Int.J.Curr.Microbiol.App.Sci (2022) *11*(02): 139-165

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 02 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1102.018

Physical and Chemical Properties of some Water Samples from Egypt (Menoufia Governorate), the Efficiency of some Actinomycetes in Bioremediation of some Heavy Metals and Aflatoxin from Contaminated Water Samples

Amany A. A. Sharshar[1*], Sabha Mahmoud EL-Sabbagh[2] and Raafat. M. Elsanhoty[3]

[1]Department of Botany and Microbiology, Faculty of Sciences, Menoufia University, Shebin El-Kom, Egypt
[2]Department of Industrial Biotechnology, Sadat City University, Institute of Genetic Engineering and Biotechnology, Egypt
[3]Department of Microbiology, Faculty of Sciences, Menoufia University, Egypt

**Corresponding author*

Keywords

Environmental pollution, *Aspergillus flavus* and *Aspergillus parasiticus*

Article Info

Received:
06 January 2022
Accepted:
05 February 2022
Available Online:
10 February 2022

A B S T R A C T

The study aimed to see the ability of some actinomycetes in removing of some toxic heavy metal ions (Cd^{2+}, Mn^{2+}) and some aflatoxins (AFG1, AFG2, AFB1, AFB2) from contaminated water samples. Out of 57 isolates of actinomycetes were isolated from different locations of Egypt. The most five isolates removed studied heavy metals, (Cd^{2+}, Mn^{2+}) singly above 65% were selected to remove studied metal ions from binary mixtures. The highest removing percentage was recorded by A26 isolate which was removed 71.4 %, and 70.0 % from Cd^{2+}, and Mn^{2+} respectively. The 16S rRNA analyses and phylogenetic data of A26 concluded that it was member of *Streptomyces* genus, A26 was deposited in Gene Bank Data base under accession number MW947066. The results of the dead biomass of *Streptomyces albus A*26 for Cd^{2+}, and Mn^{2+} under the optimized conditions pH-8 at 40°C for 3 hours with 0.3% bio sorbent dosage was found (Cd^{2+}79.5 ± 2.37%, $Mn^{+2,}$81.6±2%). All studied heavy metals were removed from aqueous solutions when the biosorbent dosage increased to 0.6% by dead biomass of A26 isolate. This confirmed the potential use of *Streptomyces albus* A26 as an inexpensive and efficient technology for removal of Cd^{2+} and Mn^{2}. The isolate A33 was recorded as high percentage also which was removed 68.3 %, and 69.4 % from Cd^{2+}, and Mn^{2+} respectively and phylogenetic data of strain concluded that it was N. *vermiculate* under accession numbers MZ229469.1 in Gene Bank. The results of the dead biomass of *A*33 *N.vermiculate* for Cd^{2+}, and Mn^{2+} under the optimized conditions for studied heavy metals were removed from aqueous solutions (Cd^{2+} 76.2 ± 1.17%, Mn^{+2} 72.6 ± 0.3%). The isolates A26 and A33 showed high removing effect for some aflatoxins *(AFG1- AFG2-AFB1-AFB2)* extracted from fungi group (*Aspergillus flavus* and *Aspergillus parasiticus)* under the optimized conditions (pH-8 at 40°C for 3 hours with 0.3% biosorbent dosage**).**

Introduction

The problem of environmental pollution has become more critical with increasing human population and associated rapid industrialization (Liu & Lin, 2019). The water pollutants cause undesirable changes which in turn affect the ecological balance of the environment (Madhav *et al.,* 2020). Heavy metals are non-degradable compounds that may exist in different inorganic and organic forms (Yadav *et al.,* 2019). When discharged into the water, heavy metals represent a serious threat to the human health (Bolisetty *et al.,* 2019). Also, accumulation of heavy metals in marine ecosystems is of global importance due to their impact on human health (Al Naggar *et al.,* 2018). Some heavy metals such as Fe, Cu and Zn are essential trace elements but others such as Cd, Pb, Hg and as are toxic even in traces (Dong *et al.,* 2020).

Based on human studies involving chronic exposures, the Integrated Risk Information System (IRIS) of United States Environmental Protection Agency (EPA) set the threshold of reference Cd dose at 0.5 mg/kg/day in water and 1 mg/kg/day in food (Zhai *et al.,* 2015). To date, no specific treatment for heavy metals poisoning has been confirmed (Nordberg *et al.,* 2014), On the other hand, aflatoxins are a group of carcinogenic mycotoxins causing various acute or chronic intoxications and liver cancer (Williams *et al.,* 2004). Toxicological studies showed that among four naturally occurring aflatoxins including (AFB1, AF2, AFG1 and AFG2) AFB1 is the most common and toxic one (Zoghi *et al.,* 2014).

Water and food are the key routes for heavy metals contamination in the biological systems. Aquatic animals, especially fish, are the major sources of heavy metals contamination in human body (Cheng and Gobas, 2007). Therefore, removal of heavy metals from water should be the priority measure to control the problem of bioaccumulation (Joseph *et al.,* 2019). Different methods for removal of heavy metals from water including flocculation, precipitation, ion exchange and membrane filtration were introduced (Renu *et al.,* 2017). However, these methods are claimed to have disadvantages such as incomplete heavy metals removal, expensive equipment and monitoring system requirements, high reagent or energy requirements, and generation of toxic sludge or other wastes that require disposal (Zouboulis *et al.,* 2004; Halttunen *et al.,* 2007).

The removal of toxic heavy metals using algae, fungal and bacterial biomass was introduced as an inexpensive and novel method (Qin *et al.,* 2020). Mechanisms involved in heavy metals biosorption include complex formation, ion exchange, adsorption, chelation and microprecipitation (Halttunen, 2007; Elsanhoty *et al.,* 2016).

High-level of aflatoxin exposure produces an acute hepatic necrosis (acute aflatoxicosis), resulting later in cirrhosis or carcinoma of the liver (Dhakal and Sbar, 2020). Acute liver failure is made manifest by bleeding, edema, alteration in digestion, changes to the absorption and/or metabolism of nutrients, and mental changes (Long *et al.,* 2018).

The expression of aflatoxin-related diseases is influenced by factors such as species, age, nutrition, sex, and the possibility of concurrent exposure to other toxins (Ledda *et al.,* 2017). A regular diet including apiaceous vegetables, such as carrots, parsnips, celery, and parsley may reduce the carcinogenic effects of aflatoxin (Long *et al.,* 2018).

There is no specific antidote for aflatoxicosis (Ugbaja *et al.,* 2020). Symptomatic and supportive care tailored to the severity of the liver disease may include intravenous fluids with dextrose, active vitamin K, B vitamins, and a restricted, but high-quality protein diet with adequate carbohydrate content (Naseem *et al.,* 2018).

Actinomycetes, comprises a large group of soil bacteria that are important in the recycling of carbon in polymeric macromolecules (Timková *et al.,* 2018). They may be exposed to heavy metals treatment with sewage sludge (Agunbiade *et al.,* 2019). The ways in which they interact with heavy

metals are unknown (Bankar & Nagaraja, 2018). It has been reported that actinomycetes as a group are more tolerant to cadmium than other species (Mawang *et al.*, 2021).

Therefore, there is a dramatic increase in the interest on studying the interactions of heavy metals with these microorganisms. Therefore, this study aimed to removal the heavy metals, Cd2+ and Mn2+ from contaminated water samples by eco-friendly and low cost-effective biological method.

Materials and Methods

Sample collection

Different samples of drinking water (50 samples of 100 ml in sterile bottles) collected from different places of Egypt. 20 samples of ground water, 19 tape water samples, 4 samples of mineral water and 7 filtered water samples were collected for measuring some metal ions and some physical properties by ICP. Soil samples were collected from different farms from Egypt, EL-Menoufia farms and Kafr Elshekh farms. The collected samples were transferred in sterile plastic containers to the laboratory. The soil samples were collected by stainless steel sampler and transferred in sterile polyethylene bags into laboratory for maintained at 4 for °C further processing.

Chemical preparation

Stock solutions of Cd^{2+} and Mn^{2+} were prepared from $CdCl_2.H_2O$, and $MnCl_2.4H_2O$, respectively. These stocks were used in the preparation of different dilutions for further experiments (25, 50, 100, 150, 200 and 300 mg/L). The pH value of the metal solutions was adjusted by using 1M (HCl and/or NaOH).

Actinomycetes isolation

Different morphologically isolates of actinomycetes were isolated from different location in Egypt, soil samples were taken after the removal of about 5 cm of the surface. The samples were collected under sterile condition according to the standard methods, these isolates were used to perform the experiment by dead biomass for removal studied heavy metal ions, such as ($Cd^{2+}$and Mn^{2+}). The ability of isolates to remove some aflatoxins were tested.

Physical treatment

The physical treatment was used for isolation of actinomycetes from soil. Where samples were dried at 100°C for one hour (Hayakawa *et al.*, 1997) and serial dilution was prepared from dried samples then inoculated in an inorganic salt-starch agar and incubated at 30°C for 7 days. After incubation isolated actinomycetes was purified and stored in slants at 4°C for further studies. Chemical and physical properties of water samples were measured at Sadat City University, institute of Genetic Engineering and biotechnology by ICP.

Gram staining method

A smear of the selected strain (A-4) was prepared on a clean glass slide and the smear was allowed to air-dry 1min then heat- fixed. The heat-fixed smear was flooded with crystal violet and after one minute, it was washed with water and flooded with mordant Gram's iodine after one minute. The smear was decolorized with 95 % ethyl alcohol, washed with water and then counter-stained with safranin for 45 seconds. After washing with water, the smear was dried with tissue paper and examined under oil immersion (100 x), (Williams *et al.*, 2004).

Preparation the dead biomasses of actinomycetes

Biomass of isolated actinomycetes were used as natural biosorbent to removal the Cd^{2+} and Mn^{2+} from aqueous solutions. One ml (2×10^6 CFU/ ml) from each isolated actinomycetes transferred into 250 mL Erlenmeyer flask containing 100 mL broth media (inorganic salt-starch agar) and incubated at 30 °C on shaker at 150 rpm for 7 days. Thereafter, the biomass of each isolated actinomycetes was pelletized by centrifugation at 4500 rpm for 20

minutes. supernatant was removed and pellets washed with 0.1 M NaCl to removal non biomass particles. Dead biomass was performed by drying the living biomass at 70°C overnight). To confirm completely dead of dried mycelium, the sample inoculated in agar media and incubate at 30°C for 7days, the absence of any growth referred to positive results (Simeonova *et al.,* 2008).

Evaluation the efficiency of dead biomass of the isolated actinomycetes in biosorption of the studied heavy metal from aqueous solution

To evaluate the biosorption efficiency of dead biomass of actinomycetes, 250 ml Erlenmeyer flask was prepared for every isolate to assay a single metal. One hundred ml from metal dilution 100 mg/ L was added in flask and inoculated by dead biomass, 3g/l). The flasks were incubated on rotary shakers, 150 rpm. at 30°C for 3h. Then, the samples were filtrated by 0.45µm cellulose membrane filter, the filtrate was analyzed for determine residual heavy metals using, ICP- OES.

Inductively Coupled Argon Plasma-Optical Emission Spectroscopy, Perkin Elmer Optima-3000 Redial. USA). The following equation used to determine the percentage of heavy metals that adsorbed by isolated actinomycetes, R), (Abd El-Motaleb *et al.,* 2020).

$(R = C_I – C_F)/ C_I \times 100$, Where the C_I refer to the initial concentration of heavy metals in the solution, C_F refer to the residual concentration of heavy metals in the solution.

Binary metals system

The isolates which were removed any studied heavy metals cd^{2+}, and Mn^{2+} singly above 65% was selected to remove metals from binary mixture for studied metals ions. The concentration of each metal ion in the mixture was 100 g/l in aqueous solution (Timková *et al.,* 2018) and the experiment was done as above (dead biomass 3gm/l 150rpm, at 30°C for 3 hours the flasks incubated on rotary shakers).

