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Biosorption of Cu^{2+}, Pb^{2+} and Cd^{2+} from Wastewater by Dead Biomass of *Streptomyces cyaneus* Kw42

Maryam Mustafa Abd El-Motaleb[1], Sabha Mahmoud El-Sabbagh[2], Walaa Salah El-din Mohamed[1]* and Karam Rabee Wafy[1]

[1]*Central Laboratory for Environmental Quality Monitoring, National Water Research Center, El-kanater El-khairia, P.M.B 13621, Egypt*
[2]*Department of Botany and Microbiology, Faculty of Sciences, University of Menoufia, Shibin El-kom, P.M.B 32511, Egypt*

**Corresponding author*

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A B S T R A C T

Environmental contamination by toxic heavy metals is causing a serious problem worldwide. The objective of the study was the utilization of eco-friendly and low-cost effective biomass of actinomycetes in the removal the toxic heavy metals (Cu^{2+}, Pb^{2+}, and Cd^{2+}) from wastewater. Out of sixty-six isolates of actinomycetes were isolated from different drains in Egypt, eleven isolates removed studied heavy metals (Cu^{2+}, Pb^{2+}, and Cd^{2+}) singly above 70% were selected to remove metals from ternary mixtures. The biosorption by dead biomass for all isolates was higher than that by living biomass. The highest removal was recorded by Kw42 isolate. It was removed 81.7%, 88.6% and 69.2% from Cu^{2+}, Pb^{2+} and Cd^{2+}, respectively. The 16S rRNA analyses and phylogenetic data of Kw42 concluded that Kw42 is a member of *Streptomyces* genus and it was deposited in the GenBank database under accession number MK020765. The biosorption by the dead biomass of *Streptomyces cyaneus* Kw42 under the optimized conditions (pH-8 at 40°C for 3 h with 0.3% biosorbent dosage) was found to be;Pb^{2+} (83.4%) > Cu^{2+} (74.5%) >Cd^{2+} (68.4%). By Electronic Microscope investigation the surface of dead biomass of Kw42 became smoother after binding with metal ions. Treatment of real wastewater by dead biomass of *Streptomyces* sp. Kw42 yielded complete bio-removal for all studied heavy metal ions after 120 min.

Introduction

Freshwater bodies are the main source of water for human consumption. The large percentage from wastewater goes into freshwater bodies without treatments that affection human population and lead to death by nearly five million per year (Kanamarlapudi *et al.*, 2018). Industries are mainly responsible for the contamination of water resources where water bodies exposed to discharge large quantities of toxic pollutants. Environmental contamination by toxic heavy metals is causing a serious problem worldwide due to their incremented

accumulation in the food chain and continued persistence ecosystem. The most common heavy metal contaminants such as lead, cadmium, copper, zinc, and iron are difficult to remove from aqueous solutions at any concentrations (Oves *et al.,* 2012). The toxic heavy metals are not biodegradable and accumulate in living organisms causing damage tissues and various diseases (Wasi *et al.,* 2013). Many physicochemical treatment methods have been used for heavy metal removal from contaminated wastewater such as ion exchange, precipitation, reverse osmosis, evaporation, and sorption (Ajmal *et al.,* 2000). Most of these conventional methods have disadvantages like high energy requirements, high cost and secondary pollution. In recent years, the biosorption process is novel, efficient, and eco-friendly alternative treatment technology for the removal of heavy metals.

Biosorption can be considered as the binding of metals ions to the surface of the biological biosorbent. Biological removal includes biopolymers, plant, agriculture, and industrial wastes. Also, in many recent studies microorganisms (living or dead) have been used as biosorbent in heavy metal removal from contaminated wastewater (Zouboulis *et al.,* 2004). This research article has been focused on actinomycetes as biosorbent of heavy metal ions. The heavy metal adsorption by Streptomyces has been presumed to possess a large heavy metal binding capacity and was considered as an alternative method to recover metals from wastewater (Bailey *et al.,* 1999).

Economically, the use of dead biomass has been considered better than living biomass because it has not been required nutrients and did not affect biological and chemical oxygen demands in effluents (Low and Chase, 1999). This study aimed to the isolation of actinomycetes strains from wastewater and utilization eco-friendly and low cost-effective biological biomass in the removal the toxic heavy metals (Cu^{2+}, Pb^{2+}, and Cd^{2+}) from wastewater.

Materials and Methods

Sample collection

Wastewater samples were collected from different drains in Egypt (6 sites) (EL-Khadrawia drain, Qalubia drain, Bahr Hadus drain, 10th of Ramadan and Sadat City drain). The collected samples were transferred in sterile plastic containers to the laboratory and maintained at 4°C for further studies. The soil and sediment samples were collected by stainless steel sampler and transferred in sterile polyethylene bags into the laboratory for further processing.

