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Biosorption of Cu^{2+}, Pb^{2+} and $Cd^{2+}$from wastewater by dead biomass of *Streptomyces cyaneus* KW42

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Abstract: Environmental contamination by toxic heavy metals is causing a serious problem through the worldwide due to their incremented accumulation in food chain and continued persistence ecosystem. Sixty-six isolates of actinomycetes were isolated from different drains in Egypt. The eleven isolates which removed any studied heavy metals (Cu^{2+}, Pb^{2+} and Cd^{2+}) singly above 70% was selected to remove metals from ternary mixture. The biosorption by dead biomass for all isolates was higher than living biomass. The highest removing percentage 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 was member of streptomyces genus and kw42 was deposited in the Gen Bank Database under accession number MK020765. The results of biosorption by the dead biomass of *Streptomyces cyaneus* Kw42 for Cu^{2+}, Pb^{2+} and Cd^{2+} under the optimized conditions pH-8 at 40°C for 3 hours with 0.3% biosorbent dosage was found to be as follows: Pb^{2+} (83.4%) > Cu^{2+} (74.5%) >Cd^{2+} (68.4%). By Electronic Microscope investigation the surface dead biomass of Kw42 became more smoothly after binding with metal ions. Also, the EDX charts after biosorption had cupper peak at 8 Kev, lead peak at 2.8 Kev and cadmium peak at 3.1 Kev. Treatment of real wastewater by dead biomass of Streptomyces sp. Kw42 recorded complete bio-removal for all studies of heavy metal ions after 120 min as a maximum contact time.
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Keywords: Actinomycetes; Dead biomass; Heavy metals; Living biomass; Streptomyces

1. Introduction

Human activities such as mining, industrial production, and agriculture drain increase amount of metals in water body. Water pollution is a major problem in the world. Environmental contamination by toxic heavy metals is causing a serious problem worldwide due to their increment accumulation in food chain and continued persistence ecosystem (Uwa et al., 2011). The most common heavy metal contaminants (e.g. lead, cadmium, copper, zinc, and iron) at different concentrations are difficult to be removed from aqueous solutions. The large-scale production of a variety of chemical compounds has caused a global deterioration of environmental quality (Mata et al., 2010). Conventional physicochemical methods such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation, and sorption for heavy metal removal from waste streams are not cost effective (Kadirvelu et al., 2002). A variety of mechanisms are used for the removal of heavy metals from aqueous solution by different microorganisms such as bacteria (Mythili and Karthikeyan, 2011), algae (El-Sherif et al., 2008), mixture of dried microalgal/bacterial biomass (Loutseti et al., 2009), mold (Xiao et al., 2013), yeast (Rehman et al., 2008; Ahmad et al., 2013) and actinomycetes (Al Turk and Kiki, 2011; Amatullah et al., 2012). 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). Intact microbial cells live or dead and their products can be highly efficient bio-accumulators of both soluble and particulate forms of metals (Elouzi et al., 2012). Actinomycetes have long and branching filaments that resemble the hyphae of fungi. They are Gram positive and constitute a significant component of the microbial population in most soils. Although distributed extensively in soil, they can also be isolated from sediments, and water (Amatullah et al., 2012).

This study aimed to isolation of actinomycetes strains from wastewater and utilization eco-friendly and low cost effective biological biomass in removal the toxic heavy metals (Cu^{2+}, Pb^{2+} and Cd^{2+}) from wastewater.

2. Material 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 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 Cd SO_4 respectively. These stocks were used in preparation different dilutions for further experiments (25, 50, 100, 150, 200 and 300 mg/ L). The pH 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 5 days, the mycelium of actinomycetes was penetrated the pores of cellulose membrane filter and grown in nutrient agar media, non-actinomycetes bacteria restricted in membrane surface then removal the membrane filter and incubation of media again to allow 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 dilution was prepared from dried samples then inoculated in an inorganic salt-starch agar and incubated at 28°C for 7 days. After incubation isolated actinomycetes was purified and stored in slants at 4°C for further studies.