Factor effect on biosorption of heavy metals

To determine the impact of temperature on biosorption by the two experimental isolates no. A26 and no. A33 were carried out with different temperature, 25, 30, 35, 40, 45, 50 and 55°C under conditions in which 3 g/ L biomass was dispersed in 100mL of a solution containing 100mg/ L of interested heavy metals. The experiment was kept at continuous shaking, 150 rpm. for 3h at pH 7 after that the aqueous solutions were filtrated and each filtrate was analyzed for determine residual metal concentration. For analyzing the effect of pH, on the removal of the studied heavy metals by the two isolates, experiments were conducted at different pH values, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 under optimum temperature and 3 g/L biomass in 100 mL of a solution containing 100mg/ L of heavy metals and shaking at 150 rpm for 3h and the residual metal concentration was analyzed as described above. Different weights of biomass, ranging from 0.1% to 0.6%, were dispersed in each metal solution under optimized parameters to determine conditions for maximum metal ion biosorption. The effect of initial metal concentration, 25, 50, 100, 200 and 300 mg/ L of cd2+ and Mn2+. was studied by analyzing biosorption under conditions where all the parameters, pH, temperature, and biosorbent dosage 3g / L were optimized for the best isolates (Djinni & Djoudi, 2021).

Identification of isolate fungi (*Aspergillus flavus group*)

(*Aspergillus flavus and Aspergillus paraciticus*) fungi were brought from Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Egypt. The developed fungi were sub-cultured on PDA slants (7 days old) then identified according to their cultural, morphological and microscopical characters under steriobinoculer microscope. Identification was further confirmed according to Maren and Johan (1988) for *Aspergillus sp* then were streaked on the Czapek-Dox Agar plates and incubated for 7 days at 27±2 C°. The Czapek-Dox broth medium consist of

gm/L (sucrose 30.0, sodium nitrate 3.0 dipotassium phosphate 1.0, magnesium sulphate 0.5, potassium chloride 0.5, ferrous sulphate 0.01 and final pH 7.3±0.2 was prepared and then 1000 mL of the prepared medium was distributed equally at rate of 100 mL per each conical flask (100 mL) then they autoclaved at 121°C for 20 min. The flasks were inoculated with discs (one disk 5 mm/flask) of *Aspergillus flavus* group according to their fluorescence; under sterile conditions and then subjected for incubation at 27±2°C for 7 days. The mycelial mats were harvested by centrifugation (Biofuge – Heraeus) at 10000 xg for 10 min and filtrates were stored at 4°C until used (El-Bazza *et al.*, 1983).

Detection of aflatoxins produced by *A. flavus* group using fluorescence technique

The tested fungi produce aflatoxins was achieved for 4 plates of *Aspergillus flavi* by the fluorescence technique mentioned by Hara *et al.*, (1974). Each plate was streaked on the Czapek-Dox Agar plates and incubated at 27±2°C for 7 days in dark. Petri dishes with non-fluorescent glass were used. Diffusible zones of aflatoxins were detected visually as fluorescent zones under UV (365 nm) light with a Phoretix workstation at the documentation unit, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Egypt.

Extraction of Aflatoxins and their absorbance by dead biomass of isolates A26 and A33.

The percentage of acetonitrile: methanol (40:60 v/v) was added to 50 ml of fungi filterate, left for 30 min at 25°C and shaked for the same time. One gram of sodium chloride (NaCl) and 4 gm of magnesium sulfate (MgSO4) were added and shaked for 10 min. Subsequently, supernatant was evaporated to dryness and rinsed with 0.5 ml acetonitrile 10%. Derivation process was done using trifluoroacetic acid (TFAA) under incubation for 15 min. After cooling, the equal dried weight of extraction was subjected to equal volume of sterile water (100ml), two erlenmeyer flasks were treated with dead biomass of isolates (A26, A33) which showed high absorption of heavy metals ($Cd^{2+}$and Mn^{2+}) at optimum condition (0.3 % of dead biomass of the two isolates, pH7.0 at 30°C at 150 rpm for 3 hours) then were filtered by filter paper, Another flask was used as control without treatment (Alkhalaf *et al.*, 2010).

Determination of Aflatoxins by HPLC (High performance liquid chromatography)

Extraction and clean up procedure were done in the Pesticides Research Laboratories, National Research centre, (NRS) El Dokki, Cairo Egypt, according to Choochuay *et al.*, (2018).

Identification of the most removable isolates by 16S rRNA

Genomic DNA extraction, PCR amplification of the 16S rRNA, the purification of PCR products, gel electrophoreses and 16S rRNA sequencing were done. The 16S rRNA sequences of A26, A33 were aligned with the published representative sequences of Actinomycetes obtained from the NCBI Gen Bank database for 16S rRNA sequences. The tree topologies were evaluated by maximum likelihood and bootstrap analysis based on 1000 replications with MEGA6, and phylogenetic trees was inferred using the neighbor-joining method (Saitou and Nei 1987, Roth *et al.*, 2003). The 16S rRNA sequences of the isolate A26 *(Streptomyces albus*) and A33 *(N.vermiculate*) were deposited in the Gen Bank database under accession numbers MW947066.1 and MZ229469.1 respectively.

Phylogenetic analysis

A comparative analysis of sequences was performed using the CLUSTAL W multiple sequence alignment program, version 1.83 of Meg Align module of Laser gene DNA Star software Pairwise, which was designed by Thompson *et al.*, (1994). Sequence alignments and phylogenetic comparisons of the aligned sequences for the gene were also

performed with the Meg Align module of Lasergene DNA Star software to determine nucleotide and amino acid sequence similarities and relationships.

Scanning Electron Microscope (SEM) and Energy Dispersive X- ray analysis (EDX)

Dead biomass of actinomycetes were examined before and after biosorption of cadmium and manganese and aflatoxins (AFB1-AFB2-AFG1-AFG2) at optimum conditions as temperature, pH and (3 g/L biomass in 100 mL of a solution containing100 mg/L of heavy metal ions) by using scanning electron microscope (JEOL JSM- 5500 LV) at the faculty of science, Alexandria University, Egypt. Also, dead biomass of actinomycetes was examined by EDX analysis (Module Oxford 6587 INCA x-sight) attached to SEM before and after biosorption. This technique was used to know the elements that are present on their wall and the mechanism involved in biosorption process (Mende *et al.*, 2016)

Statistical analysis

All the experiments were carried out in triplicates. Statistical analysis was performed using Statistical Package of Social Science, SPSS. software 16.0 and computer program Microsoft Office Excel, 2010). The results were expressed as mean ± standard deviation. Differences were considered significant at $p \leq 0.01$. The data were subjected to analysis of variance, ANOVA. (Ishak and Ali, 2016), $p \leq 0.01$.

Results and Discussion

Different morphological actinomycetes were isolated from collected soil samples. The dead biomass of isolates was used to perform the experiment for removal of studied heavy metal ions (Cd^{2+} and Mn^{2+}) and Aflatoxins

Determination of some metal ions concentrations of 50 drinking water samples collected from different locations in Menoufia governorate (ground water, tap water, mineral water, filtered water), the samples determined by means of inductively coupled plasma- mass spectroscopy (ICP-MS) results are in tables no (1,2,3 and 4).

The isolates A1, A15, A26, A33, and A46 were recorded the highest biosorption percentage above 65% for heavy metals ions singly (one at least) as shown in table (5).

The isolates A1, A15, A26, A33, and A46 were recorded the highest biosorption percentage above 65% for heavy metals ions singly (one at least).

These isolates were selected to perform the experiment using binary mixture composed of Mn^{2+} and Cd^{2+} in aqueous solution. The highest removal percentage was recorded by A26 dead biomass, it has removed 52.7 % from Cd^{2+}, 59.4 % from Mn^{2+} according to the results in table (6).

16S rRNA sequence and phylogenetic analyses of the highest biosorbent isolate (A26)

Molecular Identification

Briefly, after culturing each microbe on its appropriate growth culture, the grown bacteria were collected by centrifugation at 4000 rpm for 10 min. After discarding the supernatant, each genomic DNA was extracted from the harvested bacterial pellet using GeneJet genomic DNA purification Kit (Thermo K0721) as described by the supplier's protocol. The extracted DNA was considered pure when the OD_{260}/OD_{280} ratio is around 1.8, and the OD_{260}/OD_{230} ratio is about 2.0. The PCR was used to amplify the 16S rDNA gene using 27F and 1492R as PCR primers. The amplicons were then purified by the PCR purification kit (QIAquick, Qiagen Inc. Valencia CA) according to the manufacturer's instructions. Sequencing analysis was performed by 27F/1492R as sequencing primers via applied biosystems 3130 automated DNA sequencer (ABI, 3130, USA) using a ready reaction bigdye terminator V3.1 cycle sequencing kit (PerkinElmer/Applied Biosystems, Foster City, CA) with Cat. No. 4336817. The chromatograms were

inspected to remove primers and low-quality sequenced regions. Then, the obtained sequences were deposited in the nucleotide database of the National Center for Biotechnology Information (NCBI Resource Coordinators 2016), and the sequence identifiers are MW947066.1 and MZ229469.1.

The 16S rDNA partial sequences MW947066.1 and MZ229469.1 were used separately as queries in a blastn algorithms with a cutoff e-value 1e-10 and with a word size of 256 to retrieve the authentic homologous 16S rDNA genes of each query. Retrieved sequences were aligned using by MAFFT version 7 (Katoh *et al.*, 2019). Maximum likelihood phylogenetic trees were inferred assuming the lowest BIC (Bayesian Information Criterion) score models (Nguyen *et al.*, 2015; Hoang *et al.*, 2018).

The Tamura-Nei (TN) substitution model with empirical frequencies (+F) with assuming that sites are evolutionary invariable (+I) under the four rate categories of discrete gamma distribution (+G) was used for the phylogenetic tree topology of the *Streptomyces albus* ASH21 (MW947066.1) homologous sequences. However, the Hasegawa-Kishino-Yano (HKY) substitution model with empirical frequencies (+F) with assuming that sites are evolutionary invariable (+I) under a discrete gamma distribution (+G) with 4 rate categories was used to construct the phylogenetic tree of *Nocardia vermiculata* ASH22 (MZ229469.1) homologous sequences (Kalyaanamoorthy *et al.*, 2017).

Factors effect on biosorption of some heavy metals

The dead biomass has several advantages greater than living biomass. These advantages include their ease of treatment and no metal toxicity that can influence on live cells. Additionally, the dead biomass doesn't required supplementation with nutrients which can increase the biological and chemical oxygen demands on the treated water (Daboor *et al.*, 2014, Al Turk and Kiki, 2011).

Effects of different temperature on biosorption efficiency by the two isolates

Reduction efficiency of dead biomass of the isolate coded *Streptomyces albus* was increased with increasing temperature from 20 °C to 40 °C. The effect of temperatures on removal the studied heavy metals was significant $p < 0.01$. On the other hand, bio removal efficiency was decreased when the temperature was increased from 40°C to 60 °C. Efficiency of dead biomass of *Streptomyces albus* and *N. vermiculate* was increased with increasing temperature from 20 °C to 40 °C. Then, bio removal efficiency was decreased when the temperature was increased from 40 °C to 60 °C. as shown in fig (3,4). The increase in the biosorption percentage with elevation in temperature can be attributed to the several factors such as a change in the pore size of the biosorbent leading to a greater inter particle diffusion within the pores, the creation of new active sites on the sorbent, a temperature based acceleration of some slow sorption steps, an enhancement in the mobility of metal ions from the bulk solution toward the adsorbent surface, and/or an enhancement in the chemical affinity of the metal cations for the surface of adsorbent (Wassel *et al.*, 2014).

Effects of different pH on biosorption efficiency by the two isolates

The pH of the aqueous solution has been considered as one of the most important factors influencing the biosorption process. It was influenced not only the dissociation of functional groups on the active sites of the biosorbent but also the solution ion chemistry. Different metals were shown different pH optima for their biosorption as shown in fig (5). The maximum biosorption by the isolate coded *Streptomyces albus* for Mn^{2+} and Cd^{2+} ions were found at pH 8 with removal efficiency 76.3±1.8%, and 87.5±1.6% respectively. Also, the maximum biosorption by the isolate coded *N. vermiculate* for Mn^{2+} and Cd^{2+} ions at pH 8 were 80.3±0.8% and 75.5±0.88% respectively as shown in fig. (6)

Table.1 Different samples of drinking water (50 samples) collected from different places of Egyptian area, Menoufia Governorate.