Chemical preparation

Stock solutions of Cu^{2+}, Pb^{2+} and Cd^{2+} were prepared from $CuSO_4.5H_2O$, $Pb(NO_3)_2$ and $CdSO_4$ 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

Actinomycetes were isolated from wastewater by membrane filter technique. This method was described for isolation of branched actinomycetes from water without using antibiotics or specific media (Hirsch and Christensen, 1983). Samples were filtrated by 0.45µm pore size sterile cellulose membrane filter then inoculated in Nutrient agar media and incubated at 28°C for 7 days. The mycelium of actinomycetes has penetrated the pores of the cellulose membrane filter and was grown in nutrient agar media. Non-actinomycetes bacteria restricted in membrane surface then the membrane filter was removed. Finally, the media was incubated

again to allow the growth of actinomycetes.

Physical treatment was used for isolation of actinomycetes from sediment and soil, where samples were dried at 100°C for one hour (Hayakawa *et al.,* 1997) and serial dilutions were prepared from dried samples then inoculated in an inorganic salt-starch agar and incubated at 28°C for 7 days. After incubation, the isolated actinomycetes were purified and stored in slants at 4°C for further studies.

Preparation of living and dead biomasses

Biomass of isolated actinomycetes was used as natural biosorbent, to removal Cu^{2+}, Pb^{2+}, and Cd^{2+} from aqueous solutions. Ten days old culture spores (10^8 CFU) from each isolated actinomycetes were transferred into a 250- mL Erlenmeyer flask containing 100 mL broth media (peptone 4 g/L, yeast extract 2 g/L, glucose 10 g/L) and incubated at 28°C in a shaker at 150 rpm for 7 days. Thereafter, the biomass of each isolated actinomycetes was pelletized by centrifuging at 4500 rpm for 20 min. After that, the supernatant was removed and the pellets was resaved then washed with 0.1 M NaCl to remove non biomass particles. Dead biomass was prepared by drying the living biomass at 70°C overnight (figure 1). To confirm that dried myceliumare completely dead, the sample was inoculated in agar media and incubated at 28°C for 7 days, the absence of growth indicated positive results (Simeonova *et al.,* 2008).

Heavy metal biosorption capacity of isolated actinomycetes (living and dead biomass)

To evaluate the biosorption efficiency of each isolate (living and dead biomass) two 250 mL Erlenmeyer flasks were prepared for every isolate to assay a single metal. 100 mL from metal dilution 100 mg/L was put in each flask. One of them was inoculated by live biomass (3 g/L) and the other by dead biomass (3 g/L). The flasks were incubated on rotary shakers (150 rpm) at 28°C for 3 h. Then, the samples were filtrated by 0.45µm cellulose membrane filter. The filtrate was analyzed for determination of residual heavy metals using (ICP-OES) Inductively Coupled Argon Plasma-Optical Emission Spectroscopy (Perkin Elmer Optima-3000 Redial. USA).

The following equation was used to determine the percentage of heavy metals that were adsorbed by isolated actinomycetes (R).

$$(R) = (C_I - C_F)/ C_I \times 100$$

Where the (C_I) referred to the initial concentration of heavy metals in the solution and (C_F) referred to the residual concentration of heavy metals in the solution.

Ternary metals system

The isolates that removed any studied heavy metals (Cu^{2+}, Pb^{2+} and Cd^{2+}) singly above 70% were selected to remove studied metal ions from ternary mixture. The initial concentration of each metal ion in the mixture was 100 mg/L in aqueous solution.

Identification of the most removable isolate by 16S rRNA sequencing

PCR amplification of the 16S rRNA and sequencing were performed for the most removable isolate. The 16S rRNA sequence of kw42 was 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 with MEGA6, and phylogenetic trees was inferred using the neighbor-joining method (Roth *et al.,* 2003; Saitou and Nei, 1987).

Optimization of biosorption conditions

To determine the impact of temperature on biosorption by most removable isolate, experiments 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 100 mL of a solution containing 100 mg/L of interested heavy metals.

The experiment was kept at continuous shaking (150 rpm) for 3 h. at pH 7. After that, the aqueous solutions were filtrated and each filtrate was analyzed for the determination of residual metal concentration. To study the effect of pH, experiments were conducted at different pH values (4.0, 5.0, 6.0, 7.0, 8.0and 9.0) under optimum temperature and 3 g/L biomass in 100 mL of a solution containing 100 mg/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 and 100mg/L of Cu^{2+}, Pb^{2+} and Cd^{2+}) was studied by analyzing biosorption under conditions where all the parameters (pH, temperature, and biosorbent dosage 3 g/L) were optimized for the best isolate.

