ISOLATION AND IDENTIFICATION OF BACTERIAL STRAINS RESISTANT TO FE, MN AND AL METAL IONS FROM RIVER NILE WATER IN EGYPT

Tamer T. El-Tokhy [(1)], Hoda Mahrous [(2*)], Ibrahim E.Mousa[(1)] and Abdel-Hameed M.Othman [(1)]
[(1)] Environment Biotechnology Dept., Genetic Engineering and Biotechnology Research Institute (GEBRI) Sadat City University, **EGYPT**
[(2)] Industrial Biotechnology Dept., Genetic Engineering and Biotechnology Research Institute (GEBRI) Sadat City University, **EGYPT**

ABSTRACT

The Nile River often thought of as the backbone of Egypt, has been source of great sustenance and mobility throughout the years. The heavy-metals are carried into streams, rivers, lakes and oceans and may be deposited in sediments at the bottom of the water body or they may evaporate and be carried elsewhere as rainwater. In our study hundred water samples were collected from River Nile in Rod El-farag region, Cairo, Egypt. To make isolation and identification of bacterial strains have the ability to bear and resist high concentrations of Al, Fe and Mn metals up to 5 mg/l. The isolated bacterial strains were *Pseudomonas putida biotype A, Citrobacter freundii* and *Aeromonas hydrophila DNA Group1*.The first and second strains had the ability to resist up to 5 mg/l Fe and Mn separately; while the last one had the ability to resist up to 5 mg/l Fe, Mn and Al, separately. The strains were identified by IMVIC test and confirmatory tests were done by Biolog GeneIII MICROPLATE technique.

Keywords: Nile River; Pseudomonas putida; Citrobacter freundii; Aeromonas hydrophila; Heavy metals.

INTRODUCTION

Egypt can be considered as one of the immense countries in Africa and represents the heart of the Arabic world (Mahmoud, 2002). The River Nile is the donor of life to Egypt and represents the principle .Fresh water resource that meets nearly all demands for drinking water and irrigation (Korium & Toufeek, 2008; Ali *et al*., 2008) and it supplies about 97% of Egypt's water reserves (Abdel-Shafey & Aly 2002). The revering floodplains and delta are subjected to slight flooding and Cyprus papyrus swamps, which play a significant role in elimination of pathogenic microorganisms. The area of the swamps has been decreased, however. Rare published information is, however, available on microbiological characteristics of the Nile water and all of them deal with the bacterial indicators only. Payment et al. (1985) mentioned that faecal indicators bacteria failed to judge the water safety because different opportunistic and/or pathogenic bacteria were found in the absence of such indicators. El-Abagy et al. (1999) studied the quality of River Nile water at Beni-Suif area. They detected different genera of pathogenic bacteria in the water. Ali et al. (2000) studied the biological characteristics of the River Nile water to evaluate the trophic and autotrophic state of the River. They revealed the presence of faecal bacterial indicator, high number of pathogenic bacteria and yeasts because the River body receiving big quantities of domestic, industrial and agricultural wastes. El-Taweel & Shaban (2001 a, b) studied the classical bacterial indicators of the River Nile water at Cairo segment and Ismailia canal. They reported that the total coliforms, faecal coliforms and faecal *Streptococcus*

were high in the water. They also detected the presence of *Aeromonas, Staphylococcus* and *Listeria* genera which revealed the high pollution of the water.

Heavy metals are among the most common environmental pollutants, and their occurrence in waters and biota indicate the presence of natural or anthropogenic sources. This comes under the inorganic division. Some metal ions such as Fe, Mn and Al are toxic especially when its values exceed the guide values which are 0.3, 0.4 and 0.2 respectively according to WHO. The main natural sources of metals in waters are chemical weathering of minerals and soil leaching. The anthropogenic sources are associated mainly with industrial and domestic effluents, urban storm, water runoff, and landfill, mining of coal and ore, atmospheric sources and inputs rural areas. Among toxic substances reaching hazardous levels are heavy metals (Vieira &Volesky, 2000).

Aeromonas hydrophila is a primary (Esteve *et al*., 1993), secondary (Joice *et al*., 2002) and opportunistic pathogen (Dooley & Trust, 1988; Lio-Po *et al*., 1996) of a variety of aquatic and terrestrial animals, including humans. It is a ubiquitous, free living, Gram-negative bacterium, mainly found in water and water-related environments and causes a wide variety of symptoms (Hazen *et al*., 1978a). The disease caused by *A. hydrophila* is called motile Aeromonad septicaemia (MAS) and this pathogen is associated with number of other diseases in fish, for example, epizootic ulcerative syndrome (EUS) as a secondary pathogen (Roberts, 1993; Pathiratne *et al*., 1994; Lio-Po *et al*., 1998). The clinical signs in fish vary from tissue swelling, necrosis, ulceration and haemorrhagic septicaemia (Hazen *et al*., 1978a; Karunasagar *et al*., 1986; Angka, 1990; Aguilar *et al*., 1997; Azad *et al*., 2001).