Type of sample	Sample source
20 Ground water samples	**Tala** 1-Babel- 2-Kfr El-Aarab 3-Kfr Sanaded 4- Kafr Krshom 5- Zenara- 6-El-Zawya **Brkt Elsabea** 7- Ganzor 8-Shentena Elhagar **Elshohadaa** 9- Denshway **Qwesna** 10- Kafr Amros 11-Kafr El- Raml- 12- Shamandeel **Ashmon** 13- Greese 14- Barasheem 15-El-Kawady **ShebenElkom** 16-El-Batanon 17-Kafr El-Meselha 18- Betebs **Elbagor** 19- Meet Afeef 20- Garwan .
19 tape water samples	**Tala** 1-Babel 2-Kafr El-Aarab 3- Kafr Sanaded 4-Kafr Krshom 5-Zenara 6- El-Zawya **Brkt Elsabea** 7- Ganzor 8- Shentena El-Hagar **Elshohadaa** 9- Denshway **Qwesna** 10- Kafr Amros 11-Kafr El-Raml 12-Shamandeel. **Ashmon** 13- Greese 14-Barasheem 15-El-Kawady **ShebenElkom** 16-El-Batanon 17- Kafr El-Meselha **Elbagor** 18- Meet Afeef **19- Elsadat City**
7 Filtered Water samples	**Tala** 1-Babel 2-Kfr El-Aarab 3-Kfr Sanaded 4 -Kafr krshom 5-Zenara 6-El-Zawya **Brkt Elsabea** 7- Ganzor
4 mineral water samples	**Tala 1- Baraka 2- Aquafina 3- Nestle 4 - Dasany**

Table.2 Sites used in sample collection and their description.

Sample code	Type of sample	(Place of sample)	Type of plant
A1	Soil	Kafr El -Shekh, Khadmia	Beans
A2	Soil	Kafr El- Shekh, Khadmia	Beet
A3	Soil	Kafr El -Shekh,,Eshak	Cabbage
A4	Soil	Kafr El Shekh,Elarada	Guava
A5	Soil	Kafr El –ShekhElhamrawy	Wheat
A6	Soil	Menoufia, Tala, Babel	Beans
A7	Soil	Menoufia, Tala, Kafr El-arab	Potato
A8	Soil	Menoufia, Talam El Zawya	Corn
A9	Soil	Kafr El-Shekh, Sedy Ghazy	Onion
A10	Soil	Menoufia, Berket El-Sabea	Grapes
A11	Humus	Menoufia, Tala, kafr Sanaded	--------
A12	Soil	Menoufia, Tala, Kafr Sanaded	Grapes
A13	Soil	Menoufia, Sheben El-Kom , Betebs	Ornamental trees
A14	Soil	Menoufia, Sheben EL-Kom Batanon	Ornamental trees
A15	Soil	Kafr El-Shekh, Elhamrawy	Wheat
A16	Soil	Menoufia, Tala, Kfr Krshom	Cucummber
A17	Soil	Menoufia,Tala, Barawy	Wheat
A18	Soil	Kafr El-Shekh Sedy Salem, kelen	Clover
A19	Soil	Kafr El-Shekh, Sedy Salem, Damaro	Garlic
A20	Soil	Menoufia, Tala,Kafr Askar	Potato
A21	Soil	Menoufia,Tala,Kafr Rabea	Clover
A22	Soil	Menoufia Sheben El-Kom Kafr El-Sokaria	Ornamental trees
A23	Soil	Menoufia, El-Shohada,Denshway	Onion
A24	Soil	Menoufia,El-Shohada, Kafr Amros	Beans
A25	Soil	Kafr El-Shekh, SedySalem Kafr El-Masharka	Wheat
A26	Soil	Menoufia,Ashmon Shatanof	Corn
A27	Soil	Menoufia,Ashmon,Samadon	Wheat
A28	Soil	Kafr-El-Shekh, Sedy Salem, El-Hadady	Grapes
A29	Soil	Kafr El-Shekh, Ezbet Kamees	Wheat
A30	Humus	Menoufia, Tala, Zenara	-------
A31	Soil	Menoufia ,Qwesna ,Kafr El-Raml	Wheat
A32	Soil	Menoufia, Qwesna, Shamandeel	Garlic
A33	Humus	Menoufia, Tala,Kafr Askar	-------
A34	Soil	Menoufia, Qwesna, Meet Eleaz	Onion

A35	Soil	Kafr El-Shekh, Albakhanse	Garlic
A36	Soil	Menoufia, Tala, Tabloha	
A37	Soil	Menoufia, Tala, Zenara	Corn
A38	Soil	Menoufia, Tala, Babel	Onion
A39	Soil	Menoufia, Tala ,Zawyt Bemm	Clover
A40	Soil	Menoufia, Tala, Kafr El-Sokaria	Botato
A41	Soil	Menoufia, Menouf, Damleeg	Beans
A41	Soil	Menoufia, Menof ,Damleeg	Clover
A42	Soil	Menoufia, Menouf, Alhamoul	Onion
A43	Soil	Menoufia , Qwesna Omkhanan	Garlic
A44	Soil	Menoufia, Sheben El-Kom, Kafr El-Meselha	Beans
A45	Soil	Menoufia, Sheben ElkomM Batanon	Clover
A46	Soil	Menoufia, Ashmoon, Greese	Corn
A47	Soil	Menoufia, Ashmoon, Barasheem	Corn
A48	Soil	Menoufia Ashmoon, Elkwady	Wheate
A49	Soil	Menoufia, El-Bagor, Meetafef	Ornamental trees
A50	Soil	Menoufia, El-Bagor, Grawn	Ornamental trees
A51	Soil	Menoufia, El-Bagoor, Astanha	Corn
A52	Soil	Menoufia, El-Bagoor Astanha	Botato
A53	Soil	Menoufia, Brkt EL-Sabea ,El-Roda	Corn
A54	Soil	Menoufia, Brkt El-Sabea, Shentena El-Hagar	Beans
A55	Soil	Menoufia, Brkt El-Sabea Ganzoor	Clover
A56	Soil	Kafr-El-Shekh, kelen, Nashart	Clover
A57	Soil	Kafr-El-Shekh kelen, Hourine	Clover

Table.3 Metal ions concentrations (mg/L) and physical properties of mineral water collected from Menoufia governorate.

Metal ions:	**Samples No.**			
	1	2	3	4
Na	18.202	23.177	3.639	20.325
Mg	2.837	4.555	0.076	0.532
K	1.585	1.231	1.533	11.56
Ca	41.496	28.039	1.731	12.665
Mn	0.011	0.011	0.005	0.271
Cd	0.037	0.043	0	0.001
Ph	7.95	7.90	6.86	6.9
Ec	178	178	143	194
TDS	129	268	217	295

Table.4 Metal ions concentrations (mg/L) and physical properties of tap water collected from Menoufia governorate.

Metal ions:	Samples No.																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Na	35.84	20.15	16.21	26.01	39.34	37.77	40.64	25.24	35.88	33.33	45.73	69.53	23.17	57.94	52.15	22.25	62.21	45.81	84.974
Mg	8.101	3.888	5.089	12.45	1.032	0.989	1.064	0.661	1.145	1.277	1.197	13.665	4.555	11.38	10.249	4.373	27.62	10.643	36.29
K	0.843	0.347	0.469	0.592	22.43	21.5	23.12	14.36	18.19	23.13	26.02	3.693	1.231	3.078	2.770	1.182	2.875	0.578	0.157
Ca	13.39	33.98	37.17	26.63	24.57	23.53	25.33	15.73	27.27	25.35	28.50	84.1	28.04	70.09	63.09	26.92	45.66	81.07	90.248
Mn	0.011	0.013	0.085	0.151	0.065	0.062	0.067	0.041	0.584	0.651	0.61	0.033	0.011	0.028	0.025	0.011	0.038	0.070	0.147
Cd	0.044	0.044	0.032	0.029	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.046	0.015	0.038	0.035	0.015	0.150	0.099	0.084
pH	7.62	7.41	6.98	7.04	6.75	7.53	7.39	7.02	7.04	7.19	7.21	7.68	7.58	7.90	7.61	7.58	7.23	7.60	7.2
Ec	450	391	504	1.24	511	479	515	675	380	373	371	1332	419	1112	1123	428	405	331	260
TDS	326	283	365	90	336	687	339	444	578	567	564	747	235	624	630	240	790	645	507

Table.5 Metal ions concentrations (mg/L) and physical properties of filter water collected from Menoufia governorate.

Metal ions:	Samples No.						
	1	2	3	4	5	6	7
Na	19.007	7.975	12.36	4.574	16.5	48.56	62.687
Mg	4.416	1.086	0.027	0.12	1.868	9.37	30.005
K	0.240	0.032	3.586	2.602	8.575	0.836	1.427
Ca	33.640	13.364	6.342	2.85	84.49	81.90	64.138
Mn	0.029	0.006	0.75	0.061	0.005	0.031	0.364
Cd	0.041	0.044	0.01	0	0.018	0.106	0.070
PH	7.02	6.98	7.26	7.06	6.10	7.20	8.04
EC	210	33	111.9	107.2	65.6	282	335

Table.6 Metal ions concentrations (mg/L) and physical properties of ground water collected from Menoufia governorate

Metals ions:	**Sample No.**																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Na	55.535	44.489	78.043	74.987	70.724	41.78	30.87	31.18	36.38	53.75	37.19	55.625	71.849	61.419	61.651	35.322	98.47	87.67	43.145	44.94
Mg	15.109	19.000	0.946	0.908	1.851	1.094	0.808	0.881	0.762	1.407	0.973	10.932	14.121	12.071	12.116	6.942	15.35	27.53	5.87	39.43
K	0.375	0.082	19.033	18.28	40.241	23.77	17.56	19.14	15.33	18.51	21.16	2.954	3.816	3.262	3.274	1.876	8.575	2.537	0.173	0.0979
Ca	30.075	47.250	21.487	20.64	44.071	26.03	19.23	20.96	17.31	24.68	23.17	67.29	86.921	74.303	74.58	0.052	84.49	61.09	72.299	78.49
Mn	0.132	0.077	0.136	0.131	0.116	0.069	0.051	0.055	0.046	0.717	0.0496	0.026	0.034	0.029	0.029	0.017	0.271	0.460	0.032	0.345
Cd	0.006	0.044	0.005	0.005	0.004	0.002	0.002	0.002	0.004	0.003	0.002	0.037	0.048	0.041	0.041	0.023	0.200	0.173	0.238	0.016
pH	7.84	7.06	7.42	6.99	6.62	7.7	7.21	7.2	7.08	7.32	7.38	7.61	7.70	7.51	7.69	7.49	7.59	7.22	7.60	7.44
Ec	1063	963	1087	1044	1891	1117	825	842	579	898	801	1080	1340	1182	1184	679	1085	760	780	345

Table.7 The percentage of biosorption (%) of (cd2+ and Mn2+) by dead biomass actinomycetes isolates.