The flasks were allowed to attain equilibrium on the rotary shaker and the samples were collected at regular time intervals (30, 60, 120, 180, 240 and 300 min.) in order to determine biosorbent efficiency (%).

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

Dead biomass of actinomycetes were examined before and after biosorption of copper, lead and cadmium at optimum conditions as temperature, pH and (3 g/L biomass in 100 mL of a solution containing 100 mg/L of heavy metal ions) by using scanning electron microscope (JEOL JSM-5500 LV) at the Regional Center of Mycology and Biotechnology, El- Azhar University, Cairo, 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).

Bio-removal of heavy metals (Cu^{2+}, Pb^{2+} and Cd^{2+}) from real wastewater by the dead biomass

The experiments were performed on raw wastewater samples that were collected from different drains in Egypt (EL-Khadrawia drain, Qalubia drain, Bahr Hadus drain, 10^{th} of Ramadan city drain and Sadat City drain). The heavy metals ions concentrations were measured before and after their treatment with the best actinomycetes isolate of the dead biomass.

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 Microsoft Office Excel (2010). The results were expressed as mean ± standard division. The data were subjected to analysis of variance (ANOVA) (Le *et al.*, 2008) (P-value< 0.05).

Results and Discussion

Different morphological actinomycetes were isolated from collected wastewater (27

isolates), sediment and soil samples (39 isolates). The living and dead biomass of isolates were used to perform the experiment for removal of studied heavy metal ions (Cu^{2+}, Pb^{2+} and Cd^{2+}).

The isolates Kw9, Kw14, Kw27, Kw28, Kw36, Kw40, Kw42, Kw49, Kw52, Kw58 and Kw66 were recorded the highest biosorption percentage above 70% for heavy metals ions singly (one at least).

These isolates were selected to perform the experiment using ternary mixture composed of Cu^{2+}, Pb^{2+} and Cd^{2+} in aqueous solution. The highest removal percentage was recorded by Kw42 isolate. Living biomass of Kw42 has removed 58.4 % from Cu^{2+}, 67.7 % from Pb^{2+} and 52.0 % from Cd^{2+}, while the dead biomass has removed 76.6 % from Cu^{2+}, 82.7 % from Pb^{2+} and 68.3 % from Cd^{2+} (Table 1). According to the results in figure 2 (A, B and C), the biosorption by dead biomass for all isolates was higher than living biomass.

16S rRNA sequence and phylogenetic analyses of the highest biosorbent isolate (Kw42)

According to phylogenetic comparison 16S rRNA sequence of Kw42 isolate for similarity with the sequences of valid species in Gen Bank using Blast analysis and Mega 6 software, Kw42 isolate was identified as *Streptomyces cyaneus* where the percentage of similarity 99% between *Streptomyces cyaneus* Kw42 MK020765 and *Streptomyces cyaneus* strain TU11. Phylogenetic tree analysis was constructed based on neighbor joining tree method and illustrated in Figure 3. The database was deposited in NCBI Gen Bank under the accession number MK020765.

Optimization of biosorption conditions

Biosorption efficiency of dead biomass of *Streptomyces cyaneus* kw42 has increased with increasing temperature from 20 °C to 40 °C as shown in Figure4. Bio-removal capacities for Cu^{2+}, Pb^{2+} and Cd^{2+}were 21.2 %, 17.7 % and 12.6 % respectively at 20°C and were increased to 86.6 %, 90.2 % and 78.5 % respectively at 40°C. The effect of temperature on removal of the studied heavy metals was significant, where factor of temperature evidencing was low (P-value (0.00)<0.05) with confidence level of 95%. On the other hand, bio-removal efficiency was decreased when the temperature was increased above 40°C.

As shown in Figure 5 the maximum biosorption using *Streptomyces cyaneus* Kw42 for Cu,Pb and Cd ions (87.3 %, 93.1 %and 79.1 %,respectively) was recorded at pH-8. The effect of pHvalue on removal of the studied heavy metals was significant with P-value (0.02)<0.05.

The effect of biosorbent dosage (0.1 %– 0.6 %) on sorption efficiency in aqueous solutions under optimized temperature and pH was recorded in Figure 6. The results have indicated that when a biosorbent dosage was increased from 0.1% to 0.3%, the removal of Cu^{2+}, Pb^{2+} and Cd^{2+} by *Streptomyces cyaneus* Kw 42 increased from 32.4%, 36.5% and 27.7% to 89.7%, 91.8% and 77.3%, respectively. Moreover, when the biosorbent dosage increased to 0.5% the metals were completely removed from aqueous solution. The effect of biosorbent dosage on removal the studied heavy metals was significant (P-value (0.00)<0.05 with confidence level 95%).