Preparation of live and dead biomasses

Biomass of isolated actinomycetes were used as natural biosorbent to removal Cu^{2+}, Pb^{2+} and Cd^{2+} from aqueous solutions. Ten days old culture spores (10^6 CFU) from each isolated actinomycetes transferred into 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 30°C on shaker at 150 rpm for 7 days. Thereafter, the biomass of each isolated actinomycetes was pelletized by centrifuge at 4500 rpm for 20 minute. After that, supernatant was removed and the pellets was resaved then washed with 0.1 M NaCl to removal non biomass particles. Dead biomass was performed by drying the living biomass at 70°C overnight (fig.1). To confirm completely dead of dried mycelium, the sample inoculated in agar media and incubated at 30°C for 7 days, the absence of any growth was referred to positive results (Simeonova et al., 2008).

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

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. One hundred ml from metal dilution 100 mg/ L was put in each flask and one was inoculated by live biomass (3 g/l) and other by dead biomass (3 g/l). The flasks were incubated on rotary shakers (150 rpm) at 30°C for 3 hours. 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 was used to determine the percentage of heavy metals that adsorbed by isolated actinomycetes (R).

$(R) = (CI - CF)/ CI \times 100$

Where the (CI) referred to the initial concentration of heavy metals in the solution and (CF) 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 concentration of each metal ion in the mixture was 100 g/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 (Kw42). 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 (Saitou and Nei, 1987; Roth et al., 2003).

Optimization of biosorption conditions

To determine the impact of temperature on biosorption by Streptomyces Kw42, 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 100ml of a solution containing 100 mg/l of interested heavy metals. The experiment was kept at continuous shaking (150 rpm) for 3 hours 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, 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 0.5%, 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 300mg/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. Flasks were allowed to attain equilibrium on the rotary shaker and samples were collected at regular time intervals (30, 60, 120, 180, 240, 300 and 360 min.) in order to determine biosorbent efficiency (%).

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

Dead biomass of actinomycetes were examined before and after biosorption of copper, lead and cadmium at optimum conditions from 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 which present on their wall and the mechanism involved in biosorption process (Mende et al., 2016).

Bio-removal of heavy metals (Cu2+, Pb2+ and Cd2+) from real wastewater by the dead biomass

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

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 division. The data were subjected to analysis of variance (ANOVA) (Ishak and Ali, 2016) ($p < 0.05$).

3. Results

Different morphologically 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 studied heavy metal ions (Cu^{2+}, Pb^{2+} and Cd^{2+}).

The isolates Kw9, Kw14, Kw27, Kw28, Kw36, Kw40, Kw42, Kw49, 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 from Cu^{2+}, Pb^{2+} and Cd^{2+} in aqueous solution. The highest removal percentage was recorded by Kw42 isolate. Living biomass of Kw42 was removed 58.4 % from Cu^{2+}, 67.7 % from Pb^{2+} and 52.0 % from Cd^{2+} but the dead biomass was removed 76.6 % from Cu^{2+}, 82.7 % from pb^{2+} and 68.3 % from cd^{2+} (Table 1).

According to the results in Fig. 2 (A, B and C), the biosorption by dead biomass for all isolates was higher than living biomass.

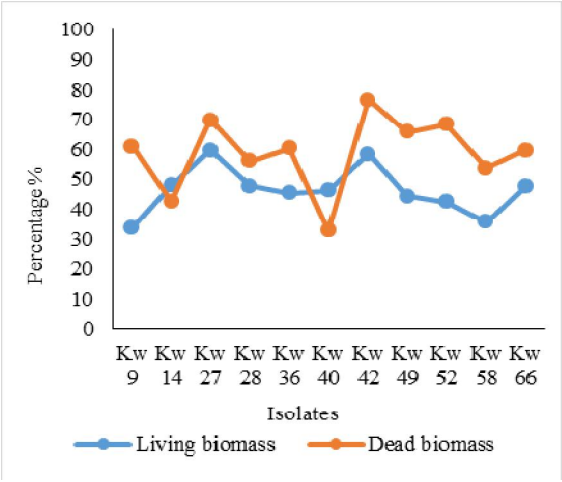
16S rRNA sequence and phylogenetic analyses of the highest biosorbent isolate (kw42)

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 Fig. 3. The database was deposited

