IJIRR

International Journal of Information Research and Review
Vol. 06, Issue, 02, pp.6146-6150, February, 2019

RESEARCH ARTICLE

BIOREMOVAL OF SOME HEAVY METALS FROM AQUEOUS SOLUTIONS BY DEAD BIOMASS OF NOCARDIA TAKEDENSIS KW27

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ARTICLE INFO

Article History:
Received 29th November, 2018
Received in revised form
06th December, 2018
Accepted 01st January, 2019
Published online 28th Februarv. 2019

Keywords:
Actinomycetes, Dead biomass,
Heavy metals,Living biomass,
Streptomyces, Wastewater.

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ABSTRACT

Environmental contamination by toxic heavy metals is causing a serious problem worldwide due to their incremented accumulation in food chain and continued persistence ecosystem. Sixty-five isolates of actinomycetes were isolated from different regions in Egypt. The eleven isolates which were removed any studied heavy metals, cu^{2+}, pb^{2+} and cd^{2+}. singly above 70% was selected to remove metals from ternary mixture for studied metals ions. The highest removing percentage was recorded by kw27 isolate which was removed 70.8 %, 76.3 %, and 61.7 % from Cu^{2+}, Pb^{2+} and Cd^{2+} respectively. The 16S rRNA analyses and phylogenetic data of kw27 concluded that Kw27 was member of *Nocardia* genus and kw27 was deposited in the Gen Bank Database under accession number MK014899. The results of the dead biomass of *Nocardia takedensis* Kw27 for Cu^{2+}, Pb^{2+}, and Cd^{2+} under the optimized conditions pH-8 at 45^0C for 3hours with 0.3% biosorbent dosage was found to be as follows: Pb^{2+}, 87.2 %. > Cu^{2+}, 79.7 %. >Cd^{2+}, 71.3 %). All studied heavy metals were removed from aqueous solutions when the sorbent dosage increased to 0.6% or when contact time increased from 5 to 6 hours by dead biomass of Kw27 isolate. This work conformed the potential use of *Nocardia takedensis* Kw27 as an inexpensive and efficient technology for removal of cu^{2+}, pb^{2+} and cd^2 from aqueous solutions.

INTRODUCTION

Pollution of water is a major problem in the world. Human activities such as mining, industrial production, and agriculture release increase the amount of metal ions into water. Environment contamination by toxic heavy metals is causing a serious problem worldwide due to their incremented accumulation in food chain and continued persistence ecosystem (Uwah *et al.,* 2011). The most common heavy metal contaminants, e.g. lead, cadmium, copper, zinc, and iron. at any concentrations are difficult to remove 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 (Kadirvelu *et al.,* 2001) for heavy metal removal from waste streams are 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 (Ahmad *et al.,* 2013, Rehman *et al.,* 2008) 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 bioaccumulators 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 removal the toxic heavy metals, Cu^{2+}, Pb^{2+}, and Cd^{2+}. from wastewater by eco-friendly and low cost effective biological method.

MATERIALS AND METHODS

Sample collection: Wastewater samples were collected from different drains from Egypt, EL-Khadrawia drain, Qalubia drain, Bahr Hadus drain, 10th of Ramadan and Sadat City). The collected samples were transferred in sterile plastic containers to the laboratory and maintained at 4^0C 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.

Actinomycetes isolation: Actinomycetes were isolated from wastewater by membrane filter technique, this method

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 5days, 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 100^0C 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^oC for 7days. After incubation isolated actinomycetes was purified and stored in slants at 4 °C for further studies.

Prepare the dead biomasses of actinomycetes: Biomass of isolated actinomycetes were used as natural biosorbent to removal the Cu^{2+}, Pb^{2+}, and Cd^{2+} from aqueous solutions. ten days old culture spores, 10^6 CFU. from each isolated actinomycetes transferred into 250mL Erlenmeyer flask containing 100 mL broth media [peptone 4 g/l, yeast extract 2 g/l, glucose 10 g/l] and incubated at 30^0C, 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. 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 70^0C overnight, fig-1). To conform completely dead of dried mycelium, the sample inoculated in agar media and incubate at 30^0C for 7days, the absence of any growth referred to positive results (Simeonova *et al.,* 2008)