Citrobacter are Gram-negative bacilli of the family Enterobacteriaceae. These microorganisms are facultatively anaerobic and typically motile by means of flagellae; they can use citrate as the sole carbon source. The genus *Citrobacter* is taxonomically most closely related to *Salmonella* and *Escherichia coli*. *Citrobacter* strains are normal inhabitants of human and animal intestine, and are also commonly distributed in natural environment such as soil, water, sewage and food Sedlak, (1973).

Pseudomonas putida is a Gram-negative rod-shaped bacterium with multitrichous flagella. While this species is not pathogenic, it can make a capsule, like many of its pathogenic cousins; this capsule is made of complex polysaccharides and plays a role in helping the bacterium attach to surfaces, and may provide the cell with protection from desiccation and phagocytosis (Ghiorse & Kachlany, 2003).

The Biology identification system is based on the simultaneous examination of utilization of 95 different carbon sources. The utilization of the carbon source in a well was demonstrated by formation of purple colour or turbidity changes as compare to a negative control. Computer software and a database enabled the species level identification. The carbon source utilization pattern of a strain investigated was compared with the results in database. The computer displays the closest matches on screen along with a numerical similarity index. A strain can be clustered in relation to its closest relatives, or entire user-constructed library can be clustered. The strains were streaked on nutrient agar and grown for 12–18 hours. With cotton swab 1–2 colonies were suspended in physiological saline, and by using the Biology turbidimeter, the desired cell density of about 3×10^{8} cells ml^{-1} was adjusted. The pre- warmed microplate was used for Gram-negative

strains, were inoculated promptly by a multichannel repeating pipette with precisely 150 µl suspension. The inoculated microplates were covered, and incubated at 28 ^{o}C for 4 hours, read using a Uniscan II (Labsystem, Finland) microplate reader and the results were evaluated, and clustered by using the Micro Log software of Biolog (Winding 1994).

The present study aimed to collect water samples from Rod Al-farag region in Cairo City, Egypt to isolate bacterial strains have the ability to bear high concentration of Aluminium, Iron and Manganese ion Concentrations up to 5 mg/L separately and make complete identification by IMVIC tests and confirmatory test by Biolog GeneIII MICROPLATE technique for recognizing of those strains.

MATERIALS AND METHODS

Isolation of bacterial strains resistant to heavy metals

Source of sample

Samples were collected from River Nile, in the fresh water supply of Rod El-Farag Drinking Water Treatment Plant in Cairo city, Egypt.

Sample Collection.

Samples were collected from the River Nile in Rod El-Farag Region, Cairo, Egypt by microbial auto sampler at 6 meter depth from the water surface. Samples for microbiological analysis were collected in sterile containers and transported to the laboratory in an icebox immediately. The microbiological analysis was done within 3 hours of collection. The samples were maintained at 4°C for minimizing the changes in the physico-chemical properties, until used.

Analysis of sample

One hundred River Nile water samples were collected under aseptic condition and analyzed as soon as reached to lab, enriched in nutrient broth media, and incubated at 28°C for 24 hours.

Isolation of bacterial strains Resistant to Iron, Manganese and Aluminum.

Bacterial enrichment.

Nutrient broth medium was used for enrichment the bacteria from River Nile water by Inoculate 1 ml of sample in 9 ml of nutrient broth media and incubate at 28°c for 24 hours. The growth of bacteria in the medium was determined in terms of turbidity in the culture broth, by measuring absorbance at 600 nm in UV visible spectrophotometer (jenway). Growth was expressed as optical density (OD).

Total Aerobic Bacterial Count (T.A.B.C).

Spread plate technique was employed. One ml of bacterial growth was diluted to10-7. Inoculate one ml of this dilution by spread method on nutrient agar plate. After plating; the plates were incubated at 28°C for 24hours.Count the number of colonies on the plate.

Precipitation test of media containing heavy metals.