No of isolates	Cd^{2+} %	Mn^{2+} %
A1	66.7± 0.26	67.5 ±0.25
A2	40.2±0.47	39.5 ±0.87
A3	43.5 ±0.75	55.9±1.14
A4	50.4 ±1.01	41.5 ±0.33
A5	32.0 ±0.29	63.5 ±0.71
A6	26.4 ±0.42	51.5 ±0.67
A7	28.2 ±0.47	49.5 ±0.37
A8	58.6 ±0.83	51.8 ±0.58
A9	60.8 ±0.68	52.6 ±1.23
A10	47.5 ±0.90	48.7 ±1.09
A11	57.3 ±0.83	32.9 ±0.93
A12	56.2 ±0.17	62.0 ±0.66
A13	51.8 ±0.52	29.8 ±0.36
A14	54.5 ±0.82	58.1 ±0.68
A15	65.9 ±0.23	65.3±0.47
A16	48.3 ±0.89	49.4 ±0.98
A17	38.7 ±0.78	26.3 ±0.44
A18	39.2 ±1.21	55.4 ±1.08
A19	40.5 ±0.44	34.0 ±0.28
A20	39.4 ±0.66	38.2 ±0.77
A21	64.8 ±0.92	58.7 ±0.21
A22	54.0 ±0.87	72.4 ±0.34
A23	61.3 ±0.37	60.3 ±0.17
A24	33.4 ±0.47	29.4 ±0.65
A25	25.7 ±0.33	38.3 ±0.45
A26	71.4 ±1.02	70.0 ±0.28
A27	48.6 ±0.48	21.3 ±0.87
A28	39.1 ±0.85	36.5 ±0.12
A29	61.8 ±0.24	46.1 ±1.01
A30	25.9 ±0.85	28.2 ±0.85
A31	38.4 ±0.27	54.3 ±0.72
A32	55.7 ±0.78	47.4 ±0.96
A33	68.3 ±0.47	69.4 ±0.85
A34	58.7 ±0.72	47.5 ±0.63
A35	21.4 ±0.68	39.0 ±1.08
A36	56.8 ±0.38	36.1 ±0.13
A37	47.9 ±0.22	40.8 ±0.26
A38	22.5 ±1.10	59.2 ±0.79
A39	40.9 ±1.37	51.0 ±0.18
A40	57.3 ±0.92	49.6 ±0.87

A41	28.3 ±0.74	37.5 ±0.22
A42	55.8 ±0.84	50.4 ±0.69
A43	46.7 ±0.43	49.1 ±0.88
A44	49.3 ±0.47	29.3 ±0.74
A45	25.8 ±0.25	55.1 ±0.36
A46	66.2 ±0.81	60.2 ±1.04
A47	17.4 ±0.29	54.2 ±0.85
A48	56.3 ±0.11	39.9 ±0.29
A49	61.1 ±0.94	42.5 ±0.86
A50	38.5 ±0.77	58.4±0.95
A51	43.5 ±0.14	46.1 ±0.36
A52	49.0 ±0.25	34.7 ±0.28
A53	44.9 ±1.20	38.5 ±0.77
A54	58.4 ±0.84	59.4 ±0.88
A55	29.8 ±0.67	46.3 ±0.47
A56	37.6 ±0.34	42.6 ±0.62
A57	14.9 ±0.61	57.1 ±0.14

Table.8 The percentage of biosorption (%) for Mn 2+, and Cd2+ in binary metal systems by dead biomass of isolated actinomycetes which showed the greatest activity for metals biosorption.

Sample number	Cd2+ %	Mn 2+ %
A1	46.3±1.1	54.6±0.52
A15	45.2±0.56	52.5± 0.17
A26	52.7±0.54	59.4±1.04
A33	48.3±0.63	56.7±0.87
A46	37.8 ± 0.82	42.02±0.91

*SD: standard deviation

Table.9 Efficiency of dead biomass (*Streptomyces albus* and *N. vermiculate*) for absorption of different types of aflatoxins.

Sample	Concentration (ng/g)			
	AFG_1	**AFB_1**	**AFG_2**	**AFB_2**
Control	2.0	19.0	0.25	2.85
Streptomyces Albus	0.80	11.42	ND	ND
N. vermiculate	0.40	19.04	ND	2.85

ND = 0

Fig.1 A maximum likelihood phylogenetic tree with 1000 bootstrap replicates is constructed based on the partial 16S rDNA sequences (V_{2-8}) from various bacterial strains. To show the evolutionary relationships between the homologous sequences of the *Streptomyces albus* ASH21 16s rDNA, the phylogenetic tree is rooted using the *Escherichia coli* JCM 1649 homologous sequence. The downloaded phylogenetic tree was visualized and presented using iTOL (Interactive Tree of Life) webserver (Letunic and Bork 2016).

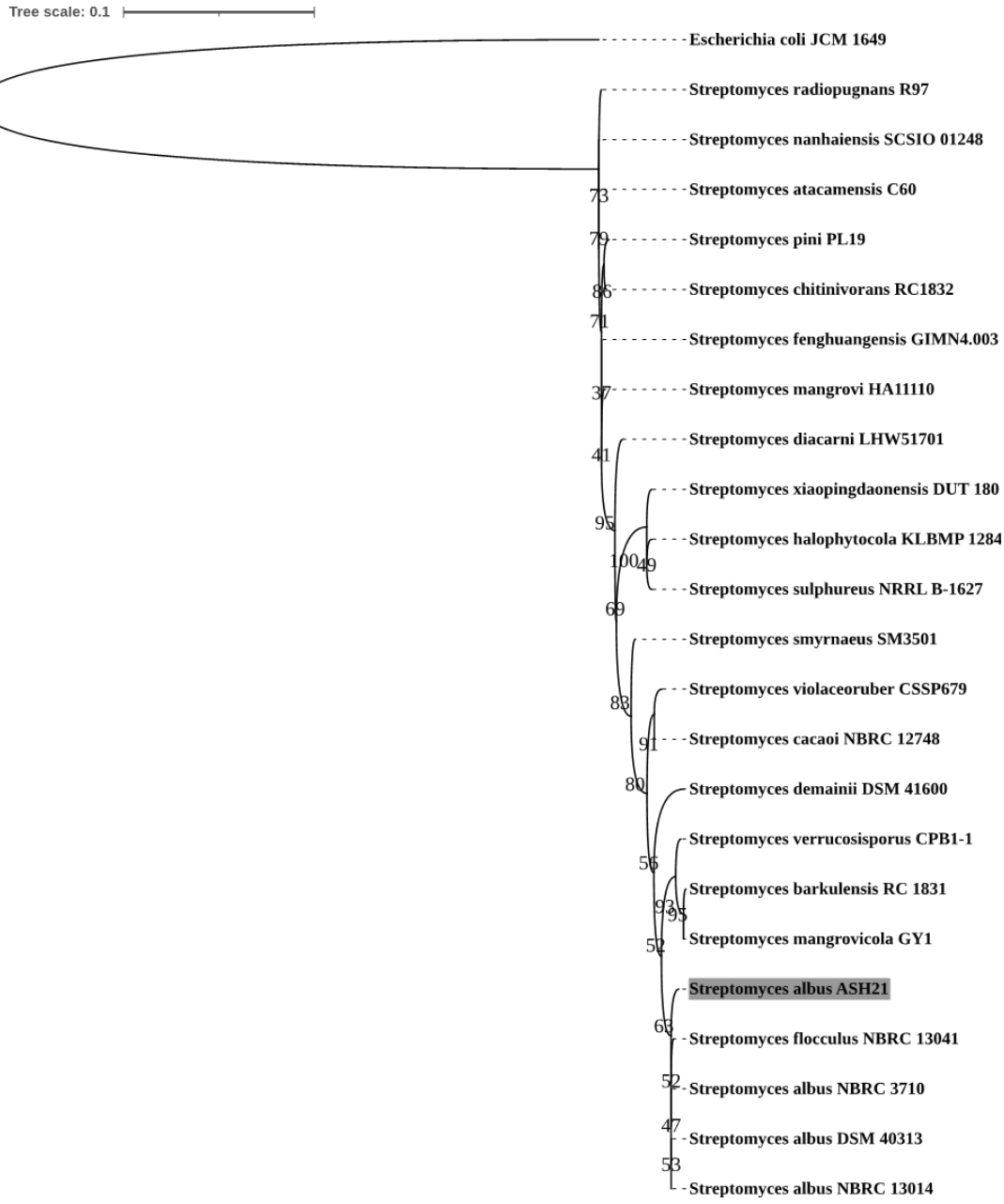


Fig.2 A maximum likelihood phylogenetic tree with 1000 bootstrap replicates is constructed based on the partial 16S rDNA sequences (V_{2-8}) from various bacterial strains. To show the evolutionary relationships between the homologous sequences of the *Nocardia vermiculata* ASH22 16s rDNA, the *Escherichia coli* NBRC 102203 16s rDNA is used as an outgroup for the phylogenetic tree. The downloaded phylogenetic tree was visualized and presented using iTOL (Interactive Tree of Life) webserver (Letunic and Bork 2016).

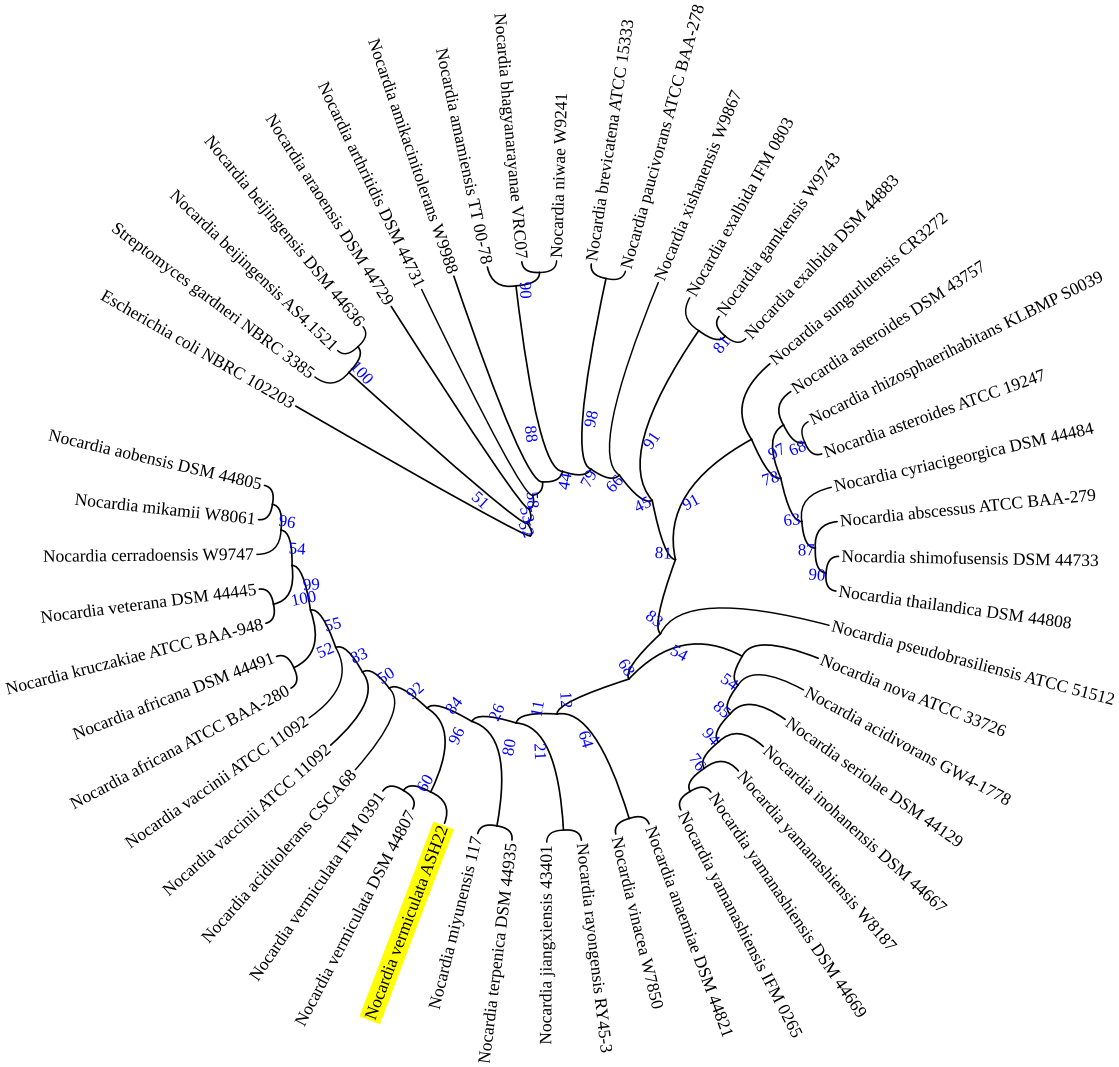


Fig.3 The effect of different temperature on the biosorbent efficiency Streptomyces *albus*.