Biosorption experiments with biomass were conducted for solutions containing 25-100 mg/L of Cu^{2+}, Pb^{2+}, and Cd^{2+}. As shown in Table 2, at low concentrations (25 mg/L) metal ions were removed completely after about 60 min. for Pb^{2+} and 120 min. for Cu^{2+} andCd^{2+}; while at higher concentration (100 mg/L), complete biosorption took about 240

min. for Pb^{2+} and 300 min. for Cu^{2+} andCd^{2+}. by *Streptomyces cyaneus* Kw42.

The effect of initial heavy metal concentrations was significant (P-value (0.03)<0.05with confidence level 95%).

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

The morphology of dead biomass of *Streptomycetes cyaneus* Kw42was analyzed byScanning Electron Microscope before and after biosorption of Cu^{2+}, Pb^{2+} and Cd^{2+}ions. An electron micrograph of dead biomass of *S.cyaneus* Kw42 was presented in Figure 7.

The figure indicates that the change in the surface where it became smoother and as red rings in the image referred to the pores on the surface were disappeared after binding with metal ions.

On the other hand, the dead biomass of *Streptomycetes cyaneus* Kw42was examined by EDX analyses before and after biosorption of Cu^{2+}, Pb^{2+} and $Cd^{2+}$from aqueous solution.

As shown in figures (8 A and B), all charts did not have any peak of Cu^{2+}, Pb^{2+} and Cd^{2+} before biosorption.

The charts after biosorption had copper peak at 8 Kev, lead peak at 2.8 Kev and cadmium peak at 3.1 Kev (Figure 8B). On the other hand, the Mg^{2+} and Ca^{2+} were the main cations that changed during biosorption.

Bio-removal of heavy metals (Cu^{2+}, Pb^{2+} and Cd^{2+}) from real wastewater by the dead biomass of *Streptomyces cyaneus* Kw42 (MK020765)

The heavy metal concentrations were determined before and after treatment by dead biomass of *Streptomyces cyaneus* Kw42MK020765 by using ICP instrument. As shown in Table 3, the results of the studied heavy metals(Cu^{2+}, Pb^{2+} and Cd^{2+}) were recorded as 1.43 mg/L, 0.91 mg/L and 3.66 mg/L, respectively in EL-Khadrawia drain. The Cu^{2+} and Pb^{2+}ions were completely removed after treatment for 90 min.,while Cd^{2+} needed 120 min for complete removal.

Table.1 The percent biosorption efficiency (%) in ternary metal systems (Cu^{2+}, Pb^{2+}, and Cd^{2+}) by living and dead biomass.Thedata are the mean of triplicates ± SD.

S.No.	Living biomass			Dead biomass		
	Cu^{2+} %	Pb^{2+} %	Cd^{2+} %	Cu^{2+} %	Pb^{2+} %	Cd^{2+} %
Kw 9	33.8±1.13	54.9±1.01	53.3±0.50	61±0.812	69.6±0.82	53.9±0.62
Kw 14	48±0.83	40.9±0.90	39.3±0.24	42.7±1.00	60.5±0.24	46.3±1.12
Kw 27	59.6±0.52	60.3±0.63	52.9±1.03	69.9±0.36	74.3±1.02	61.8±0.81
Kw 28	47.9±1.06	53.4±0.52	38±1.04	56.1±0.99	67.3±0.36	63.3±0.60
Kw 36	45.6±0.24	48.9±0.43	45±0.55	60.4±0.43	67.2±0.24	51.9±1.07
Kw 40	46.4±0.92	44.4±1.03	45.5±0.27	33.0±0.64	69.7±0.73	46.3±1.15
Kw 42	58.4±1.04	67.7±0.54	52.0±0.73	76.6±1.06	82.7±1.34	68.3±0.20
Kw 49	44.4±0.30	46±0.22	40.2±1.07	65.9±0.22	53.6±1.06	47±0.73
Kw 52	42.6±1.25	40.8±0.82	39.6±0.31	68.3±0.86	60.6±0.32	57.6±0.88
Kw 58	35.8±0.12	42.4±0.67	30.1±0.23	53.7±0.60	63.6±0.12	55.8±0.86
Kw 66	47.6±0.78	50.8±1.02	41.2±1.14	59.7±1.06	65. 6±0.52	58±1.06

Table.2 Effect of initial heavy metal concentrations on biosorption by dead biomass of *Streptomyces* sp. Kw42. Thedata are the mean of triplicates ± SD.