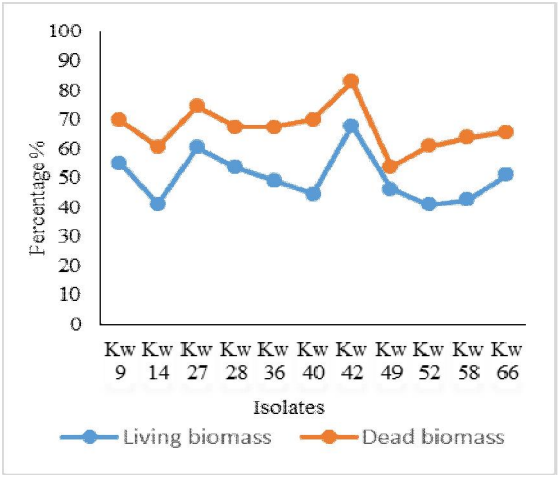
in NCBI Gen Bank under the accession number; MK020765.

Table 1. The percentage of biosorption efficiency (%) in ternary metal systems (Cu^{2+}, Pb^{2+}, and Cd^{2+}) by living and dead biomass, n=3 ± SD.

S.N.	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



A.



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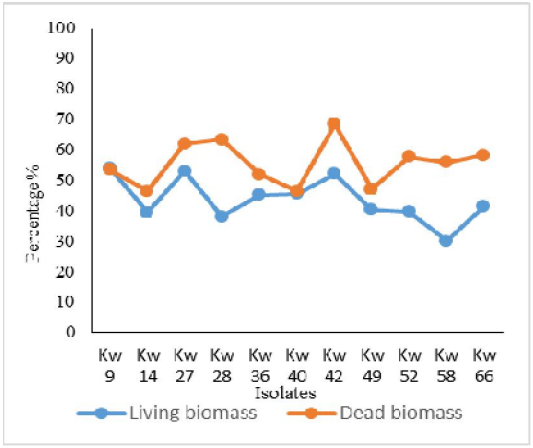

C.

Figure 2. Biosorption of Cu2+ (A), Pb2+ (B) and Cd2+ (C) by living and dead biomass of Streptomyces cyaneus KW42.

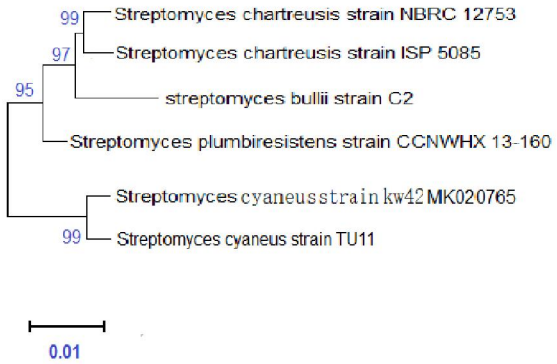

Figure 3. Phylogenetic tree based on 16S rRNA gene sequence analysis constructed with the neighbor-joining method showing the phylogenetic position of *Streptomyces cyaneus* kw42 MK020765.

Optimization of biosorption conditions

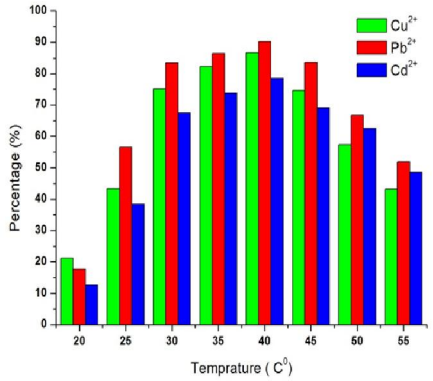

Figure 4. Effect of different temperatures on the biosorption capacity of cu2+, pb2+, and cd2+ by dead biomass of *Streptomyces cyaneus* kw42. The data are the mean of triplicates ± SD.

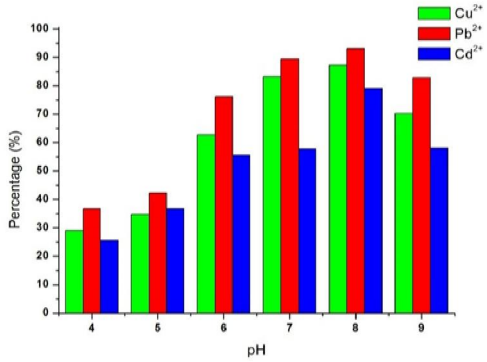

Figure 5. Effects of pH on biosorption of cu2+, pb2+, and cd2+ by dead biomass of Streptomyces cyaneus kw42. The data are the mean of triplicates ± SD.