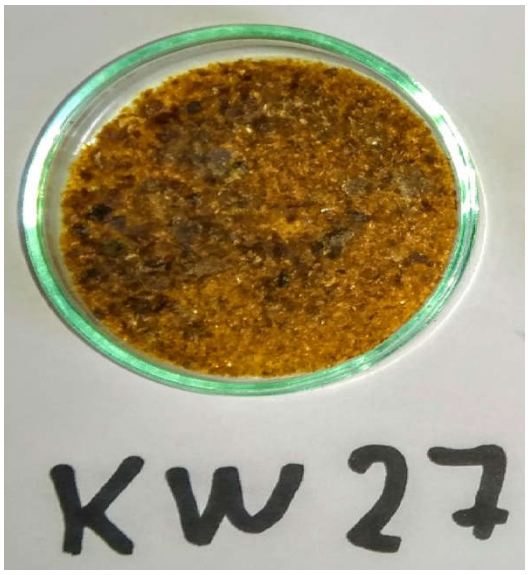

Fig. 1. Patri dish containing the dead biomass of *Nocardia* sp. Kw 27. that is used to biosorption of Cu^{2+}, Pb^{2+}, and Cd^{2+} from aqueous solutions

Evaluation of dead biomass in biosorption of heavy metal from aqueous solution: To evaluate the biosorption efficiency of dead biomass of actinomycetes, 250 ml elementary was prepared for every isolate to assay a single metal. One hundred ml from metal dilution 100 mg/ L was put in flask and inoculated by dead biomass, 3g/l). The flasks were incubated on rotary shakers, 150 rpm. at 30^0C 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).

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

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.

Ternary metals system: The isolates which were removed any studied heavy metals, cu^{2+}, pb^{2+} and cd^{2+}. singly above 70% was selected to remove metals from ternary mixture for studied metals ions. 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: 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 kw27, 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 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 kw42 strain was deposited in the Gen Bank database under accession numbers: **MK014899**

Factor effect on biosorption of heavy metals: For determine the impact of temperature on biosorption by *Nocardia* kw27, 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 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, 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 100mL 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 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 3g/ 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, %).

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 morphologically actinomycetes were isolated from collected wastewater, 27 isolates), sediment and soil samples, 39 isolates). These isolates were used to perform the experiment by dead biomass for removal studied heavy metal ions, Cu^{2+}, Pb^{2+} and Cd^{2+}). The data in table 1 revealed that the isolates kw 9, kw 14, kw 27, kw 28, kw 36, kw 40, kw 48, kw 51, kw 57, kw 65, were recorded the highest biosorption percentage above 70% for heavy metals ions singly, one at least).

Table 1. The percentage of biosorption efficiency (%) of various (Cu^{2+}, Pb^{2+}, and Cd^{2+}) heavy metals by dead biomass of Actinomycete biosorbent isolates (biosorption conditions are 100 mg/ L of each heavy metal, 0.3 % of biosorbent, pH 7.0 at 30 ◦C and shaken 150 rpm for 3h.). n=3 ± SD