Time (min)	25mg/L			50mg/L			100mg/L		
	Cu^{2+}	Pb^{2+}	Cd^{2+}	Cu^{2+}	Pb^{2+}	Cd^{2+}	Cu^{2+}	Pb^{2+}	Cd^{2+}
30	84.6±1.01	93.6±0.70	81.3±0.16	64.6±0.52	86.4±0.60	59.2±1.06	30.3±0.27	38.6±0.48	27.7±0.29
60	96.7±0.83	100±0.00	93.5±1.06	89.7±1.07	93.9±0.675	82.3±0.32	48.9±0.84	53.3±0.74	42.6±1.05
120	100±0.00	100±0.00	100±0.00	97.2±1.22	100±0.00	91.8±0.92	75.4±0.23	82.7±0.25	59.1±0.62
180	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	91.3±0.51	95.1±0.42	85.6±0.29
240	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	98.3±0.26	100±0.00	97.2±0.90
300	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Table.3 Analysis of wastewater collected from different regions of Egypt before and after treatment by using dead biomass of *Streptomyces cyaneus* Kw42

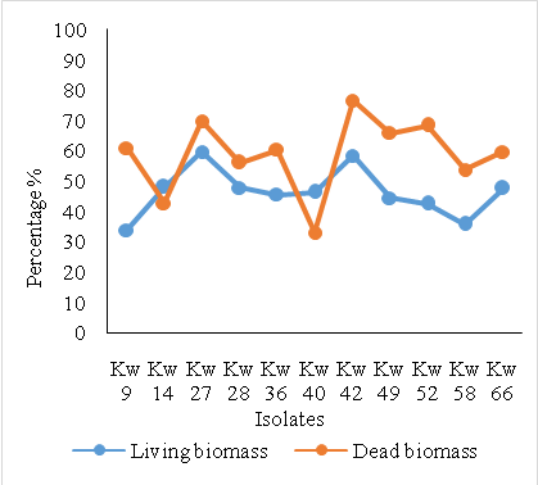
Region	Before treatment (mg/L)			After treatment (mg/L)											
				30 min.			60 min.			90 min.			120 min.		
	Cu^{2+}	Pb^{2+}	Cd^{2+}	Cu^{2+}	Pb^{2+}	Cd^{2+}	Cu^{2+}	Pb^{2+}	Cd^{2+}	Cu^{2+}	Pb^{2+}	Cd^{2+}	Cu^{2+}	Pb^{2+}	Cd^{2+}
EL-Khadrawia drain	1.43	0.91	3.66	0.86	0.26	0.86	0.11	0.03	0.23	N.D	N.D	0.10	N.D	N.D	N.D
Qalubia drain	9.71	1.62	2.10	3.42	0.31	1.32	0.69	0.02	0.72	0.02	N.D	N.D	N.D	N.D	N.D
Bahr Hadus drain	0.85	0.01	0.07	0.22	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
10th of Ramadan	0.02	1.91	3.31	N.D	0.46	0.61	N.D	0.02	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Sadat City	0.04	2.76	4.25	N.D	0.52	0.97	N.D	0.07	0.05	N.D	N.D	N.D	N.D	N.D	N.D

N.D: not detected

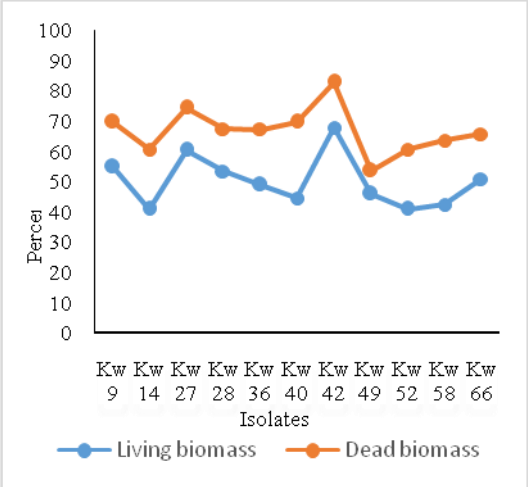
Fig.1 The dead biomass of Kw42 that was used for biosorption of Cu^{2+}, Pb^{2+} and Cd^{2+} from wastewater

Fig.2 Biosorption of A: Cu^{2+}, B: Pb^{2+} and C: Cd^{2+} by living and dead biomass. The plotted data are the mean of triplicates ± SD

A.



B.



C.