This test was carried out to ensure that all doses of heavy metals were completely soluble in broth media without any precipitation.

a. Nutrient broth media were prepared and supplemented with different doses (5, 10, 15, and 20) mg/l of Iron, Manganese and Aluminum individually and together with each dose by standard of certified reference material. The tubes of each concentration were kept standing in incubator without inoculums, at 28°C for 24 hours. Observe the probability of precipitation.
b. Nutrient broth media supplemented with dose 5 mg/l of Fe, Mn and Al individually were kept standing in incubator without inoculums at 28°C for 24hours. The supernatant of media were taken for each element and acidified with drops of nitric acid high grade (merck). The samples measured by Atomic spectroscopy (Perkinelmer A- Analyst 100), according to standard calibration curve method, in Accurate Analysis Centre of Cairo University to confirm each dose concentration.

Screening for Iron, Manganese and Aluminum resistant bacteria.

Nutrient broth media supplemented with dose 5 mg/l of Fe, Mn and Al individually were prepared, each one inoculated with one ml of bacterial inoculums after enrichment and incubated at 28° c for 24 hours. The growth of bacteria in the medium in each case was determined in terms of turbidity in the culture broth, by measuring absorbance at 600 nm in UV visible spectrophotometer (Jenway). Growth was expressed as optical density (OD).

One ml was taken from each growth and obeyed for several dilutions to 10^{-5}, one ml of each dilution was inoculated on duplicate nutrient agar medium by spread method and the plates were incubated at 28° c for 24 hours. The total viable counts were observed. The bacterial colonies were observed for their colony morphology, shape, size and colour. Each colony of different morphological shape of all plates we redoubled inoculated by streak method on nutrient agar media; the last step was repeated several times to obtain pure bacterial strains. Later, single celled colonies were picked from each different colony shapes and subcultured on nutrient agar slants and maintained at 4°C.

Identification of resistant bacterial strains.
Microbiological identification tests.

Isolates were identified according to Bergey's Manual Analyses for biochemical and microbiological characteristics (Culture dependent methods). In IMViC test there are four biochemical tests and this test are carried out individually. IMViC test is carried out to identify members of *Enterobacteriaceae* family. Each letter of IMViC test stand for individual test; I- Indole production test; M - Methyl red test;V- Voges- Proskaver test and C - Citrate utilization; i -Lower case i is used for ease of pronunciation.

Confirmatory test

Biolog GeneIII MICROPLATE Confirmatory test was done for each strain by Biolog Gene III Technique in National Centre for Research in Cairo, Egypt as the following steps:

a) Isolated bacterial strains were streaked individually on nutrient agar plates at 28°c for 24 hours to obtain a pure single colony.
b) Test Procedure

Preparation
Before starting, prewar MicroPlates.

Step 1. Culture Organism on Biology Recommended Agar Media.

* A pure culture was isolated on Biology recommended agar media (BUG+B or Chocolate Agar) and incubates at 33° C. Some species may require special culture conditions, for example both lower or higher temperature (26° - 37° C) and elevated CO_2 (6.5% - 10%).

*The cells must be freshly grown since many strains lose viability and metabolic vigor in stationary phase. The recommended incubation period for most organisms is 4-24 hours. Sporeforming gram-positive bacteria (*Bacillus* and related genera) should be grown for less than 16 hours to help minimize sporulation.

*If insufficient growth is obtained to inoculate the panel, restreak heavily (as a lawn) onto one or more agar plates. Incubate for 4-48 hours. This should give enough growth to inoculate the panel.

Step 2. Prepare Inoculums

The inoculums were prepared at the desired turbidity. The target cell density should be in the range of 90-98%T.

Step 3. Inoculate MicroPlate

* The cell suspension was poured into the multichannel pipet reservoir.

* Fasten 8 sterile tips securely onto the 8-Channel Repeating pipet or and fill the tips by drawing up the cell suspension from the reservoir.

* All wells were filled with precisely 100 µl as be careful not to carry over chemicals or splash from one well into another. The inoculating fluid will form a soft gel shortly after inoculation.

*Cover the MicroPlate with its lid and eject the pipett or tips.

Step 4. Incubate MicroPlate

*Place the MicroPlate into the OmniLog incubator/reader or into an incubator, for 3 to 36 hours. Incubate at 33° C, or use incubation conditions that were found to be optimal for the bacterium in Step 1.

Step 5. Reading and Interpretation of Results

* Read MicroPlatesusing Biolog's Microbial Identification Systems software (e.g.OmniLog® Data Collection). Refer to the User Guide for instructions.

*Biolog's Microbial Identification Systems Software performs all reading and interpretation of results.

* The color densities in wells of the carbon source utilization assays in columns 1-9 are referenced against the negative control well, A-1. All wells visually resembling the A-1 well should be scored as "negative" (-) and all wells with a noticeable purple color (greater than well A-1) should be scored as "positive" (+). Wells with extremely faint color or with small purple

ISSN 2056-5984

flecks or clumps are best scored as "borderline" (\). Most species give dark, clearly discernible "positive" reactions. However, it is normal for the "positive" reactions of certain genera to be light or faint purple.