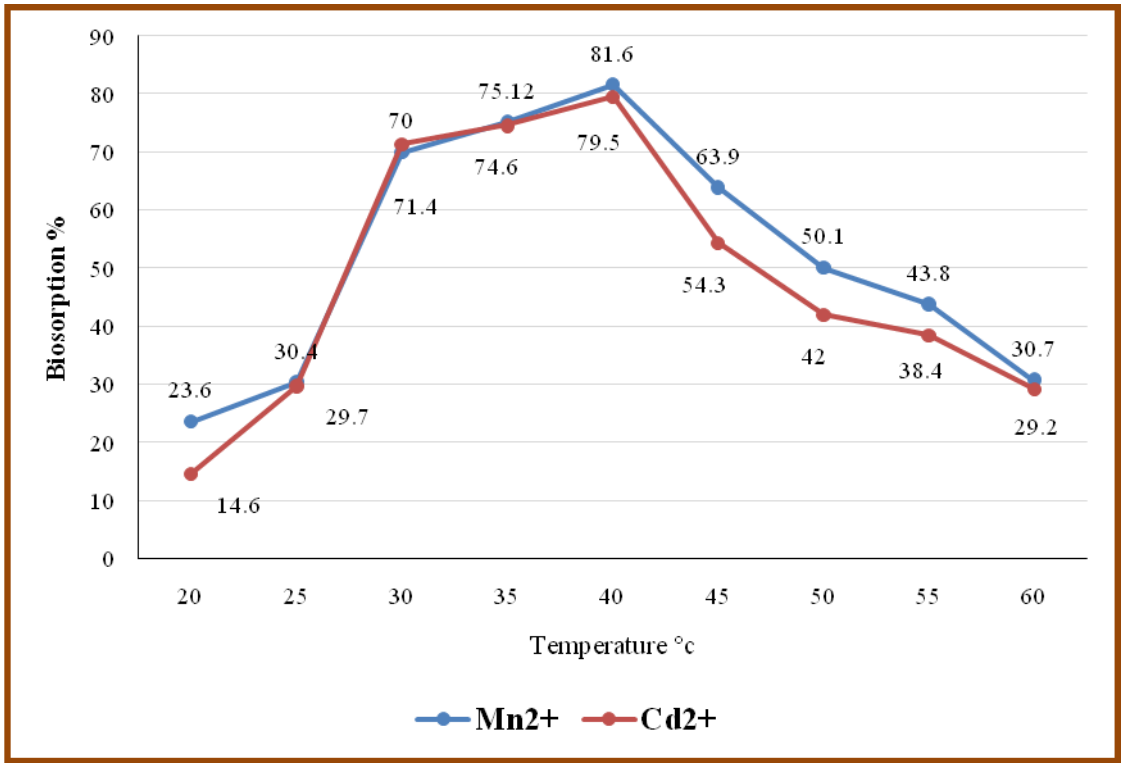


Fig.4 The effect of different temperatures on the biosorbent efficiency by *N. vermiculate.*

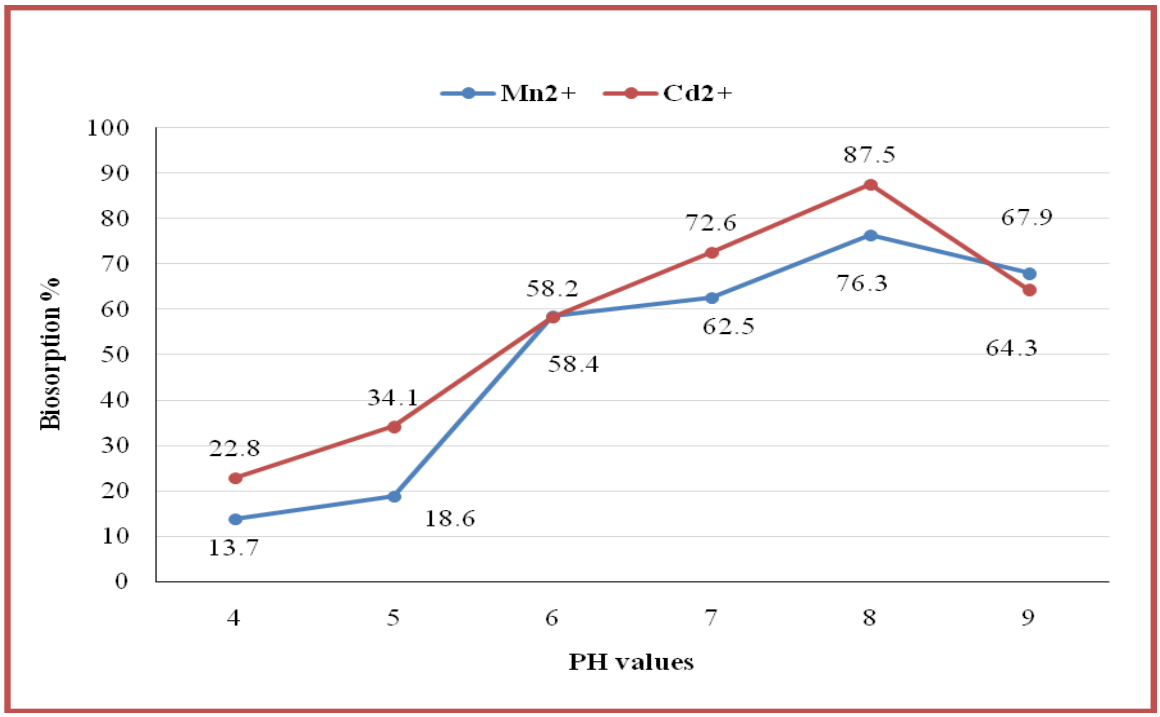


Fig.5 The effect of pH value on biosorption of Mn2+, and Cd2+by *Streptomyces albus*

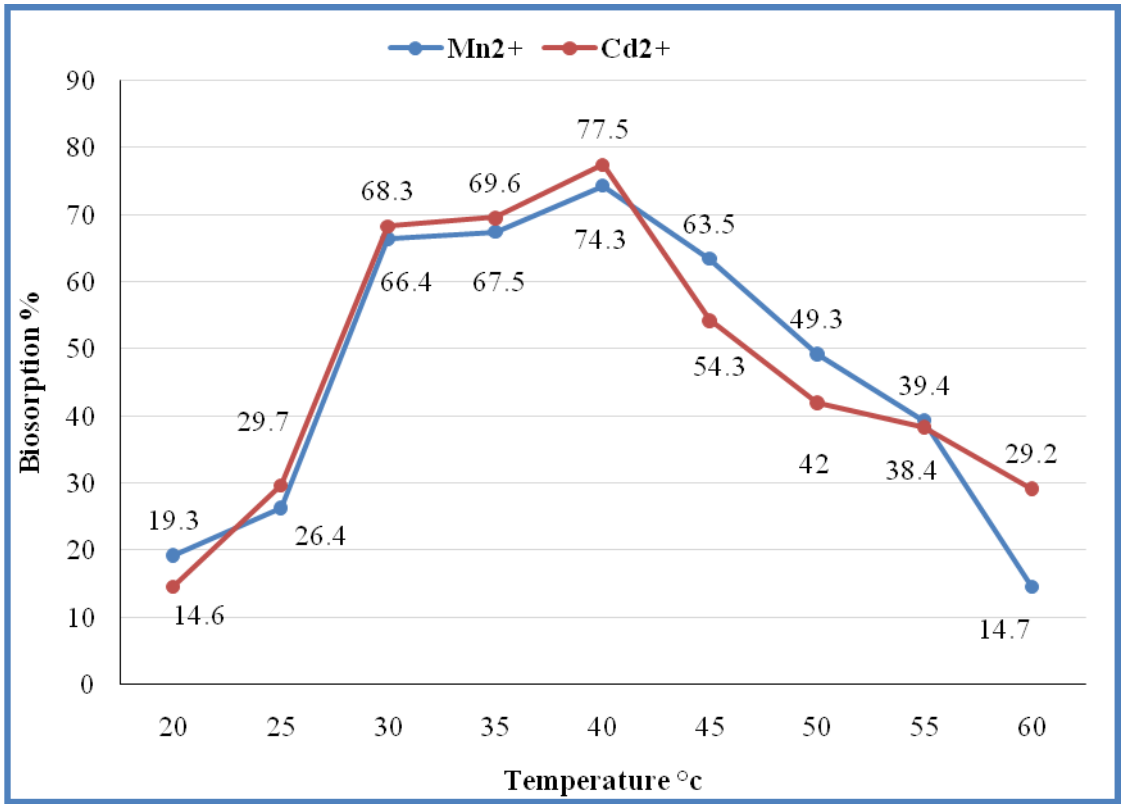


Fig.6 The effect of pH value on biosorption of Mn2+, and Cd2+ by *N. vermiculate*

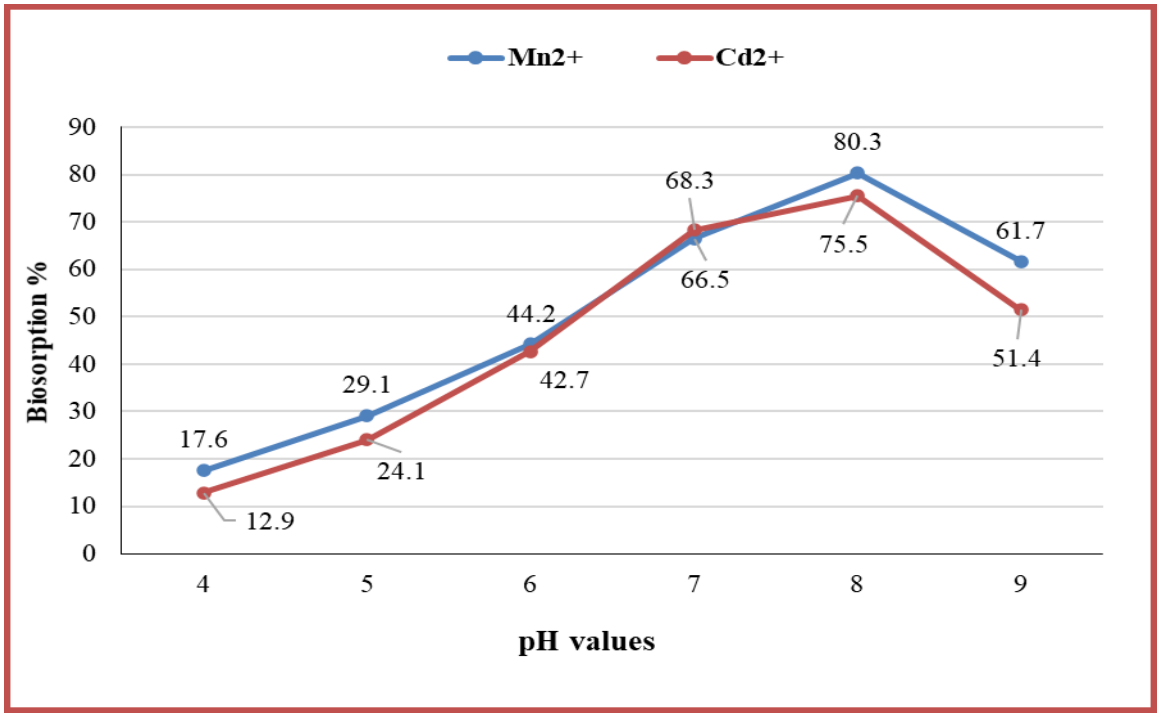


Fig.7 Effects of biosorption dosage on biosorption of Mn2+ and Cd2+ by *N. vermiculate*

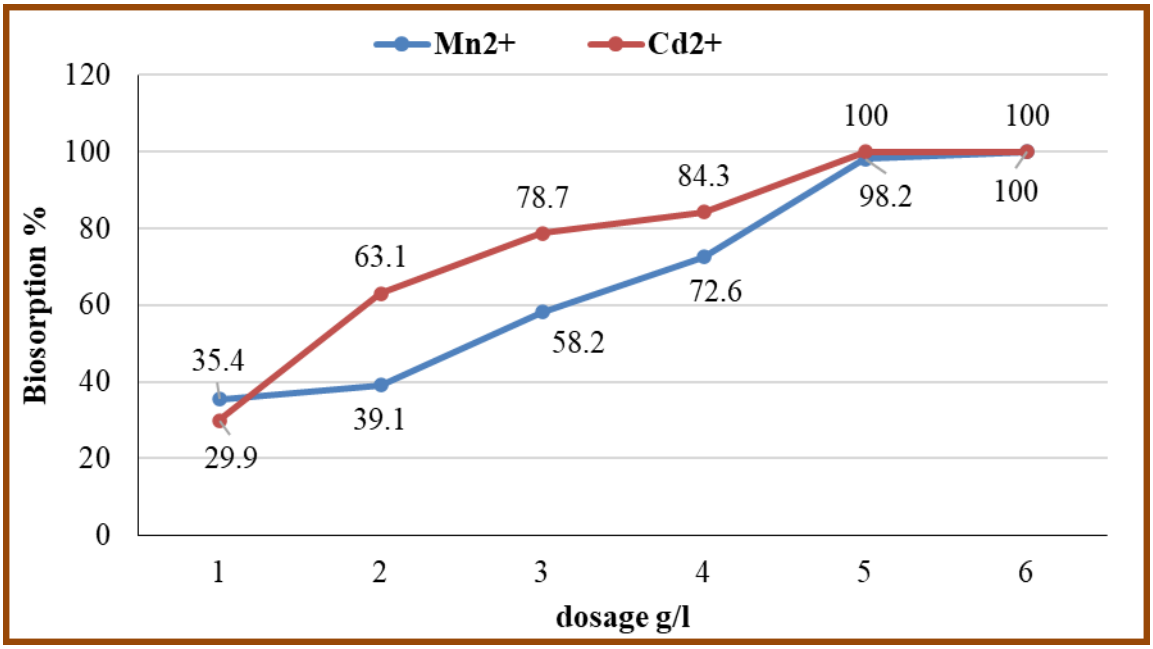


Fig.8 Effects of biosorption dosage on biosorption of Mn2+ and Cd2+ by Streptomyces *albus*.