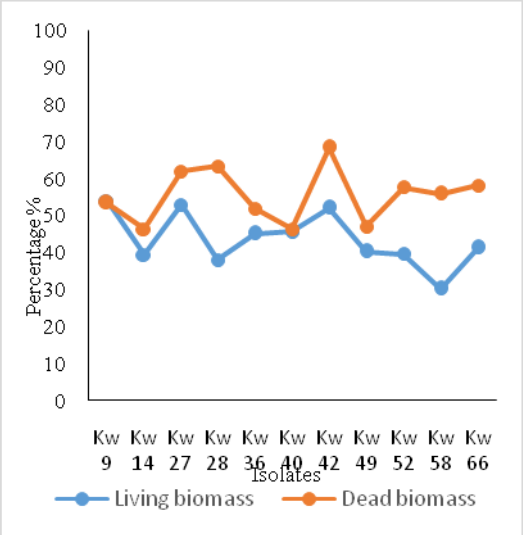


Fig.3 Phylogenetic tree based on 16S rRNA gene sequence analysis constructed with the neighbor-joining method showing the phylogenetic position of *Streptomyces cyaneus* kw42 MK020765

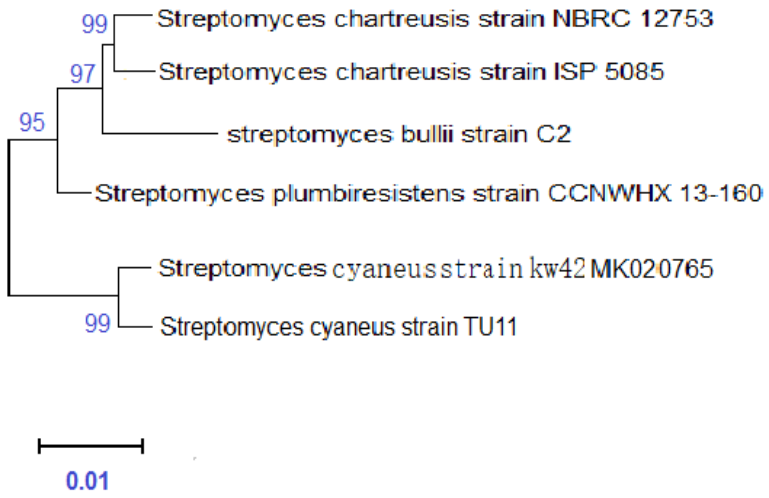


Fig.4 Effect of different temperatures on the biosorption capacity of Cu^{2+}, Pb^{2+}, and Pd^{2+} by dead biomass of *Streptomyces cyaneus* Kw42. The plotted data are the mean of triplicates ± SD

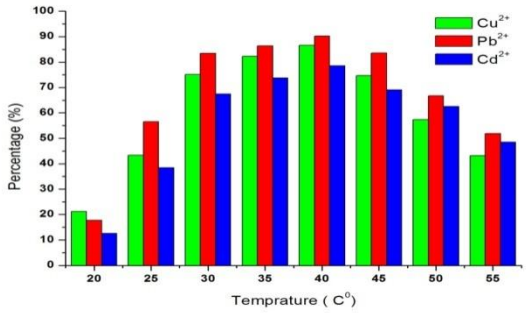


Fig.5 Effect of pH on biosorption of Cu^{2+}, Pb^{2+}, and Cd^{2+} by dead biomass of *Streptomyces cyaneus* Kw42. The plotted data are the mean of triplicates ± SD

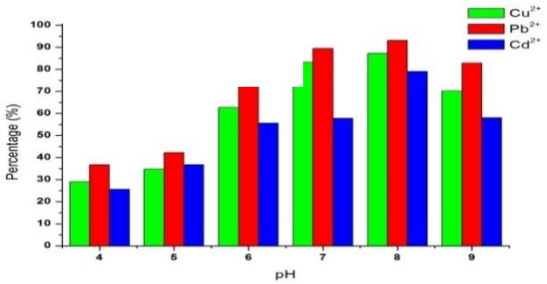


Fig.6 Effect of biosorbent dosage on biosorption of Cu^{2+}, Pb^{2+}, and Cd^{2+} by dead biomass of *Streptomyces cyaneus* Kw42**.**The plotteddata are the mean of triplicates ± SD

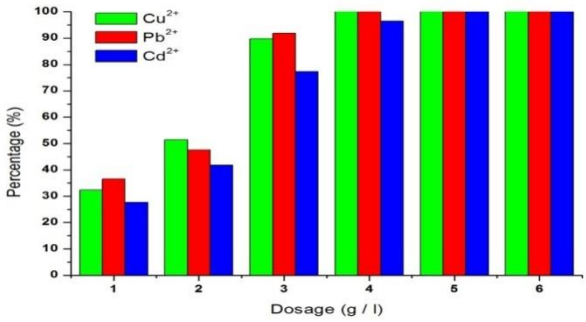


Fig.7 SEM micrograph of dead biomass of *Streptomycetescyaneus* Kw42before (A) and after (B) biosorption of Cu^{2+}, Pb^{2+} and $Cd^{2+}$from aqueous solution