Biosorption efficiency of dead biomass of Streptomyces cyaneus kw42 was increased with increasing temperature from 20°C to 40°C as shown in Fig 4. Bio-removal capacity 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 temperatures on removal the studied heavy metals were significant where factor of temperature evidencing low P-value < 0.05 with confidence level 95%. On the other hand, bio-removal efficiency was decreased when the temperature was increased more than 40°C.

As shown in Fig 5 the maximum biosorption by kw 42 for Cu, Pb and Cd ions was found at pH- 8 with removal efficacy of 87.3 %, and 93.1 % and 79.1 % respectively and biosorption by kw 42 for Cu, Pb and Cd ions was found at pH- 7 with removal efficacy of 83.2 %, 89.4 % and 75.8 % respectively. The effect of pH on removal the studied heavy metals were significant with P-value < 0.05. This result suggested that the alkaline pH was optimum for biosorption of these heavy metals using Streptomyces cyaneus Kw42.

The effect of biosorbent dosage (0.1 %– 0.6 %) on sorption efficiency in aqueous solutions under optimized temperature and pH was recorded in Fig 6. The results were 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 rapidly from 32.4 %, 36.5 % and 27.7 % to 89.7 %, 91.8 % and 77.3 %, respectively. The effect of biosorbent dosage on removal the studied heavy metals was significant (P-value < 0.05 with confidence level 95%).

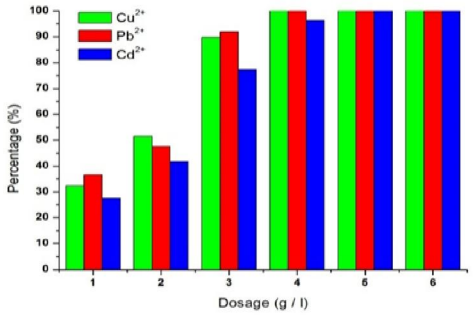

Figure 6. Effects of biosorbent dosage on biosorption of Cu2+, Pb2+, and Cd2+ by dead biomass of *Streptomyces cyaneus* kw42. The data are the mean of triplicates ± SD.

Moreover, when the biosorbent dosage increased to 0.5% the metals were completely removed from aqueous solution.

Biosorption experiments with biomass were conducted for solutions containing g 25-300 mg/l from 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 but at higher concentrations (150- 200 mg / l), complete biosorption was taken about 5 to 6 hours by Streptomyces cyaneus Kw42. The effect of initial concentrations and contact time on removal the studied heavy metals was significant (P-value < 0.05).

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

The morphology of dead biomass of *Streptomycetes cyaneus* Kw42 was analyzed by Scanning Electron Microscope before and after biosorption of Cu2+, Pb2+ and Cd2+. An electron micrograph of dead biomass of *S. cyaneus* kw42 was presented in fig.7 shown the change in the surface

where the surface became more smoothly after binding with metal ions. The dead biomass of *Streptomycetes cyaneus* Kw42 was examined by EDX analyses before and after biosorption of Cu^{2+}, Pb^{2+} and Cd^{2+} from aqueous solution. As shown in figures (25A, 26A) all charts did not have peaks of Cu^{2+}, Pb^{2+} and Cd^{2+} before biosorption. On the other hand, the charts after biosorption had cupper peak at 8 Kev, lead peak at 2.8 Kev and cadmium peak at 3.1 Kev (fig. 8B).

From fig. 8, the Mg2+ and Ca2+ were the main cations which were changed during the biosorption process by Streptomyces cyaneus Kw42.

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* Kw42 MK020765 by using ICP instrument. As shown in table 3, The studied heavy metals (Cu^{2+}, Pb^{2+} and Cd^{2+}) before treatment in EL-Khadrawia drain were recorded 1.43 mg/l, 0.91 mg/l and 3.66 mg/l respectively, and when treated for 90 min. the Cu^{2+} and Pb^{2+} completely removed but the Cd^{2+} needed 120 min. for completely removing and in Qalubia drain heavy metals recorded 9.71 mg/l, 1.62 mg/l and 2.10 mg/l respectively, but when wastewater was treated with dead biomass complete removing was taken about 90 min. for Pb2+ and 120 for Cu^{2+} and Cd^{2+}.

