea water. The measurements may provide compelling evidence that this is not the case. The lake and sea are inextricably linked.

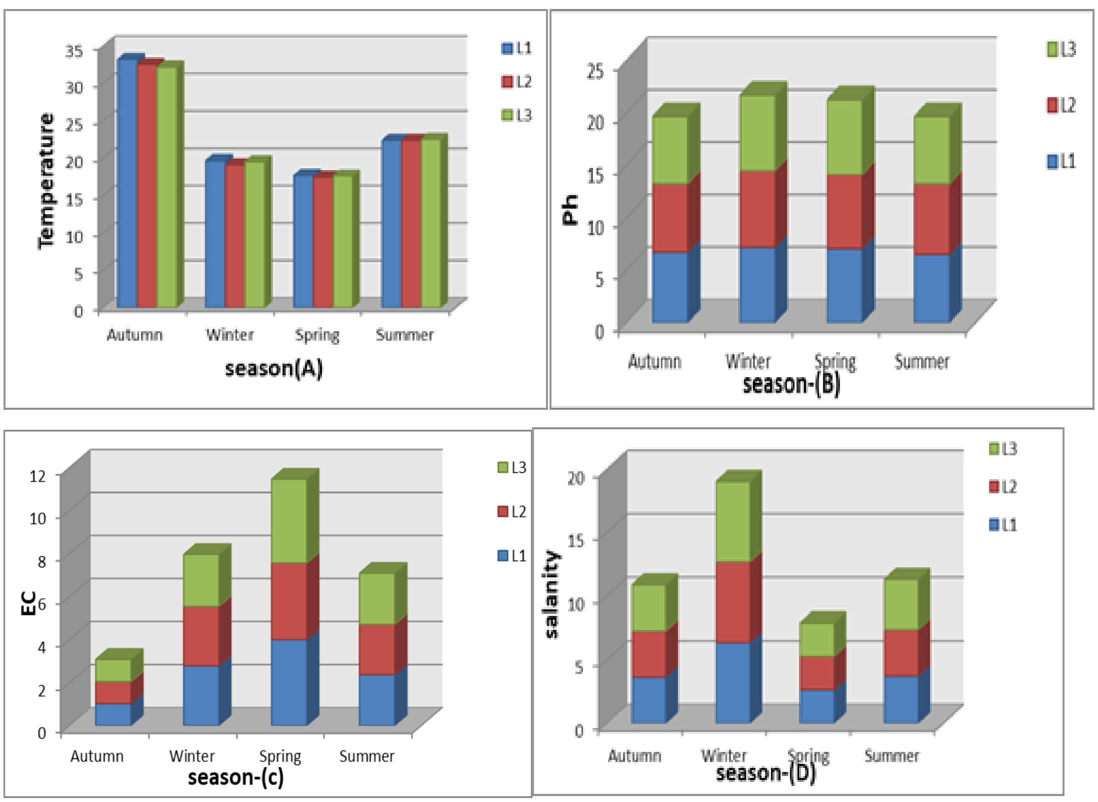
The values of total dissolved solids (TDS) varied significantly between the sampling locations. The lowest TDS value (4040 mg L^{-1}) in water was measured in L3. While, the highest value was recorded in the L (4088mg L^{-1}) throughout summer and winter (Fig. 3E). Lake Manzala according to **El-Gawady (2002)** and **Shakweer (2005)** can be dvided into two salinity-based zones. As a result of receiving high volumes of low salinity drainage water through various drains, the southern region has lower salinity values and a high amount of nutrients and heavy metals, and the second region is in the lake's north eastern area, near the lake-sea connection **(Ramadan, 2003)**, which owes its existence to geological and other natural sources. All aquatic ecosystems contain major ions. Notably, anthropogenic activities can contribute to increase salinity. Metal ions can enter ecological systems and build up in aquatic organisms, affecting their physiological state. Heavy metals are among the most dangerous pollutants, wreaking havoc on all living things.