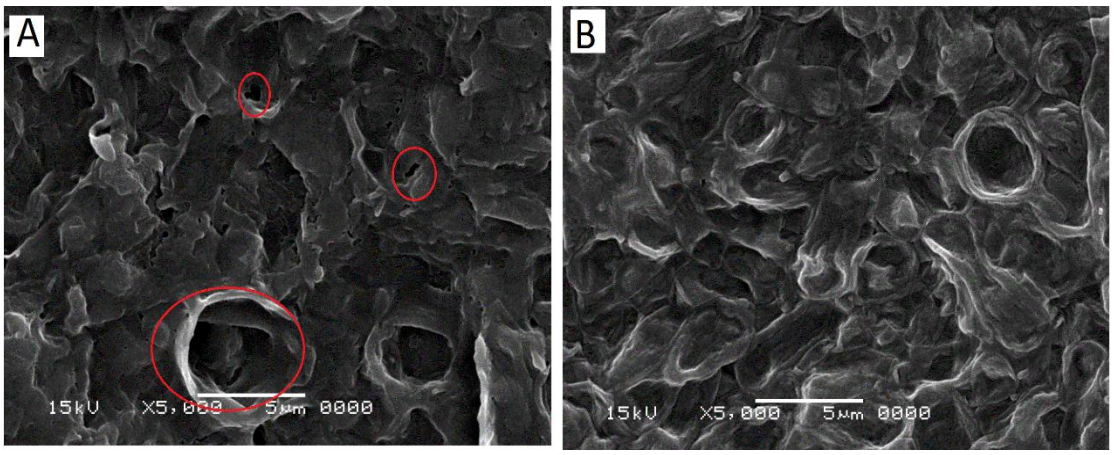
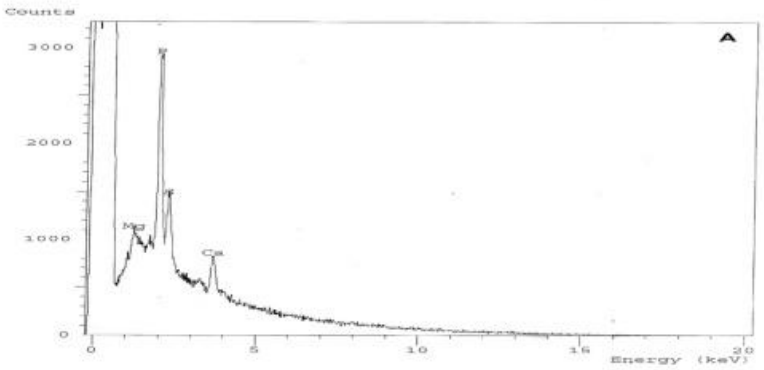
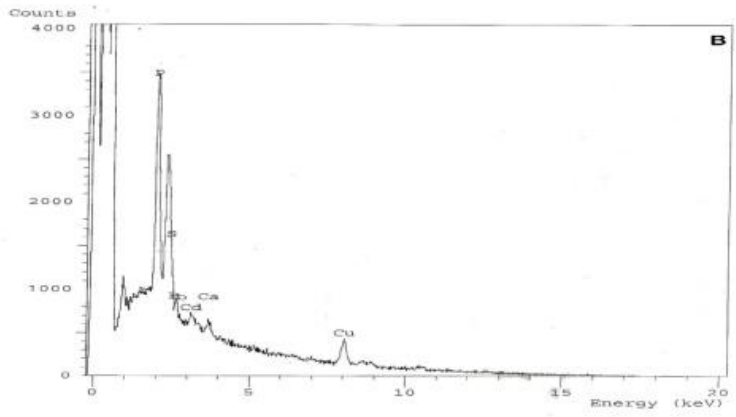


Fig.8 EDX analyses for dead biomass of *Streptomyces cyaneus* Kw42 before (A) and after (B) biosorption of Cu^{2+}, Pb^{2+} and Cd^{2+}





Also, in Qalubia drain, the studied heavy metals recorded 9.71 mg/L, 1.62 mg/L and 2.10 mg/L, respectively. When wastewater has treated with dead biomass, complete removal took place after about 90 min. for $Pb^{2+}$and $Cd^{2+}$and 120 min. for Cu^{2+}.

On the other hand, the results of studied metals in Bahr Hadus drain were initially recorded as 0.85 mg/L, 0.01 mg/L and 0.07 mg/L, respectively. The removing process needed 30 min. for complete removal of Pb^{2+} and $Cd^{2+}$and 60 min. for Cu^{2+}. In 10^{th} of Ramadan and Sadat City the studied metals needed about 90 min. for complete removal of all studied heavy metals. The contact time for removal of the heavy metals was varied depending on the initial heavy metals concentration in wastewater samples before treatment.