Table 2. Effect of versus concentrations of heavy metal at varying contact time by dead biomass of Streptomyces sp. KW42. The data 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
360	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
Time (min)	**150mg/ l**			**200mg/ l**			**300mg/ l**		
	cu^{2+}	Pb^{2+}	cd^{2+}	cu^{2+}	pb^{2+}	cd^{2+}	cu^{2+}	pb^{2+}	Cd^{2+}
30	26.4±0.77	31±0.76	21.5±0.87	22.9±1.28	28.6±1.04	20.2±1.02	18.7±0.38	21.6±1.20	12.1±1.06
60	41.8±0.56	47.8±0.43	35.9±0.23	38.8±0.60	40.5±0.43	31.5±0.93	27.4±0.92	33.5±0.37	22.8±0.51
120	58.2±1.05	61.3±0.23	51.3±0.78	51.2±1.45	58.8±0.76	46.9±0.80	48.3±0.63	50.1±0.48	38.7±0.67
180	78.5±0.68	82.4±0.28	68.7±0.46	69.5±0.29	77.4±0.43	59.6±1.34	61.5±0.59	67.8±0.35	46.2±0.46
240	90.8±0.96	96.1±0.62	83.4±0.23	83.6±0.50	83.2±0.16	76.2±0.27	76.6±1.06	75.6±1.08	61.8±0.26
300	100±0.00	100±0.00	92.3±1.09	92.8±0.69	94.6±0.52	81.2±0.54	82.3±0.58	79.6±0.30	68.4±0.23
360	100±0.00	100±0.00	97.6±0.56	100±0.00	100±0.00	87.7±0.60	86.5±0.27	84.2±0.48	69.6±0.94

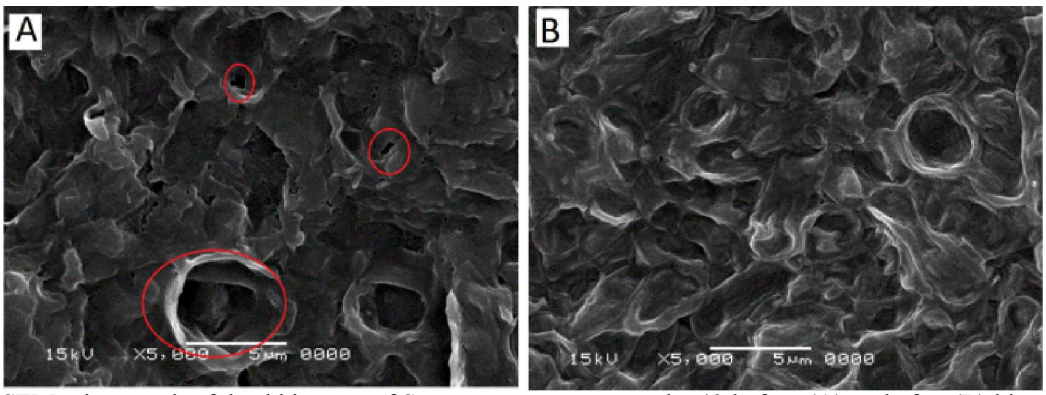

Figure 7. SEM micrograph of dead biomass of Streptomycetes cyaneus kw42 before (A) and after (B) biosorption of Cu2+, Pb2+ and Cd2+from aqueous solution.