Code of isolates	Dead biomass		
	CU^{2+} %	Pb^{2+} %	cd^{2+} %
kw 1	42.4 ± 0.20	58.0 ± 0.47	54.9 ± 0.57
kw 2	53.4 ± 1.12	62.0 ± 1.06	46.7 ± 1.06
kw 3	46.6 ± 1.08	42.4 ± 0.27	35.0 ± 1.07
kw 4	56.8 ± 0.63	55.3 ± 0.68	63.6 ± 0.91
kw 5	40.7 ± 0.46	51.8 ± 1.26	23.8 ± 0.43
kw 6	49.7 ± 0.35	56.5 ± 1.09	46.7 ± 0.54
kw 7	57.3 ± 1.23	67.0 ± 0.38	60.7 ± 0.78
kw 8	64.1 ± 0.87	53.2 ± 0.73	52.4 ± 0.32
kw 9	70.2 ± 0.16	73.7 ± 0.38	59.5 ± 1.08
kw 10	64.4 ± 0.47	62.8 ± 0.81	51.2 ±0.36
kw 11	48.8 ± 0.49	59.9 ± 0.38	45.1 ± 0.46
kw 12	60.5 ± 0.88	63.7 ± 0.17	50.4± 0.72
kw 13	33.6 ± 1.09	40. 4 ± 1.06	47.0 ± 0.89
kw 14	62.0 ± 0.13	74.0 ± 0.85	59.6 ± 0.45
kw 15	60.1 ± 0.40	48.2 ± 0.94	43.8 ± 0.80
kw 16	67.8 ± 0.32	60.4 ± 0.89	58.4 ± 0.37
kw 17	58.2 ± 0.61	62.6 ± 1.15	47.3 ± 0.73
kw 18	41.8 ± 0.26	45.6 ± 0.47	34.6 ± 1.45
kw 19	53.3 ± 0.90	60.0 ± 0.46	41.1 ± 1.21
kw 20	59.8 ± 0.49	52.0 ± 0.24	63.8 ± 0.35
kw 21	58.1 ± 1.17	48.6 ± 1.07	52.3 ± 0.32
kw 22	53.0 ± 1.07	61.0 ± 0.25	36.0 ± 0.32
kw 23	60.5 ± 0.97	66.4 ± 0.63	43.2 ± 0.73
kw 24	41.5 ± 0.48	51.7 ± 0.83	59.4 ± 0.35
kw 25	54.7 ± 0.92	43.5 ± 0.67	43.7 ± 0.92
kw 26	49.3 ± 0.90	60.9± 0.94	37.0 ± 0.25
kw 27	70.8 ± 0.47	76.3 ± 1.2	61.7 ± 0.27
kw 28	71.7 ± 0.66	74.2 ± 0.84	68.3 ± 0.92
kw 29	44.4 ± 0.16	57.9 ± 0.65	51.5 ± 0.74
kw 30	53.3 ± 0.71	60.6 ± 0.62	57.5 ± 0.32
kw 31	43.7 ± 0.42	62.2 ± 0.37	38.8 ± 0.75
kw 32	55.8 ± 0.73	34.4± 0.94	65.7 ± 0.81
kw 33	46.8 ± 0.54	51.4 ± 0.64	51.4 ± 0.49
kw 34	65.9 ± 0.71	66.7 ± 0.62	39.2 ± 0.29
kw 35	46.6 ± 1.06	44.6 ± 0.74	55.7 ± 0.75
kw 36	66.4 ± 0.82	72.8 ± 0.92	61.4 ± 0.79
kw 37	48.9 ± 0.55	56.4 ± 0.56	48.2 ± 0.85
kw 38	65.0 ± 1.08	65.4 ± 0.87	50.0 ± 0.66
kw 39	55.2 ± 1.11	42.1 ± 0.62	66.3 ± 0.35
kw 40	51.5 ± 1.08	78.8 ± 0.38	54.6 ± 0.83
kw 41	63.6 ± 0.91	55.8 ± 0.64	54.7 ± 0.74
kw 42	47.7 ± 0.16	56.3 ± 0.89	48.6 ± 0.27
kw 43	58.4 ± 1.03	51.2± 0.92	34.4 ± 1.09
Wv 44	68.8 ± 0.75	48.4 ± 0.37	39.0 ± 0.36
kw 45	40.3 ± 1.01	63.8 ± 1.16	34.3 ± 0.83
kw 46	45.1 ± 0.93	51.1 ± 0.29	25.8 ±0.34
kw 47	54.6 ± 0.17	67.7 ± 0.66	41.9 ± 0.68
kw 48	71.8 ± 0.63	55.7 ± 0.64	61.6 ± 0.43
kw 49	68.0 ± 0.71	67.0 ± 0.35	69.9 ± 0.34
kw 50	63.6 ± 1.05	58.5 ± 0.95	50.0 ± 0.98
kw 51	72.3 ± 0.82	76.2 ± 0.91	60.3 ± 1.25
kw 52	58.8 ± 0.20	47.0 ± 0.23	53.6 ± 0.27
kw 53	65.5 ± 0.96	52.4 ± 0.52	48.3 ± 0.63
kw 54	60.2 ± 0.91	35.4 ± 0.87	42.5 ± 0.86
kw 55	47.2 ± 0.63	52.7 ± 0.35	46.9 ± 0.53
kw 56	55.7 ± 0.17	65.6 ± 1.64	67.5 ± 1.09
kw 57	62.6 ± 0.34	70.0 ± 1.21	63.4 ± 1.06
kw 58	57.2 ± 0.83	46.4 ± 1.10	66.0 ± 0.65
kw 59	58.2 ± 0.71	51.8 ± 0.23	38.3 ± 0.12
kw 60	44.7 ± 0.29	64.1 ± 0.65	36.7 ± 0.35
kw 61	60.9 ± 1.07	67.1 ± 0.85	53.6 ± 0.93
kw 62	51.8 ± 1.04	51.1 ± 0.91	39.4 ± 0.43
kw 63	35.1 ± 0.75	58.3 ± 1.05	34.5 ± 0.25
kw 64	61.8 ± 0.67	55.3 ± 0.82	65.6 ± 0.67
kw 65	73.1 ± 0.25	64.7 ± 0.68	70.4 ± 0.26