* The color densities in wells of the chemical sensitivity assays in columns 10-12 are referenced against the positive control well, A-10. All wells showing significant sensitivity to the inhibitory chemical, with less than half the color of the A-10 well are considered "negative" (-) for growth. All other wells showing normal or near normal purple color (similar to well A-10) are considered "positive" (+). If there is uncertainty about the interpretation, it is best to score the well as"borderline" (\).

*"False positive" color is defined as purple color forming in the negative control well (A-1) and in other "negative" wells. This is seen with only a few species such as from the genera *Aeromonas*,*Vibrio*, and *Bacillus*. If such a result occurs, the cells are simply retested with Protocol B and IFB.

Performance Characteristics

The GENIII MicroPlate performance characteristics have been determined by establishing a database using a large collection of microorganisms from diverse sources. The database is designed to give identifications of all species in the database, in accordance with current standards of classical identification methods and current taxonomic nomenclature. To obtain accurate and reproducible results, all procedures and recommendations in these Instructions for Use must be followed precisely.

The ability of each identified bacterial strain to resist Fe, Mn and Al. separately.

A loop full of each 3 identified strains were inoculated in NB media for enrichment and incubated at 28°c for 24 hours. The growth of bacteria in the medium was determined in terms of turbidity in the culture broth, by measuring absorbance at 600 nm in UV visible spectrophotometer (jenway) for each strain. One ml of each bacterial strain growth used to inoculate NB media supplied with Fe, Mn and Al separately and incubated at 28°c for 24 hours. The growth of bacteria in the medium was determined by measuring absorbance at 600 nm in UV visible spectrophotometer (jenway) after zero time of incubation period and measured once again at the end of incubation time; the difference between the two absorbance represented the growth of each srains in each type media.

RESULTS AND DISCUSSION

Precipitation test of media containing heavy metals.

To reach the best concentration of heavy metal Al, Mn and Fe doses which completely soluble in broth media without any precipitation, tested more than concentrates as different doses (5, 10, 15, 20) mg/l of Iron, Manganese and Aluminum individually and together with each dose by standard of certified reference material (CRM®) and the heavy metals addition was visualized by eye and the results was show in table (1).

Table (1).

Incubation Time(hr)	Dose(5.0ppm)	Dose(10.0ppm)	Dose(15.0ppm)	Dose(20.0ppm)
6	No ppt	No ppt	ppt	ppt
12	No ppt	No ppt	Ppt	ppt
24	No ppt	Ppt	Ppt	ppt
36	No ppt	Ppt	Ppt	ppt
No ppt = No Precipitate, ppt = Precipitate				

The best dose of heavy metal was 5.0 mg/l which added individually to media without any precipitation. The 5, 6 and 7 ppm samples were measured by Atomic spectroscopy (Perkinelmer A- Analyst 100) and the results of actual concentration were shown in tables (2;3&4).

Table (2) Percent of solubility of different concentration of iron

Added concentration	Real concentration	Percent of solubility
5 ppm	4.95 ppm	99%
6 ppm	5.2 ppm	86.66%
7 ppm	6.1 ppm	87.14%

Table (3) Percent of solubility of different concentration of Mn

Added concentration	Real concentration	Percent of solubility
5 ppm	4.92 ppm	98.4%
6 ppm	5.65 ppm	94.16%
7 ppm	6.15 ppm	87.86%

Table (4) Percent of solubility of different concentration of Al

Added concentration	Real concentration	Percent of solubility
5 ppm	4.90 ppm	98%
6 ppm	5.75 ppm	95.8%
7 ppm	6.25 ppm	89.28%

ISSN 2056-5984

Identification of Isolates
Gram Negative Bacilli—IMViC.

There were 7 bacterial isolates could resist and survive on media supplemented with 5 mg/l Fe, Mn and Al metals individually (table 5). Two bacterial strains were isolated on media supplemented with Fe metal (*Pseudomonas sp.* and *Citrobacter sp.*). Two bacterial strains were isolated on media supplemented with Mn metal (*Pseudomonas sp.* and *Citrobacter sp.*). Three bacterial strains were isolated on media supplemented with Al metal (*Pseudomonas sp.*, *Citrobacter sp and Aeromonas sp.*).

Table (5): Pre-identification results of strains isolated from raw water samples.