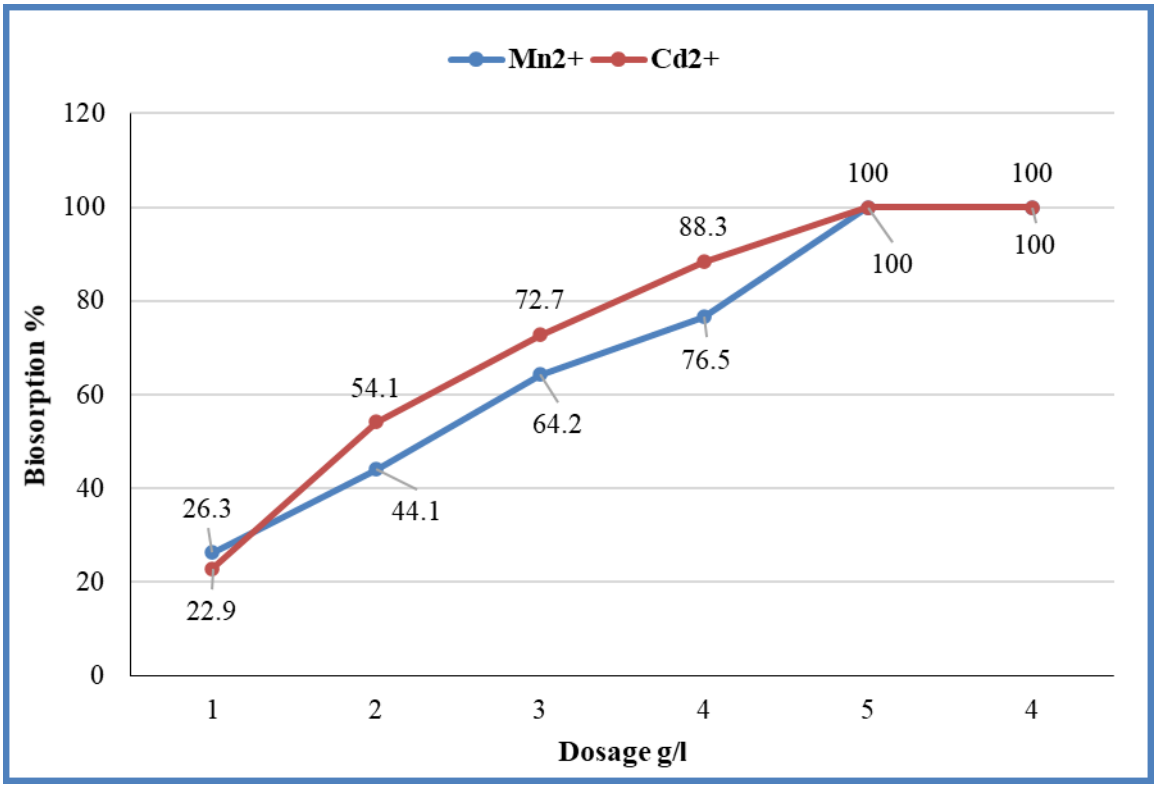


Fig.9 Detection of *Aspergillus flavus group* by fluorescence's technique

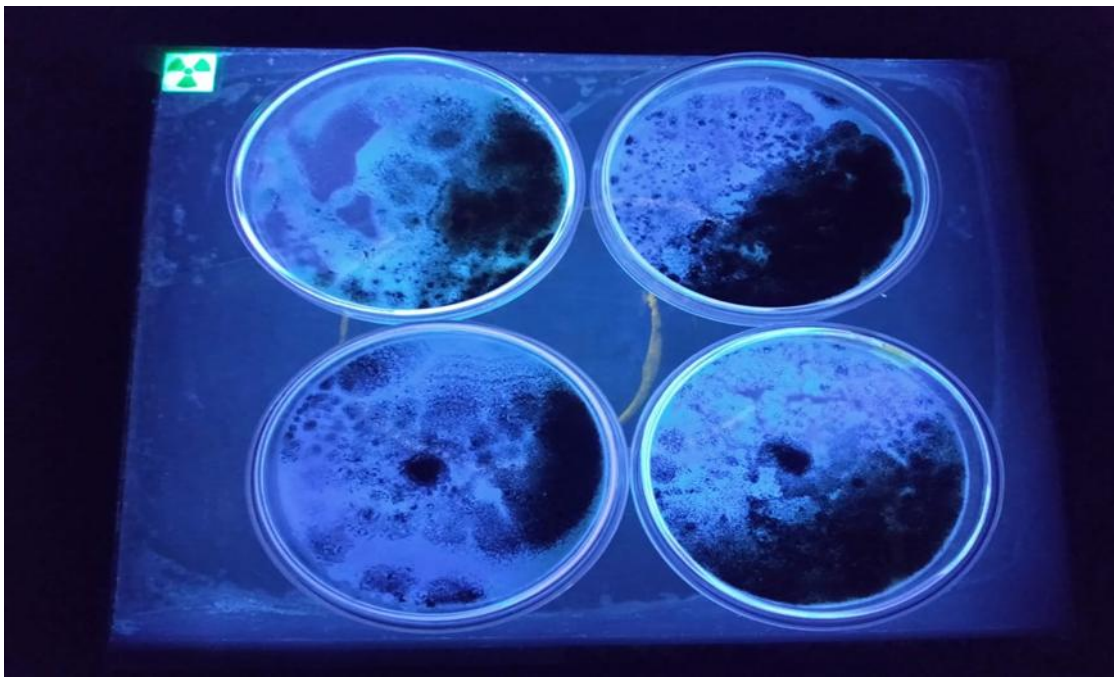


Fig.10 Chronogram analyses before treatment by dead biomass of Aflatoxins.

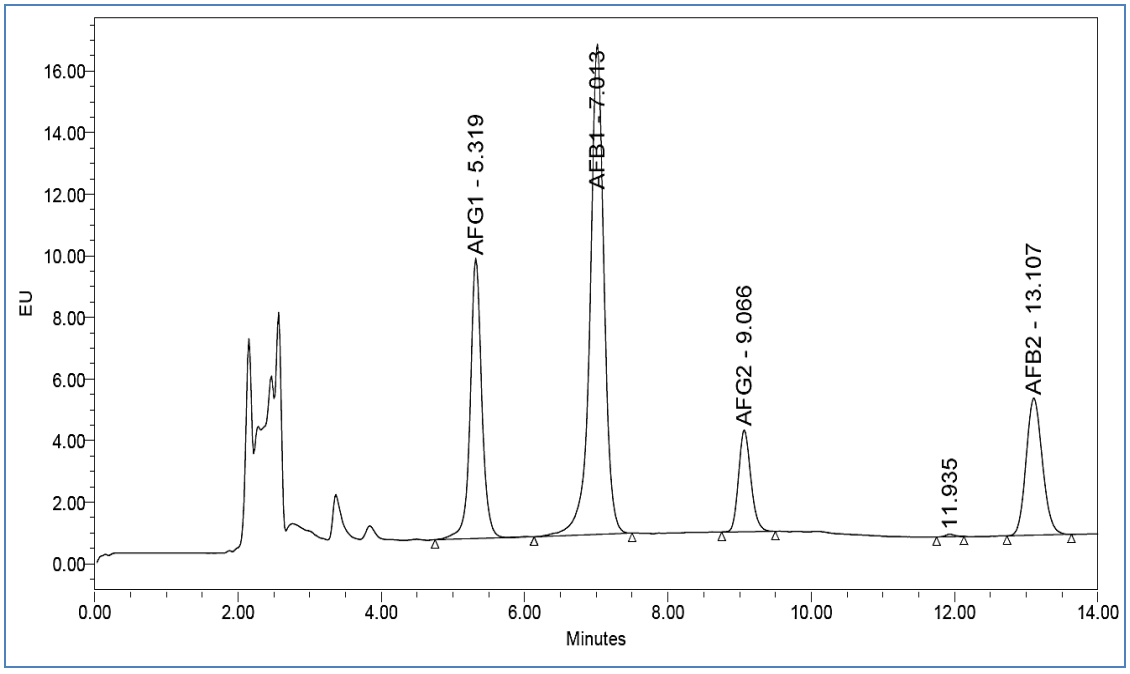


Fig.11 Chronogram analyses of Aflatoxins after treatment by dead biomass of *Streptomyces albus*.

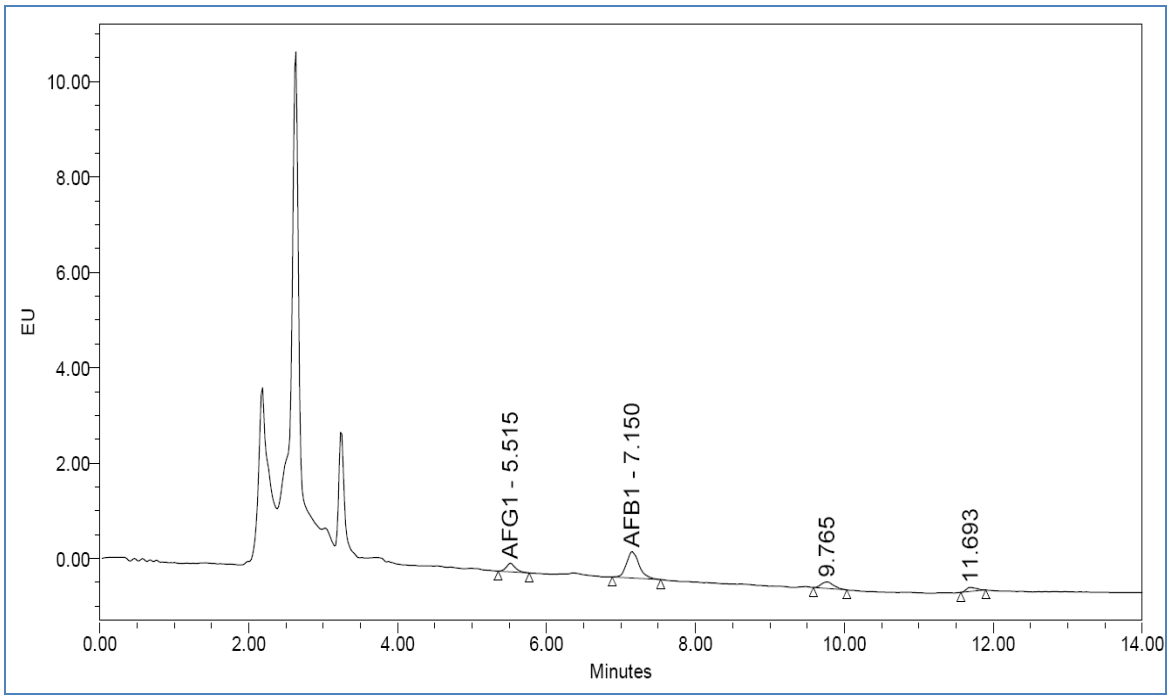


Fig.12 Chronogram analyses of Aflatoxins after treatment by dead biomass of *N. vermiculate*.