*SD: standard deviation

Isolates were selected to perform the experiment by using ternary mixture composed from Cu^{2+}, Pb^{2+} and Cd^{2+} in aqueous solution. The highest removal percentage recorded by kw 27 isolate. The dead biomass was removed 70.8 % from Cu^{2+}, 76.3 % from pb^{2+} and 61.7 % from cd^{2+} [Table 2]. Living and dead biomass of actinomycetes were used in bioremediation heavy metals. (El-Gendy and El-Bondkly 2016) recorded that the dead biomass removed the heavy metals more than living biomass. Simeonova recorded that the dead biomass of *Streptomyces fradiae* was used to biosorption Cu^{2+}, Zn^{2+}, Ni^{2+}, and Pb^{2+} 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).

Table 2. The percentage of biosorption efficiency (%) In ternary metal systems of heavy metals (Cu^{2+}, Pb^{2+}, and Cd^{2+}) by dead biomass of isolated actinomycetes. n=3 ± SD

	Dead biomass		
	Cu^{2+} %	Pb^{2+} %	Cd^{2+} %
W 9	61±0.812	69.6±0.82	53.9±0.62
W 14	42.7±1.00	60.5±0.24	46.3±1.12
W 27	69.9±0.36	74.3±1.02	61.8±0.81
W 28	56.1±0.99	67.3±0.36	63.3±0.60
W 36	60.4±0.43	67.2±0.24	51.9±1.07
W 40	33.0±0.64	69.7±0.73	46.3±1.15
W 48	65.9±0.22	53.6±1.06	47±0.73
W 51	68.3±0.86	60.6±0.32	57.6±0.88
W 57	53.7±0.60	63.6±0.12	55.8±0.86
W 65	59.7±1.06	65. 6±0.52	58±1.06

*SD: standard deviation

Identification the highest biosorbent isolate, kw42. by using 16S rRNA sequence and phylogenetic analyses of most active strains: The partially 16S rRNA sequences of strain KW27, accession number MK014899. were compared to the sequences of members of the order Actinomycetales. It was observed that the member of the genus Nocardia were the closest phylogenetic neighbors. The values were seen to range between 51, Nocardia sp. strain OS 159), 53, Nocardia asiatica strain DSM 44668, Nocardia takedensis strain MS1 3), 49, Nocardia brasiliensis strain CNM20071731 and Nocardia brasiliensis strain IFRC 713. and 43, Nocardia takedensis strain MS1-3 and Nocardia asiatica strain 2. for isolate Nocardia takedensis kw 27 MK014899, Fig. 4). Based on the 16S rRNA analyses and phylogenetic data, it was concluded that the isolate KW27 merit species status within the genus Nocardia.

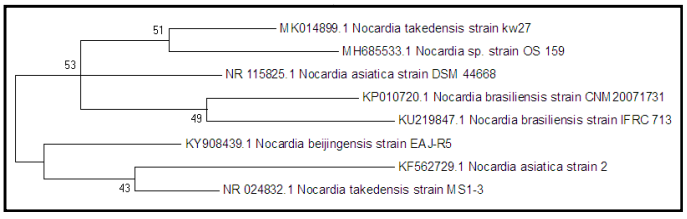

Fig. 2.Phylogenetic dendrogram, based on 16S rRNA gene sequence analysis, constructed using the neighbor-joining method, showing the phylogenetic position of *Nocardia* takedensis kw 27 MK014899