- Fe Concentration in the current study is presented in Table (2). During the autumn season, the L1 region had the highest iron concentration (0.212 mgl^{-1}). The higher Fe concentration in autumn than in winter could be attributed to temperature rise, which slows the rate of Fe assimilation by aquatic organisms, particularly macrophytes **(Berg *et al*.,1995).** With the lowest concentration recorded after bioremediation and the concentrations recorded after Psudomonas.aeruginosa (0.110 mgl^{-1}) and A.Niger (0.070 mgl^{-1}) treatment **(Fig. 3 F).** And **Mn Concentration** the outcomes are depicted in **(Fig. 3G).** That is the lake's maximum manganese concentration calculated. During the study period, water was discovered at the L2 (0.066 mgl^{-1}) level. The most likely cause was a spill of fertiliser-containing agricultural drainage water into the drain. Furthermore, near-drain fish farms are fed Mn-rich chicken farm residuals. It's almost certainly industrial waste from nearby factories. Because the lake contains many fish catches that included fish food supplied by the owners of these fish catches, unusually high determination of various in some regions at specific times may be credited to fish food residues in the sediments.These findings are in line with previous research. Lake Manzala study Abdelhamid , El-Zareef , Abdel-baky and Ibrahim ***(*Abdel-Baky *et al* .,1998; Abdel-hamid *et al*.,2003).** Following bioremediation, *Psudomonas.aeruginosa* (0.01 mgl^{-1}) and *A.Niger* (0.00).

- Cu Concentration See (Fig. 3 H) Because of springtime exposure to a nearby industrial disposal facility, this site still had the highest total Copper concentration in water (0.009mgl^{-1}). After bioremediation, *Psudomonas.aeruginosa* (0.004 mgl^{-1}) and *A.Niger* were discovered (- 0.001 mgl^{-1}). **Concentration of lead** The data and data shown in **(Fig. 3 I)**show that lead concentrations are high across the board. Locations discovered (0.009 mgl^{-1}) Location 3 yielded an unexpected result close to the sea-to-land link the lake, but after treatment with *Psudomonas.aeruginosa* reaches (0.0026 mgl^{-1}), whereas treatment with *A.Niger* reaches (0.0018 mgl^{-1}).**And Cadmium deposition**

depicts the outcomes the difference in Cd Concentration values between sites is small, but it is the most significant. Cadmium concentrations in lake water were measured and recorded in three different locations (0.005mgl^{-1}). This is most likely due to the disposal of industrial waste in the drain. Reaches (0.0003 mgl^{-1}) after treatment with *Psudomonas.aeruginosa*, whereas treatment with *A.Niger* reaches (-0.0004 mgl^{-1}). Cadmium is found in high concentrations at all locations as a result of its proximity to an industrial area. This could be due to the lake's paint factory disposal without direct treatment **(Fig. 3 J).** Previous findings are consistent with those of **(Elghobashy *et al*., 2001; Bahnasawy *et al* .,2011).**

-Zn Concentration the difference in zinc densities between the three sites is insignificant, and all three have high zinc accumulation as a result of industrial waste spilled into the lake, either directly or indirectly. Because of its exposure to indirect industrial disposal of water from Bahr Al-Bakr bacterial drains, L2 has a higher total zinc concentration (0.045 mgl^{-1}). Bioremediation with *Psudomonas.aeruginosa* (0.162 mgl^{-1}) and *A.Niger* (0.161 mgl^{-1}) **(Fig. 3 k).**