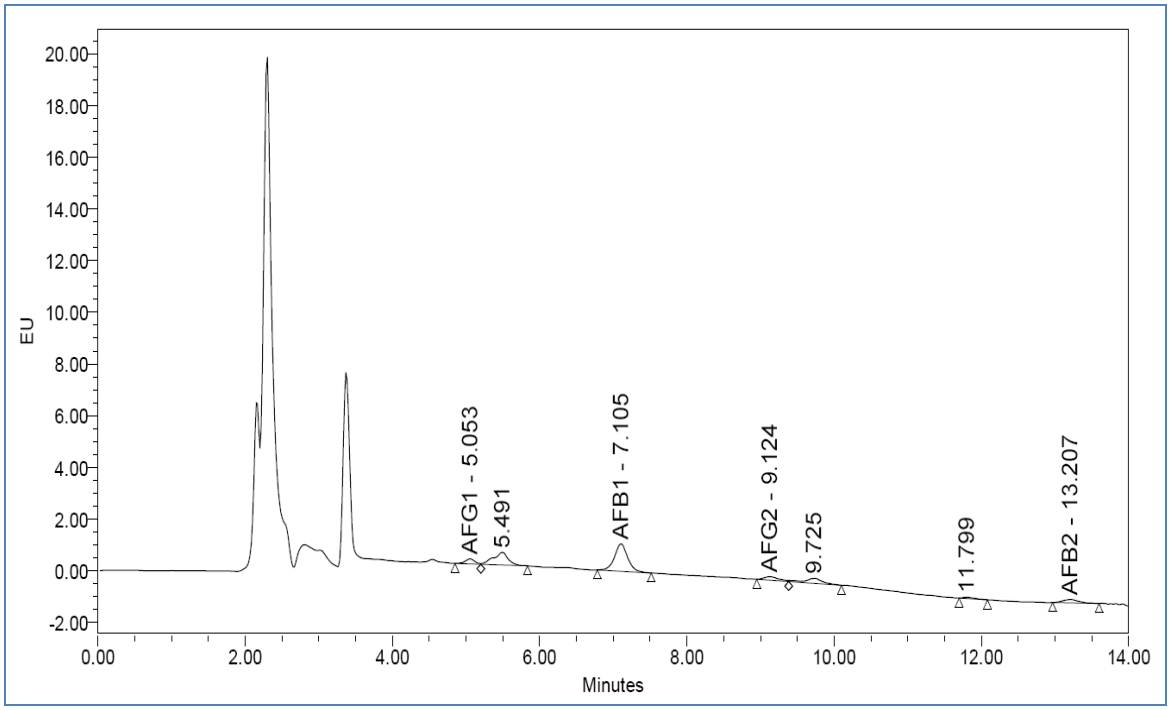


Fig.13 Scanning Electron Microscope for dead biomass *(Streptomyces albus)* (1) before biosorption of metal ions and (2) after metal ions biosorption.

()1 (2)

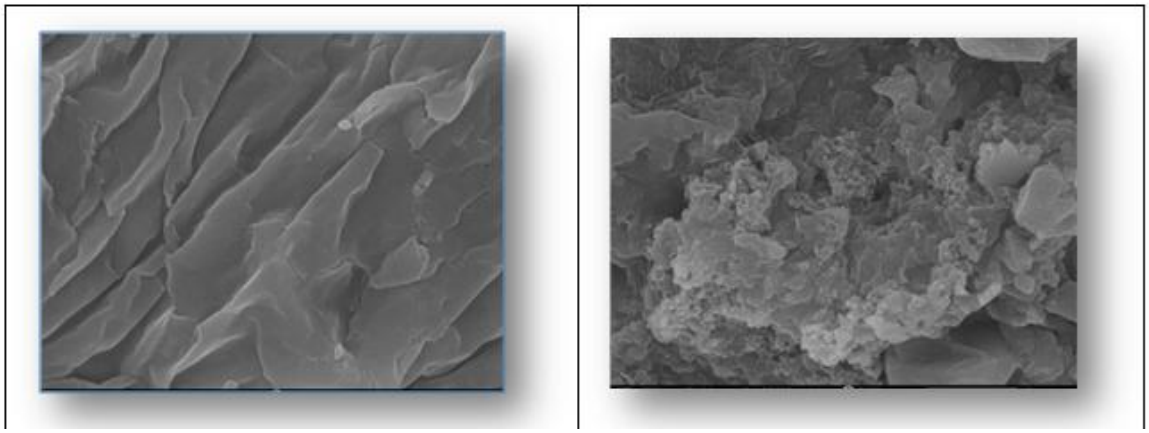


Fig.14 Scanning Electron Microscope for dead biomass (*N. vermiculate*) before (1) biosorption of metal ions and (2) after metal ions biosorption.

()1 (2)

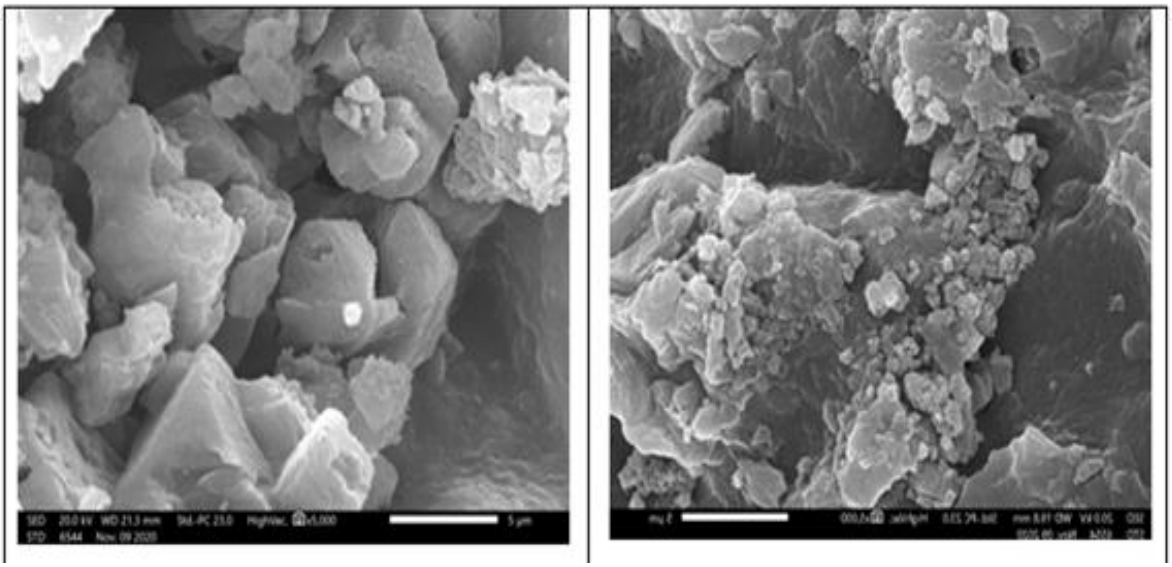
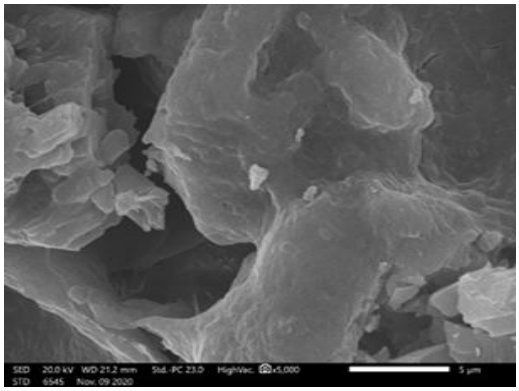
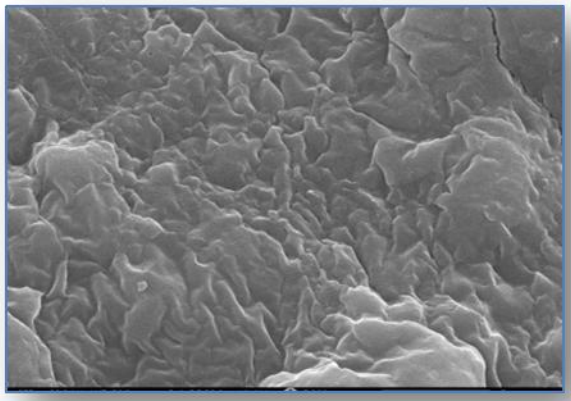


Fig.15 Scanning Electron Microscope for the two isolates (1) *(Streptomyces albus)* dead biomass before biosorption of aflatoxins (control), (2) (*N. vermiculate*) dead biomass before biosorption, (3) *Streptomyces albus* dead biomass after treatment (4) *N. vermiculate* dead biomass after biosorption of aflatoxins.

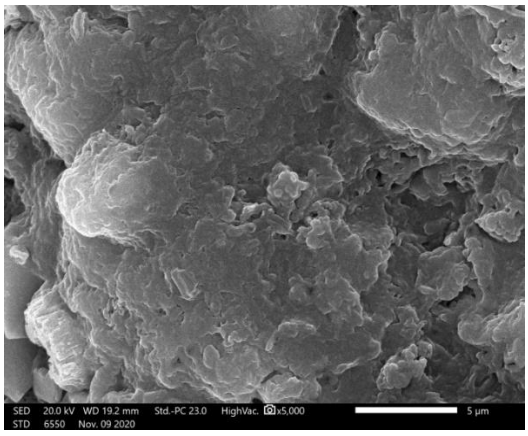
(1)



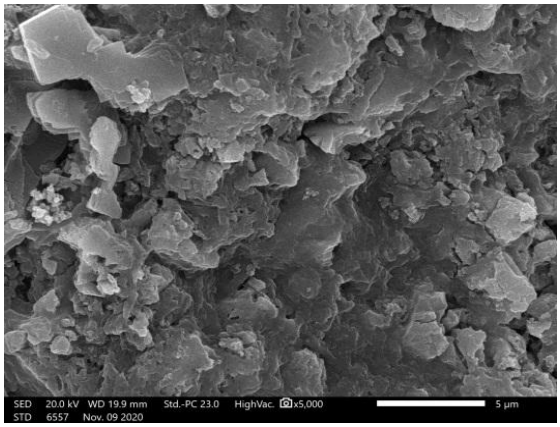
(2)



(3)



(4)



Effects of biosorbent dosage on biosorption efficiency by the two isolates

The effect of biosorbent dosage, 0.1%– 0.6%. on sorption efficiency in aqueous solutions under optimized temperature and pH was recorded in Fig. (7) The results were indicated that the removal of Mn^{+2} and $Cd^{+2}$by the isolate Streptomyces *albus* were increased rapidly by increasing the biosorption dosage from 0.1% to 0.3%. The removal of Mn^{+2} and Cd^{+2} by the isolate Streptomyces *albus* were increased from 26.3±0.12% and 22.9±0.32% to 64.2±0.38% and 72.7±0.18% respectively. Also, the results of the isolate coded *N. vermiculate* were indicated that when a biosorption dosage was increased from 0.1% to 0.3%, the removal of Mn^{+2}, and Cd^{+2} by the isolate coded *N. vermiculate* increased from 35.4±0.25% and 29.9±0.41% to 58.2±0.46% and 78.7±0.78%, respectively. Moreover, when the biosorption dosage increased than 0.6% the metal ions were completely removed from aqueous solution. The effect of biosorbent dosage on removal the studied heavy metals was significant $p < 0.01$. Moreover, when the biosorbent dosage increased than 0.6% the metals were completely removed from aqueous solution. While

the concentration of metal ions remained the same in solution, there were more biosorbent binding sites available at higher dosages than there were at lower dosage which was led to binding of all available metal ions (El-Gendy and El-Bondkly, 2016).

Determination of aflatoxin (s) production levels in control sample and after treatment with dead biomass

The dead biomass of *Streptomyces albus* and *N. vermiculate* were examined by EDX analyses before and after biosorption of different types of aflatoxin (AFG1, AFG2, AFB1, AFB2) from aqueous solution. As shown in Table (7) dead biomass (*Streptomyces albus*) had the most efficient for absorption of AFB1, and AFB2 compared *(N. vermiculate*), it recorded 11.42 ng/g and zero ng/g for AFB1 and AFB2 respectively. While dead biomass *(N. vermiculate*) had not affected on AFB1 and AFB2 doses. Regarding, AFG2 had absorbed completely by the dead biomass (*Streptomyces albus and N. vermiculate).* On the other hand, dead biomass (*N. vermiculate*) had more efficient in the removal of AFG1 (0.40 ng/g) than *Streptomyces albus* (0.8ng/g) compared with control.

Scanning Electron Microscope (SEM)

The morphology of dead biomass of isolates coded A26 and A33 were analyzed by Scanning Electron Microscope before and after biosorption of Mn^{+2}, Cd^{+2} and aflatoxins An electron micrograph of dead biomass of isolates coded *A26* and A33 were presented in Fig (13, 14 & 15) showed the change in the surface where the surface become more smoothly after binding with metal ions and filled holes by metal ions at optimum conditions of temperature 40°C, pH 8 and dosage of biomass 3gm/L.