Factors effect on biosorptionof 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 effect on live cells. Additionally, the dead biomass doesn't required supplementation with nutrients which

Table 3. Effect of different temperatures on the biosorption capacity of cu2+, pb2+, and cd2+ by dead biomass of *Nocardia takedensis* kw42. n=3 ± SD

Metal ions	Different temperatures							
	20 ^{0}C	25 ^{0}C	30 ^{0}C	35 ^{0}C	40 ^{0}C	45 ^{0}C	50 ^{0}C	55 ^{0}C
Cu^{2+}	15.2±0.2	28.3±0.64	51.1±0.25	67.5±0.326	71.9±1.08	75.5±0.47	64.2±0.35	40.7±0.25
Pb^{2+}	19±0.84	38.6±1.05	70.6±0.74	73.2±0.723	80.2±0.34	83.2±0.44	69.9±1.47	52.8±1.20
Cd^{2+}	9.6±0.93	24.4±0.16	62.3±0.61	66.4±0.231	69.1±0.55	70.7±1.09	61.7±0.93	36.4±0.90

Table 4. Effects of pH on biosorption of cu2+, pb2+, and cd2+ by dead biomass of Nocardia takedensis KW27. n=3 ± SD

Metal ions	pH value					
	4	5	6	7	8	9
Cu2+	26.5±0.45	38.9±1.23	63.4±0.76	72.2±0.26	78.6±0.43	52.8±0.65
Pb2+	27±0.24	41.5±0.53	65.6±0.46	81.6±0.78	85.5±1.26	64.7±0.89
Cd2+	19.6±0.79	33.6±0.38	52.7±0.84	67.3±0.56	69.7±0.85	47.1±0.56

Table 5. Effects of biosorbent dosage on biosorption of Cu^{2+}, Pb^{2+}, and Cd^{2+} by dead biomass of Nocardia takedensis KW42. n=3 ± SD

Metal ions	1 g/l	2 g/l	3 g/l	4 g/l	5 g/l	6 g/l
Cu^{2+}	22.9±0.59	46.5±0.57	79.7±0.86	87.4±0.74	94.8±1.04	100±0.00
Pb^{2+}	30.1±0.45	54.7±0.65	87.2±0.73	94.3±0.45	99.6±0.42	100±0.00
Cd^{2+}	17.2±0.25	36.7±0.80	71.3±0.56	79.6±0.34	84.2±0.52	96.6±0.88

Table 6. Effect of versus concentrations of heavy metal at varying contact time by *Nocardia takedensis* KW42. n=3 ± SD

Time (min)	25 mg/L			50 mg/L			100 mg/L		
	Cu^{2+}	pb^{2+}	cd^{2+}	Cu^{2+}	pb^{2+}	cd^{2+}	Cu^{2+}	pb^{2+}	cd^{2+}
30	73.5±0.64	82.6±0.38	69.3±0.45	33.6±0.82	45.4±1.00	31.8±0.63	29.3±0.23	32±0.43	22.7±0.56
60	94.8±0.213	97.2±0.85	91.6±0.63	56.7±1.00	53.9±0.93	52.3±0.85	38.0±0.83	48.2±0.64	39.1±0.68
120	100±0.00	100±0.00	100±0.00	84.2±0.80	78.5±0.34	69.5±0.73	51.4±0.47	59.7±0.15	58.6±0.26
180	100±0.00	100±0.00	100±0.00	97.7±1.04	93.2±0.95	85.4±0.86	76.6±0.36	87.9±0.69	70.3±1.03
240	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	96.6±0.76	89.3±0.73	94.7±1.15	84.4±1.14
300	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	96.5±0.53	100±0.00	92.6±0.63
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	99.1±0.93
Time (min)	**150 mg/L**			**200 mg/L**			**300 mg/L**		
	Cu^{2+}	pb^{2+}	cd^{2+}	Cu^{2+}	pb^{2+}	cd^{2+}	Cu^{2+}	pb^{2+}	cd^{2+}
30	23.4±0.83	27.1±1.57	20.5±0.34	20.1±0.86	21.6±1.23	16.2±1.44	15.2±1.37	17.8±1.33	15.1±1.34
60	35.8±0.83	43.9±0.60	32.8±0.76	31.4±1.34	38.3±0.37	23.5±0.16	26.3±1.50	32.1±2.08	18.7±0.58
120	48.2±0.53	53.4±0.74	51.4±1.54	43.6±1.38	45.9±0.48	37.8±1.28	38.5±0.97	40.2±1.63	29.3±0.93
180	67.5±1.22	72.5±0.23	65.8±1.25	59.3±0.76	66.4±1.15	50.4±1.25	44.7±0.83	53.6±0.34	41.6±0.84
240	75.8±0.85	86.2±1.13	73.2±0.18	64.2±1.45	73.5±0.92	64.8±1.75	53.6±0.75	62.8±0.63	49.8±2.24
300	88.3±1.14	91.7±0.76	81.2±0.57	71.5±1.83	83.5±1.51	74.8±1.32	61.1±1.35	69.1±0.83	56.2±1.52
360	90.5±1.88	93.2±0.84	84.6±0.84	76.1±0.96	86.0±1.35	75.5±0.45	63.4±0.78	70.4±1.21	57.4±1.34

