

## Accumulation of Heavy Metals by Living and Dead Bacteria as Biosorbents: Isolated from Waste Soil

Iqra Batool<sup>a</sup>, Saiqa Andleeb<sup>a\*</sup>, Shaukat Ali<sup>b</sup>, Kalsoom Akhtar<sup>c</sup> and Nazish Mazhar Ali<sup>d</sup>

<sup>a</sup>Microbial Biotechnology Laboratory, Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad-3100, Pakistan

<sup>b</sup>Toxicology Laboratory, Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad-3100, Pakistan

<sup>c</sup>Department of Chemistry, University of Azad Jammu and Kashmir, Muzaffarabad-13100, Pakistan

<sup>d</sup>Microbiology Laboratory, Department of Zoology, Government College University, Lahore, Pakistan

(received February 3, 2016; revised June 22, 2016; accepted July 12, 2016)

**Abstract.** In the present study *Enterococcus luteus*, *Escherichia coli* and *Staphylococcus aureus* have been used for biosorption of cadmium and chromium from aqueous solution of various concentrations. Bacteria were isolated from waste soil and identified through various morphological features, biochemical tests, and staining procedure. Biosorption capacity (both dead and live biomass) was observed through broth technique and absorbance values were measured using atomic absorbance spectrophotometer. Different parameters were optimised for metal biosorption, including incubation periods (24, 48, 72 and 96 h) and pH (4, 6, 7, 8, 9, and 10) at 37 °C. Agar well and agar disc diffusion methods were used for resistogram and antibiogram analysis. Through agar well diffusion method, *S. aureus* showed complete resistance against all concentrations of chromium and cadmium (50 to 300 µg/mL). *E. luteus* showed resistance on 50 µg/mL and 100 µg/mL of both metals while *E. coli* exhibited resistance against all cadmium concentrations (50 to 300 µg/mL) while sensitivity was observed in case of chromium (12.0 ± 0.0 mm to 24.0 ± 0.0 mm). Through broth method, *E. luteus* showed good cadmium absorbance capacity at acidic pH 4 and 6, *E. coli* at pH 4, 6 and 7 and *S. aureus* at pH 6, 7 and 8. In case of chromium, *S. aureus* showed maximum absorbance at pH 6; *E. coli* at pH 7 and 8 and *E. luteus* showed minimum absorbance for chromium at pH 6 and 8. All bacterial isolates showed maximum biosorption of both metals after 24 h of incubation. Results suggested that pH 6 and incubation period 24 h could be better for biosorption of cadmium and chromium removal. Dead biomass of *E. coli* and *S. aureus* was more efficient for cadmium removal while both dead and live biomass (*E. luteus*, *E. coli* and *S. aureus*) have potential for chromium removal. These microbes could be used as potential source of heavy metal biosorbent, biosorbent

**Keywords:** heavy metals, resistogram analysis, antibiogram assay, biosorbent

### Introduction

In developing countries like Pakistan, the risk of heavy metal exposure to the environment is increasing day by day. Such heavy metals can cause impact on human health and are toxic to animals too (Tokar *et al.*, 2011; Jomova and Valko, 2011). A considerable amount of heavy metals is present in waste water coming from different sources, i.e., electroplating, paint, leather, metal and tanning industries. Heavy metals are removed from the waste water through the process of biosorption and bioremediation using microorganisms and it has been proved as a very cost effective and environmental friendly process (Elekwachi *et al.*, 2014; Joshi *et al.*, 2011). Biosorption is a process, in which there may be some chemical relationships between the metals and

microbes used (Shumate and Stranberg, 1985). Significance of biosorption process over conventionally used method is that it is cost effective, very efficient and shows decreased chemical and biological waste products. Recovery of biomass used and possibility of metal recovery is also possible by biosorption (Kratochvil and Volesky, 1998). Biosorbents are a large subclass of low-cost adsorbents that can be subdivided into biomass (dead or living), agricultural wastes, and industrial solid wastes (Bhatnagar and Minocha, 2006). Dead biomass has been utilised by many researchers as a functional biosorbent to remove different pollutants (Li *et al.*, 2010; Saraswat and Rai, 2010). Dead biomass is more readily desorbed than its living counterpart. Living biomasses including fungi (Kumar, 2014; Ismael *et al.*, 2004; Fu and Viraghavan, 2002; 2000), algae (Navarro *et al.*, 2012; Bishnoi and Pant, 2004), actinomycetes