Cadmium and manganese were considered as toxic heavy metals and removal them from water samples was an important target to improve the quality of wastewater before releasing into the environment. Dead biomass of actinomycetes can be used as a natural sorbent for the removal of heavy metals and aflatoxins from aqueous solutions. Biosorption by dead biomass (*Streptomyces albus* and *N. vermiculate*) for studied heavy metals (Cd^{+2} and Mn^{+2}) were recorded. The removal of metal ions is affected by temperature, pH, biomass dosage, contact time, initial concentration of metal ions. The maximum removal of metal was at a pH 8 and temperature between 40 °C and 60 °C. Out of dead cells for removal studies heavy metals, use of dead cells is economical because it does not require any maintenance of sterile conditions and the dead biomass can be regenerated. Therefore, there is plenty of scope for large-scale application of non-living biomass for metal ion removal.

The dead biomass of *Streptomyces albus* and *N. vermiculate* were examined by EDX analyses before and after biosorption of different types of aflatoxin (AFG1, AFG2, AFB1, AFB2) which produced by *A. flavus & A. parasiticus* fungi from aqueous solution. Dead biomass (*Streptomyces albus*) efficient for absorption of AFB1, AFB2 more than the efficient of *N. vermiculate* and, so the dose of aflatoxins were decreased by (*Streptomyces albus*) dead biomass treatment but *N. vermiculate* dead biomass absorbed AFG1 more than (*Streptomyces albus*).

References

Abd El-Motaleb M, El-Sabbagh S, Mohamed W, Wafy K. Biosorption of Cu 2+, Pb 2+ and Cd 2+ from wastewater by dead biomass of Streptomyces cyaneus Kw42. Int. J. Curr. Microbiol. Appl. Sci. 2020; 9:422-35.

Agunbiade M O, Pohl C, Heerden E V, Oyekola O, Ashafa A. Evaluation of fresh water actinomycete bioflocculant and its biotechnological applications in wastewaters treatment and removal of heavy metals. International journal of environmental research and public health. 2019 Jan;16(18):3337.

Al Naggar Y, Khalil M S, Ghorab M A. Environmental pollution by heavy metals in the aquatic ecosystems of Egypt. Open Acc.

J. Toxicol. 2018; 3:555603.

Al Turk I M, Kiki M J. Heavy metals removal from raw industrial wastewater by halophilic actinomycetes isolated from Saudi saline soil. Journal of International Environmental Application and Science. 2011 Dec 1;6(4):606.

Alkhalaf N A, Osman A K, Salama K A. Monitoring of aflatoxins and heavy metals in some poultry feeds. African Journal of Food Science. 2010 Apr 30;4(4):192-9.

Bankar A, Nagaraja G. Recent trends in biosorption of heavy metals by Actinobacteria. In New and future developments in microbial biotechnology and bioengineering 2018 Jan 1 (pp. 257-275). Elsevier.

Bolisetty S, Peydayesh M, Mezzenga R. Sustainable technologies for water purification from heavy metals: review and analysis. Chemical Society Reviews. 2019;48(2):463-87.

Cheng W W, Gobas F A. Assessment of human health risks of consumption of cadmium contaminated cultured oysters. Human and Ecological Risk Assessment. 2007 Mar 20;13(2):370-82.

Choochuay S, Phakam J, Jala P, Maneeboon T, Tansakul N. Determination of aflatoxin B1 in feedstuffs without clean-up step by high-performance liquid chromatography. International journal of analytical chemistry. 2018 Jun 24;2018.

Daboor S M, Haroon A M, Esmael N A, Hanona S I. Heavy metal adsorption of Streptomyces chromofuscus K101. J Coast Life Med. 2014;2(6):431-7.

Dhakal A, Sbar E. Aflatoxin toxicity. StatPearls [Internet]. 2020 May 31.

Djinni I, Djoudi W. Streptomyces sp. WR1L1S8 a potent endophytic marine strain for heavy metal resistance and copper removal enhanced by RSM modeling. Acta Ecologica Sinica. 2021 Apr 17.

Dong W, Zhang Y, Quan X. Health risk assessment of heavy metals and pesticides: a case study in the main drinking water source in Dalian, China. Chemosphere. 2020 Mar 1; 242:125113.

El-Bazza Z E, Zedan H H, Toama M A, El-Tayeb O M. Isolation of aflatoxin-producing fungi from Egyptian food and feed commodities.1983.

El-Gendy M M, El-Bondkly A M. Evaluation and enhancement of heavy metals bioremediation in aqueous solutions by Nocardiopsis sp. MORSY1948, and Nocardia sp. MORSY2014. brazilian journal of microbiology. 2016 Jul 1;47(3):571-86.

Elsanhoty R M, Al-Turki I A, Ramadan M F. Application of lactic acid bacteria in removing heavy metals and aflatoxin B1 from contaminated water. Water science and technology. 2016 Aug 10;74(3):625-38.

Halttunen T, Salminen S, Tahvonen R. Rapid removal of lead and cadmium from water by specific lactic acid bacteria. International journal of food microbiology. 2007 Feb 28;114(1):30-5.

Hara S, Fennell D I, Hesseltine C W. Aflatoxin-producing strains of Aspergillus flavus detected by fluorescence of agar medium under ultraviolet light. Applied microbiology. 1974 Jun;27(6):1118.

Hayakawa M, Iino H, Takeuchi S, Yamazaki T. Application of a method incorporating treatment with chloramine-T for the selective isolation of Streptosporangiaceae from soil. Journal of fermentation and bioengineering. 1997 Jan 1;84(6):599-602.

Hoang D T, Chernomor O, Von Haeseler A, Minh B Q, Vinh LS. UFBoot2: improving the ultrafast bootstrap approximation. Molecular biology and evolution. 2018 Feb 1;35(2):518-22.

Ishak W R, Ali SM. Assessment of Malaysian cornsilk bioactive compounds and its cytotoxicity test on brine shrimp (*Artemia salina*). Health (NY). 2016; 7:124-38.

Joseph L, Jun B M, Flora J R, Park C M, Yoon Y. Removal of heavy metals from water sources in the developing world using low-cost materials: A review. Chemosphere. 2019 Aug 1; 229:142-59.

Kalyaanamoorthy S, Minh B Q, Wong T K, Von Haeseler A, Jermiin L S. Model Finder: fast model selection for accurate phylogenetic estimates. Nature methods. 2017 Jun;14(6):587-9.

Katoh K, Asimenos G, Toh H. Multiple alignment of DNA sequences with MAFFT. In Bioinformatics for DNA sequence analysis 2009 (pp. 39-64). Humana Press.

Ledda C, Loreto C, Zammit C, Marconi A, Fago L, Matera S, Costanzo V, Fuccio Sanzà G, Palmucci S, Ferrante M, Costa C. Noninfective occupational risk factors for hepatocellular carcinoma: A review. Molecular medicine reports. 2017 Feb 1;15(2):511-33.

Liu K, Lin B. Research on influencing factors of environmental pollution in China: A spatial econometric analysis. Journal of Cleaner Production. 2019 Jan 1; 206:356-64.

Long X D, Deng Y, Huang X Y, Yao J G, Su Q Y, Wu X M, Wang J, Xu Q Q, Zhu X Y, Wang C, Huang B C. Molecular mechanisms of hepatocellular carcinoma related to aflatoxins: an update. Liver Research and Clinical Management. 2018 May 2; 1:113-36.

Madhav S, Ahamad A, Singh A K, Kushawaha J, Chauhan J S, Sharma S, Singh P. Water pollutants: sources and impact on the environment and human health. In Sensors in Water Pollutants Monitoring: Role of Material 2020 (pp. 43-62). Springer, Singapore.

Maren, A. K. and I.P. Johan, 1988. A Laboratory guide to the common *Aspergillus* spp. and their teleomorph. Commonwealth Scientific and Industrial, pp. 116.

Mawang C I, Azman A S, Fuad A S, Ahamad M. Actinobacteria: An eco-friendly and promising technology for the bioaugmentation of contaminants. Biotechnology Reports. 2021 Oct 5: e00679.

Mende M, Schwarz D, Steinbach C, Boldt R, Schwarz S. Simultaneous adsorption of heavy metal ions and anions from aqueous solutions on chitosan. Investigated by spectrophotometry and SEM-EDX analysis. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2016 Dec 5; 510:275-82.

Naseem M N, Saleemi M K, Khan A, Khatoon A, Gul S T, Rizvi F, Ahmad I, Fayyaz A. Pathological effects of concurrent administration of aflatoxin B1 and fowl adenovirus-4 in broiler chicks. Microbial pathogenesis. 2018 Aug 1; 121:147-54.

NCBI Resource Coordinators. Database resources of the national center for biotechnology information. Nucleic acids research. 2016 Jan 4;44(D1): D7-19.

Nguyen L T, Schmidt H A, Von Haeseler A, Minh B Q. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular biology and evolution. 2015 Jan 1;32(1):268-74.

Nordberg G F, Fowler B A, Nordberg M, editors. Handbook on the Toxicology of Metals. Academic press; 2014 Aug 7.

Qin H, Hu T, Zhai Y, Lu N, Aliyeva J. The improved methods of heavy metals removal by biosorbents: A review. Environmental Pollution. 2020 Mar 1; 258:113777.

Renu, Agarwal M, Singh K. Methodologies for removal of heavy metal ions from wastewater: an overview. Interdisciplinary Environmental Review. 2017;18(2):124-42.

Roth A, Andrees S, Kroppenstedt R M, Harmsen D, Mauch H. Phylogeny of the genus Nocardia based on reassessed 16S rRNA gene sequences reveals under speciation and division of strains classified as Nocardia asteroides into three established species and two unnamed taxons. Journal of clinical microbiology. 2003 Feb;41(2):851-6.

Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution. 1987 Jul 1;4(4):406-25.

Simeonova A, Godjevargova T, Ivanova D. Biosorption of heavy metals by dead Streptomyces fradiae. Environmental

engineering science. 2008 Jun 1;25(5):627-34.

Thompson, J. D., D G Higgins, T J Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22(22):4673-80.

Timková I, Sedláková-Kaduková J, Pristaš P. Biosorption and bioaccumulation abilities of actinomycetes/streptomycetes isolated from metal contaminated sites. Separations. 2018 Dec;5(4):54.

Ugbaja R N, Okedairo O M, Oloyede A R, Ugwor E I, Akinloye D I, Ojo O P, Ademuyiwa O. Probiotics consortium synergistically ameliorates aflatoxin B1-induced disruptions in lipid metabolism of female albino rats. Toxicon. 2020 Oct 30; 186:109-19.

Wassel M A, Swelam A A, Awad M M, El-Zaref A S. Studies on the removal of cobalt (Co 2+) and Nickel (Ni 2+) ions from aqueous solution by using macroporous cation exchange resin. Int J Environ. 2014;3(1):42-53.

Williams J H, Phillips T D, Jolly P E, Stiles J K, Jolly C M, Aggarwal D. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. The American journal of clinical nutrition. 2004 Nov 1;80(5):1106-22.

Yadav V B, Gadi R, Kalra S. Clay based nanocomposites for removal of heavy metals from water: A review. Journal of environmental management. 2019 Feb 15; 232:803-17.

Zhai Q, Yin R, Yu L, Wang G, Tian F, Yu R, Zhao J, Liu X, Chen Y Q, Zhang H, Chen W. Screening of lactic acid bacteria with potential protective effects against cadmium toxicity. Food Control. 2015 Aug 1; 54:23-30.

Zoghi A, Khosravi-Darani K, Sohrabvandi S. Surface binding of toxins and heavy metals by probiotics. Mini reviews in medicinal chemistry. 2014 Jan 1;14(1):84-98.

Zouboulis A I, Loukidou M X, Matis K A. Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils. Process biochemistry. 2004 Apr 30;39(8):909-16.

How to cite this article:

Amany A. A. Sharshar, Sabha Mahmoud EL-Sabbagh and Raafat. M. Elsanhoty. 2022. Physical and Chemical Properties of some Water Samples from Egypt (Menoufia Governorate), the Efficiency of some Actinomycetes in Bioremediation of some Heavy Metals and Aflatoxin from Contaminated Water Samples. *Int.J.Curr.Microbiol.App.Sci.* 11(02): 139-165. doi: https://doi.org/10.20546/ijcmas.2022.1102.018