can increase the biological and chemical oxygen demands on the treated water (Daboor *et al.,* 2014, Al Turk and Kiki 2011).

Effects of temperature on biosorbent: Reduction efficiency of dead biomass of *Nocardia* sp. kw27 was increased with increasing temperature from 20 ^{0}C to 45 ^{0}C as shown in Table 3. Bioremoval capacity for Cu^{2+}, Pb^{2+} and Cd^{2+}were 15.2 %, 19 % and 9.6 % respectively at 20 ^{0}C and were increased to 75.5 %, 83.2 % and 70.7 % respectively at 45 ^{0}C. The effect of temperatures on removal the studied heavy metals was significant $p< 0.01$. On the other hand, bioremoval efficiency was decreased when the temperature was increased more than 45 ^{0}C.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).

Effects of different pH on biosorption capacity: 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 Table 4. The maximum biosorption by kw 27 for Cu, and Pb and Cd ions was found at pH- 8 with removal efficacy of 78.6 %, and 85.5 % and 69.7 % respectively and biosorption by kw 42 for Cu, Pb and Cd ions was found at pH- 7 with removal efficacy of 72.2 %, 81.6 % and 67.3 % respectively. The effect of pH on removal the studied heavy metals was significant $p< 0.01$. This result suggested that the alkaline pH was optimum for biosorption of these heavy metals using *Nocardia takedensis* Kw27.

Effects of biosorbent dosage: The effect of biosorbent dosage, 0.1%– 0.6%. on sorption efficiency in aqueous solutions under optimized temperature and pH was recorded in Table 5. The results were indicated that when a biosorbent dosage was increased from 0.1% to 0.3%, the removal of Cu2+, Pb2+ and Cd2+ by Nocardia takedensis Kw27 increased rapidly from 22.9 %, 30.1 % and 17.2 % to 79.7 %, 87.2 % and 71.3 %, respectively. 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 lead to

binding of all available metal ions (El-Gendy and El-Bondkly 2016).

Effect of initial concentrations of heavy metal versus contact time: Biosorption experiments with biomass were conducted for solutions containing g 25-300 mg/l from Cu^{2+}, Pb^{2+}, and Cd^{2+}. As seen in table 6, at lower concentrations, 25 mg/ l. biosorption was complete in about 60 min but at higher concentrations, 150- 200 mg / l), it was taken about 6 hours for biosorption by *Nocardia takedensis* Kw42 and when it was increased the concentration needed time more than it. The effect of initial concentrations and contact time on removal the studied heavy metals was significant $p< 0.01$.

Conclusion

Copper, lead and cadmium were considered as toxic heavy metals and removal them from wastewater was an important target to improve the quality of wastewater before releasing into the environment. *Nocardia takedensis* kw27 was isolated from contaminated wastewater and it was identified as potent active biosorbents and from interestingly. As shown in the study, *Nocardia takedensis* kw27 can be used to completely remove toxic heavy metals from aqueous solution when treated for 60 to 280 minutes.

Acknowledgements: The authors thank the Central Laboratory for Environmental Quality Monitoring, National Water Research Center for their technical support.

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