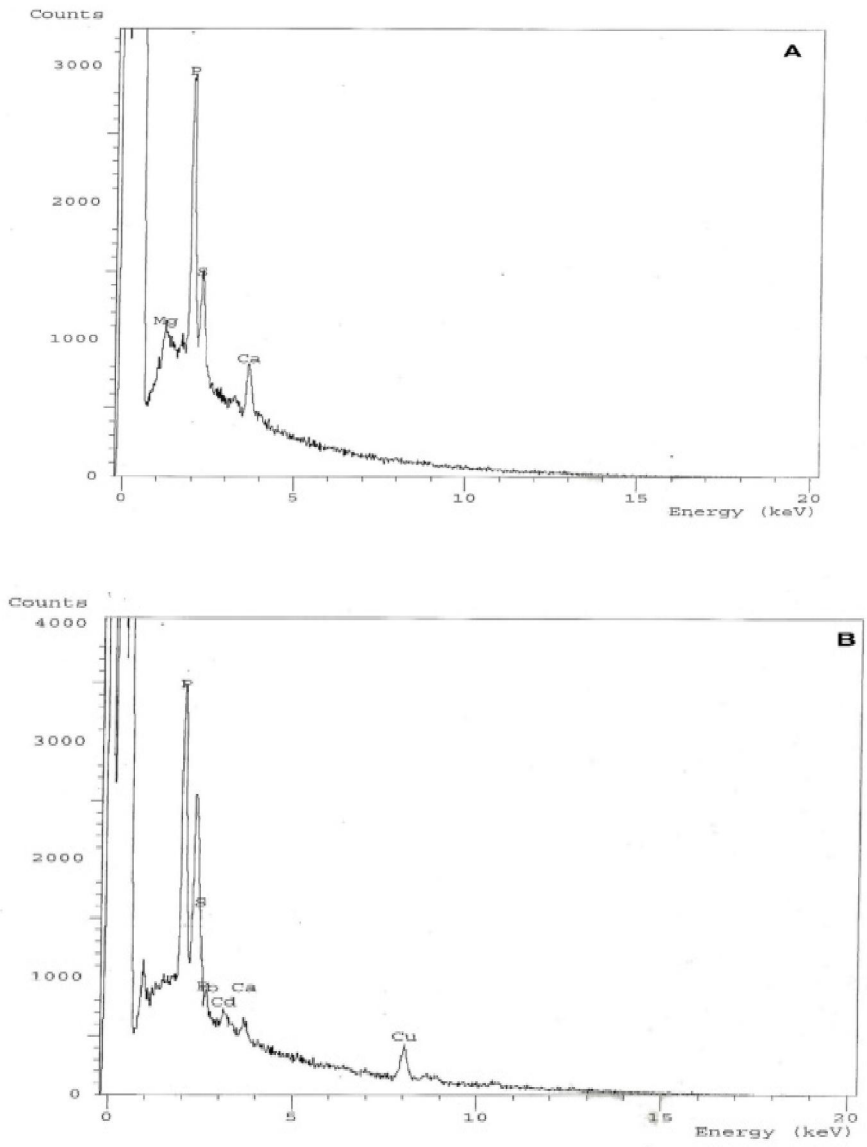

Figure 8. EDX analyses for dead biomass of Streptomyces cyaneus Kw42 before (A) and after (B) biosorption of Cu^{2+}, Pb^{2+} and Cd^{2+}.

On the other hand, the results of studied metals in Bahr Hadus drain were recorded 0.85 mg/l, 0.01 mg/l and 0.07 mg/l respectively, the removing process was needed 60 min. In 10th of Ramadan and Sadat City the studied metals needed about 90 mints for completely removal heavy metals. The contact time for removal heavy metals was changed as a result of changing the heavy metals concentration in wastewater samples.

4. Discussions

Biological removal of heavy metal 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 found in contaminated wastewater. The biosorption by dead biomass for all isolates was higher than living biomass. The dead biomass has several advantages to offer than living biomass. These advantages include their ease of treatment and no metal toxicity that can result in cell death in live cells. Additionally, the dead biomass did not require supplementation with nutrients which can increase the biological and chemical oxygen demands on the

treated water (Al Turk and Kiki, 2011; Daboor et al., 2014). Many early studies were recorded that the dead biomass removed the heavy metals more than living biomass (El-Gendy and El-Bondkly, 2016). Simeonova used the dead biomass of Streptomyces fradiae in biosorption of Cu2+, Zn2+, Ni2+, and Pb2+ from aqueous solutions. In fact, cell walls of biomass are made of large molecules (peptidoglycan) linked with teichoic acids and polysaccharides. These molecules possess functional groups which can adsorb heavy metals (Simeonova et al., 2008).

The increase in adsorption percentage with elevation in temperature can be attributed to the 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, a temperature based acceleration of some slow adsorption 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).

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

When the biosorbent dosage was increased more biosorbent binding sites were available at higher dosages than at lower dosage which was lead to binding of all available metal ions (El-Gendy and El-Bondkly, 2016).