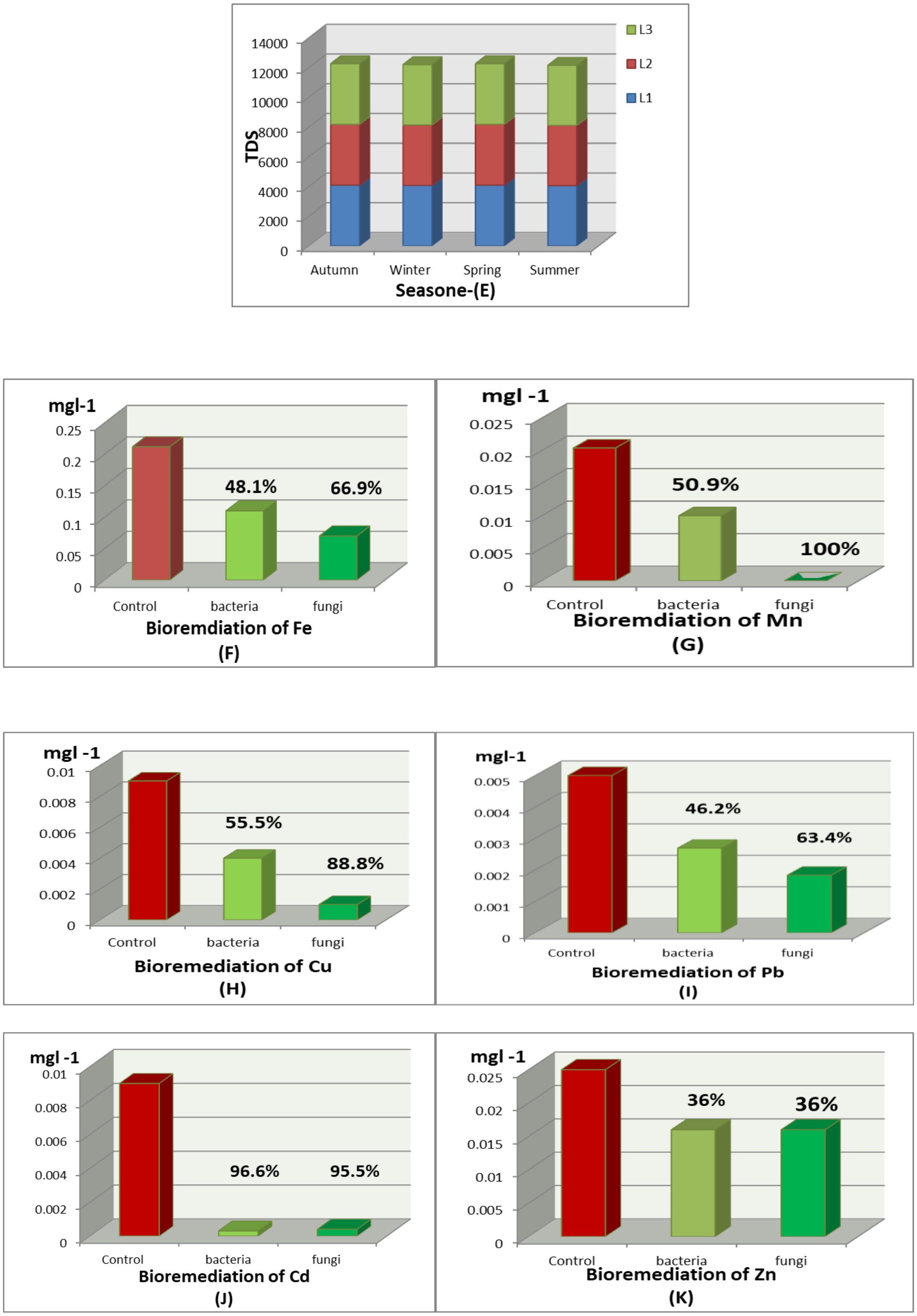


Fig. 3: Physical , chemical properties and heavy metal bioremediation of El- Kaboty area water samples: (A) Temperature of the water (T), (B) pH, (C) Electrical conductivity (Ec), (D) Salanity , (E) Total Dissolved Solids (TDS), (F) Fe bioremediation , (G) Mn bioremediation ,(H) Cu bioremediation,(I)Pb

bioremediation ,(J) Cd bioremediation, (K) Zn bioremediation (. Data is presented as a mean ±SE.

Table 1. The wastewater main drains discharge into Lake Manzala.

Drains	Type of wastewater	Serving area (feddans *)	Total wastewater flow in the Lake (%)	References
Bahr El-Baqer	Mainly domestic and industrial	536000	25	6
Hadous	Mainly agricultural	790000	49	
Ramsis	Mainly agricultural	NA	4	
El-Serw	Mainly agricultural	68700	13	
Faraskour	Mainly agricultural	20000	4	
Matariya	Mainly agricultural	NA	2	

***Approximately 4,200.833 square meters (about 1.038 acres).**

Table 2. Physicochemical parameters collected from EL-Kapoty in Lake Manzala at various sites during the study period.