Biological removal of heavy metal ions from wastewater had several advantages that were the major limitations for the conventional methods such as simple operation, no additional nutrient requirement, no increasing in the chemical oxygen demand (COD), low quantity of sludge and high efficiency.

Results of this study indicated that metal biosorption may be widespread among actinomycetes that are found in contaminated wastewater.

In this study, the focus of the work was to isolate actinomycetes from the wastewater and soil environments and rather study their capabilities for removal of heavy metal ions. For this purpose, 66 actinomycetes isolates were isolated from the collected wastewater samples. Then, out of these isolates, 11 different cultural appearance isolates were recorded the highest biosorption percentage above 70% for heavy metals ions singly. The biosorption by dead biomass for all isolates was higher than that by living biomass. The dead biomass has several advantages over living biomass. These advantages include their ease of treatment and no metal toxicity that can result in the death of living cells. Additionally, the dead biomass did not require supplementation with nutrients that can increase the biological and chemical oxygen demands on the treated water(Low and Chase, 1999). Many early studies statedthat the dead biomass removed the heavy metals more than living biomass (Ahluwalia andGoyal, 2007). Simeonova used the dead biomass of *Streptomyces fradiae* in biosorption of Cu^{2+}, Zn^{2+}, Ni^{2+}, and Pb^{2+} from aqueous solutions. Microbial population uptakes toxic metals from environments actively by bioaccumulation and/or passively by biosorption. Biosorption is more applicable than bioaccumulation because living organisms need addition of nutrient and lead to increase biological and chemical oxygen demand in effluent. On the other hand,

biosorption is a low cost and eco-friendly manner for removal of toxic heavy metal ions. In fact, cell walls of biomass are made of large molecules (peptidoglycan) linked with teichoic acids and polysaccharides. These molecules possess functional groups that can adsorb heavy metal ions.16S rRNA sequence of Kw42 was deposited in Gen Bank under the accession number MK020765 and was confirmed as *Streptomyces cyaneus*. The heavy metal adsorption by Streptomyces has been presumed to possess a large heavy metal binding capacity and was considered as an alternative method to recover metals from waste liquid (Simeonova *et al.*, 2008).

The increase in adsorption with elevation in temperature till 40°C and gradual decrease was observed after that. This increase can be attributed to several factors such as a change in the pore size of the adsorbent leading to a greater inter particle diffusion within the pores, the creation of new active sites on the sorbent and an enhancement in the mobility of metal ions from the bulk of solution toward the adsorbent surface. On the other hand, the shrinking of cells at lower and higher temperature decrease the biosorption where reducing the surface area of contact (Srinivasan and Viraraghavan, 2010).

The pH of the aqueous solution has been considered as one of the most important factors influencing ions uptake, cell surface metal binding sites and availability of metal in solution. The removal capacity has shown to increase with increasing pH value. At low pH value, the cell surface sites are closely linked to H^+ ion making these sites unavailable for other cations. With increasing pH value there is increasing in negative charges that yield to increase binding of cations on cell surface sites.

When the biosorbent dosage was increased more biosorbent binding sites were available at higher dosages than at lower ones, which leads to binding of all available metal ions (Ngah and Hanafiah, 2008).

The initial metal ions concentration was reported to be important factor for ions removal. The recorded results revealed that decreasing in removal capacity with increasing metal concentrations at the same biosorption time. The metal binding sites was reached to saturation with increasing heavy metal concentration in solution.

Applying Scanning Electron Microscope before and after biosorption indicated a surface change in biomass after binding of the metal ions where the pores on the surface were disappeared and the surface became smoother. On the other hand, the charts of EDX analyses indicated that the mechanism responsible for biosorption was ionic-exchange due to change in different cations peaks.

Moreover, the obtained results from the Bio-removal of heavy metals from real wastewater by the dead biomass of *Streptomyces cyaneus* Kw42 was indicated to complete removal of heavy metal ions from wastewater needed different times depending on the site and concentration of metal ions.

Copper, lead and cadmium ions are considered as toxic heavy metals and the removal of them from wastewater was an important target to improve the quality of wastewater before releasing into the environment. *Streptomyces cyaneus* Kw42 was isolated from contaminated wastewater and it was identified as a potent active biosorbent. Interestingly, the results of dead biomass were more effective than living biomass. As shown in the study, *Streptomyces cyaneus* Kw42 can be used to completely remove some toxic heavy metals from waste water when treated for 60 to 120 min.

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