No.	Shape	Ox.	Cat.	Starch	Nit.	Indol	MR.	V/P	Cit.	Gelatin	Urease	Motil.	Strain
St.1 on Fe	Rod-	+	+	-	-	-	-	-	+	-	+	+	*Pseudomonas sp.*
St.2 on Fe	Rod-	-	+	-	+	-	+	-	+	-	+	+	*Citrobacter sp.*
St.1 on Mn	Rod-	+	+	-	-	-	-	-	+	-	+	+	*Pseudomonas sp.*
St.2 on Mn	Rod-	-	+	-	+	-	+	-	+	-	+	+	*Citrobacter sp.*
St.1 on Al	Rod-	+	+	-	-	-	-	-	+	-	+	+	*Pseudomonas sp.*
St.2 on Al	Rod-	-	+	-	+	-	+	-	+	-	+	+	*Citrobacter sp.*
St.3 on Al	Rod-	+	+	+	+	+	+	+	+	+	-	+	*Aeromonas sp.*

Confirmatory test of strains

The confirmation of identified isolates was accomplished using Biolog GeneIII MICROPLATE(table 6). This technique was done in National Centre for Research, Giza, Egypt.

Table (6): Confirmatory test

Isolate	Identification by IMViC	Confirmation by Biolog Gene III
St.1 on Fe	***Pseudomonas sp***	***Pseudomonas putida biotype A***
St.2 on Fe	***Citrobacter sp.***	***Citrobacter freundii***
St.1 on Mn	***Pseudomonas sp***	***Pseudomonas putida biotype A***
St.2 on Mn	***Citrobacter sp.***	***Citrobacter freundii***
St.1 on Al	***Pseudomonas sp.***	***Pseudomonas putida biotype A***
St.2 on Al	***Citrobacter sp.***	***Citrobacter freundii***
St.3 on Al	***Aeromonas sp.***	***Aeromonas hydrophila DNA Group1***

There were three bacterial strains in water samples collected from the River Nile in Rod El-Farag water treatment plant intake were isolated and had the ability to resist metals concentrations, *Pseudomonas putida biotype A,Citrobacter freundii and Aeromonas hydrophila DNA Group1*. That was agreed with (Hala, 2007) who found that the third phenon of Rod El-Farag samples comprises two isolates representing the genus *Aeromonas*. Such genus is Gram-negative, rod shaped with rounded ends, oxidase and catalase positive and reduce nitrate to nitrite. (Szczuka & Kaznowski, 2004) indicated that these bacteria are widely spread in the environment, especially in surface water and sewage. The presence of different *Aeromonas* species indicates increasing pollution. Additionally, the existence of the single member's phena is the possible representatives of different sewage originating microbes. The same results we obtained also by (Hala, 2007) who found that The fourth group, representing water from El- Gezera, is identified as members of the genus *Pseudomonas* which are described as Gram-negative, non-spore forming and straight or slightly curved rods. They are typically motile by means of one or more polar flagella. It was assumed that their position were the facultative H2-autotrophic methylotrophic species, which were delineated into new genera like *Hydrogenophaga,Acidovorax* and *Hydrogenomonas* (Stover *et al*., 2000). Members of the genus are found abundantly as free-living organisms in soils, fresh water and marine environments and in many other natural habitats (Purohit *et al*., 2003).were delineated into new genera like *Hydrogenophaga*, *Acidovorax* and *Hydrogenomonas* (Stover *et al*., 2000). Members of the genus are found abundantly as free-living organisms in soils, fresh water and marine environments and in many other natural habitats (Purohit *et al*., 2003).Our results were approach with the results of (Arafat, 2013) who found that the most frequent coliforms isolated from Nile were *Enterobacter aerogenes, Enterobacter cloacae, Enterobacter agglomerans, Klebsiella oxytoca, Klebsiella planticola, Serratia spp. and Citrobacter spp.*

Incidence and frequency distribution of bacterial strains resistant to metals
Incidence and frequency distribution of bacteria resistant to Fe

Between one hundred of Nile water sample collected from - the River Nile in Rod El-Farag water treatment plant intake under aseptic condition and subjected to microbiological analysis, enrichment, precipitate to select the best concentration of metal to be added in media to become selective media for isolate the main microorganism. Microorganisms were grown in Fe selective media (5 mg/l), table 7 showed that these microorganisms were identified biochemically and by using biolog geneIIIas confirmatory test, this microorganism was *Pseudomonas putida biotype* Ain10 samplesand *Citrobacter freundii* in6 samples.

Table (7): Number and identified isolates that grown in Fe selective media (5 mg/l).