16S rRNA sequence of Kw42 was deposited in Gen Bank under the accession number; MK020765 and was confirmed as Streptomyces cyaneus. Scanning Electron Microscope before and after biosorption was referred to surface change in biomass after binding the metals and on the other hand the charts of EDX analyses were indicated that the mechanism which is responsible for biosorption was ions exchange. Complete removal of heavy metal ions from wastewater with dead biomass of Streptomyces cyaneus Kw42 MK020765 was needed different times depending on the sites and concentration of metal ions.

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	N.D	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

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References

1. Uwah E, Ndahi N, Abdulrahman F, Ogugbuaja V. Distribution of some trace metals in soils, and fruit vegetables grown in Maiduguri, Nigeria. *Continental J Appl Sci.* 2011;6:31-42.
2. Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. *Renewable and sustainable energy reviews.* 2010;14(1):217-232.
3. Kadirvelu K, Senthilkumar P, Thamaraiselvi K, Subburam V. Activated carbon prepared from biomass as adsorbent: elimination of Ni (II) from aqueous solution. *Bioresource technology.* 2002;81(1):87-90.
4. Mythili K, Karthikeyan B. Bioremediation of Cr (VI) from Tannery effluent using Bacillus spp and Staphylococcus spp. *International Multidisciplinary Research Journal.* 2011;1(6).
5. El-Sherif IY, Ashmawy A, Badr S. Biosorption of cadmium and nickel by Nile water algae.

Journal of Applied Sciences Research. 2008;4(4):391-396.
6. Loutseti S, Danielidis D, Economou-Amilli A, Katsaros C, Santas R, Santas P. The application of a micro-algal/bacterial biofilter for the detoxification of copper and cadmium metal wastes. *Bioresource technology.* 2009;100(7):2099-2105.
7. Xiao G, Zhang X, Su H, Tan T. Plate column biosorption of Cu (II) on membrane-type biosorbent (MBS) of Penicillium biomass: optimization using statistical design methods. *Bioresource technology.* 2013;143:490-498.
8. Rehman A, Farooq H, Hasnain S. Biosorption of copper by yeast, Loddermyces elongisporus, isolated from industrial effluents: its potential use in wastewater treatment. *Journal of basic microbiology.* 2008;48(3):195-201.
9. Ahmad MF, Haydar S, Quraishi TA. Enhancement of biosorption of zinc ions from aqueous solution by immobilized Candida utilis and Candida tropicalis cells. *International Biodeterioration & Biodegradation.* 2013;83:119-128.
10. Al Turk IM, Kiki MJ. Heavy metals removal from raw industrial wastewater by halophilic actinomycetes isolated from Saudi saline soil. *Journal of International Environmental Application and Science.* 2011;6(4):606.
11. Amatullah A, Rajib SA, Reza HM. Antibiotic resistance and chromium reduction pattern among actinomycetes. 2012.
12. Simeonova A, Godjevargova T, Ivanova D. Biosorption of heavy metals by dead Streptomyces fradiae. *Environmental Engineering Science.* 2008;25(5):627-634.
13. Elouzi AA, Akasha AA, Elgerbi AM, El-Baseir M, El Gammudi BA. Removal of heavy metals contamination by bio-surfactants (rhamnolipids). *Journal of Chemical and Pharmaceutical Research.* 2012;4(9):4337-4341.
14. Hirsch C, Christensen D. Novel method for selective isolation of actinomycetes. *Applied and environmental microbiology.* 1983;46(4):925-929.
15. 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;84(6):599-602.
16. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution.* 1987;4(4):406-425.
17. Roth A, Andrees S, Kroppenstedt RM, Harmsen D, Mauch H. Phylogeny of the genus Nocardia based on reassessed 16S rRNA gene sequences reveals underspeciation and division of strains classified as Nocardia asteroides into three established species and two unnamed taxons. *Journal of clinical microbiology.* 2003;41(2):851-856.
18. 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;510:275-282.
19. Ishak WRW, Ali SM. Assessment of Malaysian Cornsilk Bioactive Compounds and Its Cytotoxicity test on Brine Shrimp (Artemia salina). *Health.* 2016;7(1):124-138.

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