Sites / Parameter	L1				L2				L3				Permissile Limits*
	Autumn	winter	Spring	Summer	Autumn	winter	Spring	Summer	Autumn	Winter	Spring	Summer	
Temperature °C	33.2	19.6	17.6	22.3	32.٥	19.١	1٧.٤	22.3	32.١	19.٥	17.٦	22.٥	< 35
pH	6.7	7.1	7.0	6.5	6.5	7.3	7.0	6.7	6.4	7.2	7.1	6.4	6.0-9.0
EC(µS/cm)	1.0	2.7	4	2.3	1.0	2.7	3.5	2.3	1.0	2.4	3.9	2.3	> 4
Salanity	3.6	6.3	2.6	3.7	3.6	6.3	2.6	3.6	3.6	6.3	2.6	3.9	> 4
TDS (mgl^{-1})	4082	4065	4086	4053	4086	4064	4086	4045	4084	4065	4083	4046	<2000

***Permissible Limits of Egypt legislation of the national law 48/1982**

Table 3. Heavy metal concentrations in water samples collected from EL-Kapoty in Lake Manzala over the study period

Sites / Parameter	L1				L2				L3				permissible Limits*
	Autumn	winter	Spring	Summer	Autumn	winter	Spring	Summer	Autumn	Winter	Spring	Summer	
Fe(mgl^{-1})	0.2	0.082	0.1223	0.0705	0.2121	0.0850	0.1238	0.0370	0.213	0.0826	0.1243	0.074	< 1.0
Mn(mgl^{-1})	0.0204	0.0157	0.0662	0.036	0.0215	0.0184	0.0662	0.034	0.0217	0.0185	0.0656	0.0358	< 0.5
Cu(mgl^{-1})	0.0092	0.0083	0.0088	0.0055	0.009	0.0085	0.009	0.0057	0.0095	0.0083	0.0096	0.0052	< 1.0
Pb(mgl^{-1})	0.0053	0.0086	0.0097	0.0084	0.0052	0.0085	0.0095	0.0082	0.0052	0.0083	0.0095	0.0085	< 0.٠٥
Cd(mgl^{-1})	0.0096	0.005	0.0084	0.0035	0.0096	0.005	0.0083	0.0036	0.0096	0.005	0.0083	0.0039	< 0.10
Zn(mgl^{-1})	0.0253	0.0182	0.0429	0.0424	0.0257	0.0192	0.0443	0.0412	0.0251	0.0192	0.0437	0.0422	< 1.0

CONCLUSION

The pH was discovered to be lower than the bare minimum allowable, while the dissolved solids recorded values that were higher than the Egyptian law's allowable limits. The results revealed significant differences in the physical and chemical properties of water with the seasons of the year and this results from the climatic changes that occur throughout the previous years, including: temperature (17.3°C in Winter -33.4°C in autumn), pH (7.22 - 6.54), and total dissolved solids. (4088-4040mg L^{-1}), Iron (0.214-0.035 mg L^{-1}), Manganese (0.066-0.007 mg L^{-1}), Copper (0.009-0.001 mg L^{-1}), Lead (0.009-0.001 mg L^{-1}), Cadmium (0.009-0.001 mg L^{-1}), Zinc (0.045-0.016 mg L^{-1}).

It was determined that isolates can be used to remove Fe, Mn , Cu, Pb , Cd, and Zn from industrial effluents. The most effective in bioremediation were *Psudomonas.aeruginosa*. This suggests that bacteria play a critical role. Where both metals have been biologically treated at high rates (Fe 48.1%,Mn50.9%,Cu55.5%,Pb63.4%,Cd 95.5% and Zn 36%) .Involvement in the removal of heavy metals from industrial processes effluent and have the potential to provide a new improved microbiome Polluted environment bioremediation. The results correspond with the findings of a study on the efficacy of *Aspergillus niger* fungal masses in reducing heavy metal concentrations. Elements derived from polluted soils **(Omotayo *et al*.,2008)**. These isolates were found to be effective at lowering iron densities by 66.9%. The findings agreed with those of **(Iqbal and Farah, 2009)** Thus, it has proven its ability to treat other metals with high rates (Mn100%,Cu88.8%,Pb63.4%,Cd95.5% and Zn 36%).

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