Number of Strains.	Strain name
10	***Pseudomonas putida biotype A***
6	***Citrobacter freundii***

Incidence and frequency distribution of bacteria resistant to Mn

Microorganisms were grown in Mn selective media (5 mg/l).Table 8 illustrated these microorganisms were identified biochemically and by using biolog geneIIIas confirmatory test, this microorganism was *Pseudomonas putida biotype* Ain6 samples and *Citrobacter freundii*in 12 samples.

Table (8): Number and identified isolates that grown in Mn selective media (5 mg/l).

Nomber of Strains.	Strain name
6	***Pseudomonas putida biotype A***
12	***Citrobacter freundii***

Incidence and frequency distribution of bacteria resistant to Al

Microorganisms were grown in Fe selective media (5 mg/l), table 9 showed these microorganisms were identified biochemically and by using biolog geneIIIas confirmatory test, this microorganism was *Pseudomonas putida biotype* Ain8 samples,*Citrobacter freundii* in 5 samples and *Aeromonas hydrophila DNA Group1* in 13 samples.

Table (9): Number and identified isolates that grown in Al selective media (5 mg/l).

Nomber of Strains.	Strain name
8	***Pseudomonas putida biotype A***
5	***Citrobacter freundii***
13	***Aeromonas hydrophila DNA Group1***

ISSN 2056-5984

Behavior of microorganisms in media supplemented with 5 mg conc of Fe, Mn and Al individually

To study the behavior of three-microorganism pseudomonas, *Citrobacter* and *Aeromonas* must grow in three different media and calculate growth of each one at zero time and after incubation (24hr).From table10, Noted that two microorganisms, *Pseudomonas* and *Citrobacter* isolated strains were grown in all media type containing metal individually while *Aeromonas* was grown on media containing Al only.

Table (10): Behavior of microorganisms in media supplemented with 5 mg conc of Fe, Mn and Al individually

Microorganisms	Fe		Mn		Al	
	Zerotime (OD)	After 24h(OD)	Zerotime (OD)	After 24h(OD)	Zerotime (OD)	After 24h(OD)
Pseudomonas	0.084	0.742	0.094	0.690	0.05	0.404
Citrobacter	0.096	0.867	0.123	0.893	0.057	0.610
Aeromonas	0.191	0.200	0.163	0.199	0.141	0.803

Pseudomonas putida was able to resist and tolerate the Fe concentration in the media upto 5 mg/l .Our results were approach with the results of (Odokuma *et al*., 2010) who found that *Pseudomonas putida* showed resistance to Fe upto 100 mg/l when was cultured in media containing salt form of Fe and the growth decreased gradually by increasing fe concentration. *Pseudomonas putida* was able also to resist and tolerate the Mn concentration in the media upto 5 mg/lbut its growth in case of media with Mn was less than that of Fe case. Also *Pseudomonas putida* was able to resist and tolerate the Al concentration in the media upto 5 mg/l but its growth in case of media with Al was less than both of Fe and Mn case .The resistance of *Pseudomonas* sp. to the toxicity of the various salts may be due to its ability to use diverse compounds (organic and inorganic) as sole carbon source (Schlegel, 1997). In addition to its genetic make-up, complexity of its cell wall being a Gram-negative organism may have contributed toits resistance. Similar reasons can also be advanced for the resistance of *Aeromonas* sp to the toxicity of the various heavy metal salts (Odokuma *et al*., 2010).

For *Citrobacter freundii* results that were showed resistance and tolerance all media included fe, Mn and Al and also, showed different growth in each one. The results agreed with the results of (Chandrabhan Seniya *et al*., 2012) who found that *Citrobacter freundii* could survive and grow in media containing different concentration of Mn^{2+} and Fe^{2+} up to 100 mg/l .The resistance of *Citrobacter freundii*. to the toxicity of the various salts specially Mn^{2+} *and* Fe^{2+} may due to that The metal ions i.e. micro-nutrients usually act as cofactors and may lead growth, enhanced gene expressions, essential enzymatic reactions and metabolic pathways in the cell. These cofactors play essential and critical role in regulation of protein formation and other value added products. The usual cations that qualify as essential trace elements in bacterial nutrition are Mn, Co, Zn, Cu, Mo, Fe, Mg, Ni, K, Se, V, B, and Na. be due to its ability to use(Chandrabhan Seniya *et al*., 2012).*Citrobacter* spp could resist and tolerate metal ions concentration through two mechanisms. The first one is passive mechanism by extracellular complexation.The second one is active mechanism by precipitation (Rajendran *et al*., 2003).

The ability of *Citrobacter freundii* to resist metals (Fe, Mn and Al) and grow was differed. The maximum growth was on media supplemented with Mn then Fe and finally Al which was the lowest one in growth of *Citrobacter freundii*. *Aeromonas*, was grown only on media

of Al while did not grow neither in media supplemented with Fe nor media supplemented with Mn and that disagreed with (Odokuma *et al*., 2010) who found that *Aeromonas* resisted and grew on media supplemented with Fe up to 100 mg/l with the same degree of growth of *Pseudomonas putida*.

CONCLUSION

The River Nile forms very important aquatic and wetland ecosystems .Results from our in vitro analyses suggested that the Pseudomonas putida biotype A, Citrobacter freundii and *Aeromonas hydrophila DNA Group1 that isolated and identified from River Nile in Rod El-Farag Region, Cairo, Egypt have the ability to survive in fresh water and each one strains has the ability to bear and resist high concentration* of Fe; Mn and Al *rich to 5 mg/l.*

REFERENCES

Abdel-Shafey, H.I. & Aly, F.O. (2002). Water issue in Egypt: resources, pollution and protection endeavours. Central European Journal of Environmental Medicine 8: 3–21

Ali, G.H., El-Taweel, G.E., Ghazy, M.M. & Ali, M.A. (2000). Microbiological and chemical study of the Nile River water quality. International Journal of Environmental Studies 58: 47–69.

Ali, S.M.; Sabae, S.Z.; Fayez, M. and Hegazi, N.A. (2008). Sugar and starch industries as a potential source of water pollution of the River Nile south of Cairo: Microbiological and chemical studies. J. Egypt. Academic Society for Environmental development, 9: 25-45.

Angka S.L. (1990). The pathology of the walking catfish, Clarias batrachus (L.), infected intraperitoneally with Aeromonas hydrophila. Asian Fisheries Science 3, 343-351.

Arafat Mohammed Goja (2013).Bacterial Genera and Their Some Species of Nile Water. Asian Journal of Biological Sciences 6 (2): 116-123

Azad I.S., Rajendran K.V., Rajan J.J.S., Vijayan K.K. and Santiago T.C. (2001). Virulence and histopathology of Aeromonas hydrophila (Sah 93) in experimentally infected tilapia, Oreochromis mossambicus (L.). Journal of Aquaculture in the Tropics 16, 265-275 .

Chandrabhan Seniya1, Sumint Singh Trivedia, Richa Verma, HS Vijayarti,

El-Abagy, M.M., Shaban, A.M. & El-Taweel, G.E. (1999). Efficiency of water treatment systems in production of drinking water at Beni-Suif water treatment plant. Egyptian Journal of Applied Science 14: 23–33.

El-Taweel, G.E. & Shaban, A.M. (2001a). Microbiological quality of drinking water at eight water treatment plants. International Journal of Environmental Health Research 11: 285–290

El-Taweel, G.E. & Shaban, A.M. (2001b). Efficiency of water treatment plant steps in removing pathogenic and indicators bacteria. Published in the Berlin Congress, 15–19 October, 2001 under supervision of IWA.

Esteve C., Biosca E.G. and Amaro C. (1993). Virulence of Aeromonas hydrophila and some other bacteria isolated from European eels Anguilla Anguilla reared in fresh water. J. Diseases of Aquatic Organisms 16, 15-20.

Ghiorse, W. and Kachlany, S. (2003). http://www.asmusa.org/edusrc/library/ images /Wghiorse/HTMLpages/Pseudomonas.htm. Dated 10/01/2003 .

Hala Mohamed Rifaat (2007). Bacterial Quality of River Nile Water at Cairo Region in Egypt. J. SUO. 59 (1–8).

Hazen T.C., Raker M.L., Esch G.W. and Fliermans C.B. (1978a). Ultrastructure of red-sore lesions on largemouth bass (Micropterus salmoides): Association of the ciliate Epistylis sp. and the bacterium Aeromonas hydrophila. Journal of Protozoology 25, 351-355.

Joice A., Shankar K.M. and Mohan C.V. (2002). Effect of bacterial biofilm in nursery on growth, survival and resistance to Aeromonas hydrophila of common carp, Cyprinus carpio. Journal of Aquaculture in the Tropics 17, 283-298.

Karunasagar I., Rosalind G.M., Karunasagar I. and Gopal Rao K. (1986). Ulcerative form of Aeromonas hydrophila infection of Catla catla. J. Current Science 55, 1194.

Korium, M.A. and Toufeek, M.E.F. (2008): Studies of some physico-chemical characteristics of old Aswan Dam Reservoir and River Nile water at Aswan. Egypt. J. Aquat. Research, 34: 149-167.

Lio-Po G.D., Albright L.J. and Leano E.M. (1996). Experiments on virulence dose and portals of entry for Aeromonas hydrophila in walking catfish. Journal of Aquatic Animal Health 8, 340-343.

Lio-Po G.D., Albright L.J., Michel C. and Leano E.M. (1998). Experimental induction of lesions in snakeheads (Ophicephalus striatus) and catfish (Clarias batrachus) with Aeromonas hydrophila, Aquaspirillum sp., Pseudomonas sp. and Streptococcus sp. Journal of Applied Ichthyology 14, 75-79.

Mahmoud, S.A. (2002). Evaluation of toxicity effect of some pollutants on histological feature and biochemical composition of Oreochromis niloticus L. living in River Nile (Damietta branch). Ph.D. Thesis Fac. Sci. Zagazig Univ. Egypt.

Merino S., Rubires X., Aguilar A. and Tomas J.M. (1997). The role of flagella and motility in the adherence and invasion to fish cell lines by Aeromonas hydrophila serogroup O:34 strains. J. FEMS Microbiology Letters 151, 213- 217.

Murray R.G.E., Dooley J.S.G., Whippy P.W. and Trust T.J. (1988). Structure of an S layer on a pathogenic starin of Aeromonas hydrophila. Journal of Bacteriology 170, 2625-2630.

Odokuma L. O. and Akponah E.(2010). Effect of concentration and contact time on heavy metal uptake by three bacterial isolates. Journal of Environmental Chemistry and Ecotoxicology Vol. 2(6), pp. 84-97

P Rajendran, J Muthukrishnan and P Gunasekaran (2003).Microbes in heavy metal remediation. Indian Journal of Experimental Biology .vol. 41,pp. 935-944

Pathiratne A., Widanapathirana G.S. and Chandrakanthi W.H.S. (1994). Association of Aeromonas hydrophila with epizootic ulcerative syndrome (EUS) of freshwater fish in Sri Lanka. Journal of Applied Ichthyology 10, 204-208.

Payment, P., Trudel, M. & Planto, R. (1985). Elimination of viruses and indicator bacteria at each step of treatment during preparation of drinking water at seven water treatment plants. J. Applied Environmental Microbiology 49: 1418–1428.

Purohit, H.J., Raje, D.V. and Kapley, A.(2003). Identification of signature and primers to genus Pseudomonas using mismatched patterns of 16S rDNA sequences. J.BMC Bioinformatics 4: 19–26.

Roberts I.S. (1996). The biochemistry and genetics of capsular polysaccharide production in bacteria. J.Annual Review of Microbiology 50, 285-315.

Schlegel HG (1997). General Microbiology. 2nd edn. Sci. Tech. Publishers Madison. p. 1119

SEDLAK J. (1973). Present knowledge and aspects of Citrobacter .Curr. J. Top. Microbiol. Immunol., 62, 41–59.

Shridutt Vyas and Santosh Kumar Verma (2012). Effect of Fe2+, Mn2+ and Cu2+ ions on growth and soluble protein production in Citrobatcer freundii and Klebsilla pneumonia. Journal of Chemical and Pharmaceutical Research, 4(4):1895-1900.

Stover, C.K., Pham, X.Q., Erwin, A.L., Mizoguchi, S.D., Warrener, P., Hickey, M.J., Brinkman, F.S., Hufnagle, W.O., Kowalik, D.J., Lagrou, M., Garber, R.L., Goltry, L., Tolentino, E., Westbrock-Wadman, S., Yuan, Y., Brody, L.L., Culter, S.N., Folger, K.R., Kas, A., Larbig, K., Lim, R., Smith, L., Spencer, D., Wong, G.K., Wu, Z., Paulsen, I.T., Reizer, G., Saier, M.H., Hancock, R.E., Lory, S. & Olson, M.V. (2000). Complete genome sequence of Pseudomonas aeruginosa PA01, and opportunistic pathogen. J.Nature 406: 959–964.

Szczuka, E. and Kaznowski, A.(2004). Typing of clinical and environmental Aeromonas sp. strains by random amplified polymorphic DNA PCR, repetitive extragenic palindromic PCR and entrobacterial repetitive intergenic consensus sequence PCR. Journal of Clinical Microbiology 42: 220–228.

Vieira, RHSF and Volesky, B. (2000). “Biosorption: a solution to pollution”. J. Int Microbiol.; 3; 17–24.

Winding, A. (1994). Fingerprinting bacterial soil communities using Biolog Microtitre Plates. In: Ritz, K., Dighton, J. & Giller, K.E. (ds). Beyond the Biomass. J.British Society of Soil Science 85–94.