\*Author for correspondence; E-mail: drsaiqa@gmail.com

(Solecka *et al.*, 2012; Fu and Virarghavan, 2002; 2000) and other microbial cultures with different strains (Kocberber and Donmez, 2007) were also used as low-cost biosorbents. Bacterial species like *Bacillus* has been identified as having a high potential of metal absorption and so it has been commercially used in biosorbent material preparation (Ghaima *et al.*, 2013; Singh *et al.*, 2012; Brierley, 1990).

Cadmium and chromium have highly deleterious effects on plants, animals as well as on human life. Cadmium is hazardous and highly toxic metal for environment and to human beings. This biologically non-essential element accumulates in body, especially in kidneys, liver, lung and brain. It can induce several toxic effects, depending on the concentration and exposure time. Cadmium has been linked to Alzheimer's disease (AD) as a probable risk factor (Meleleo *et al.*, 2010). Cadmium is dangerous to virtually all vital systems in the animal body. On the other hand extreme usage of chromium supplements can cause stomach problems and hypoglycemia. Too much chromium uptake can also cause various injuries like liver, kidneys, and nerve problems, and it also causes irregular heart rhythm. Major sources of cadmium and chromium include refined foods, water containing foods, pipes of water, vegetables and fruits, coffee, tea, burning coal and cigarettes (Costa, 2003).

Understanding the problems occurring due to heavy metals exposure and their degradation by biological means is necessary. As according to the above discussion and literature review, it is an urgent need of time to explore different microbial strains as biosorbent to remove heavy metals from different sources. Therefore, this study was planned to isolate and identify the bacteria from waste sources which could be used to purify the contaminated water.

## Materials and Methods

**Sample collection.** The contaminated soil samples *viz.*, slaughter house waste, dairy waste, and household were collected in sterilised containers from different localities (Lower Chellah and Lower Plate: near workstation of automobiles) of Muzaffarabad, Azad Jammu and Kashmir, Pakistan. All samples were collected in triplicate.

**Isolation and culturing of bacteria.** Contaminated soil samples for isolation of bacterial strains were spread on nutrient agar and MacConkey agar media plates (NAM; Oxoid CMOO<sub>3</sub>, MAM; Oxoid CM115),

incubated at 37 °C in incubator up to colony appearance. After 24 h incubation, single colony was picked up, streaked into nutrient broth medium (NBM; Oxoid CMI), placed in shaking incubator at 37 °C for 24 h. Next day, the mixed cultures were again purified by quadrant streaking on nutrient agar and MacConkey agar plates. The pure culture was grown in nutrient broth medium for overnight at 37 °C. Next day, bacteria were preserved in 60% glycerol and then placed at -20 °C for further processing.

**Identification of bacteria.** Bacterial species were identified on the basis of colony morphology: Gram's staining and using biochemical tests (Cheesbrough, 2002; Collins *et al.*, 1999; 1989). Further clarification and identification of all isolated bacteria were carried out at Department of Zoology, Government College University, Lahore, Pakistan.

**Preparation of heavy metal concentrations.** Chromium (Cr) and cadmium (Cd) heavy metals were used for biosorption assay. For cadmium Cd(II) and chromium Cr(IV) solutions CdCl<sub>2</sub> and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> salts were used. For each metal, a range of 50, 100, 150, 200, 250 and 300 µg/mL were obtained. The metal solutions were prepared in sterilised distilled water so as to prevent any chances of contamination.

**Resistogram analysis.** Heavy metal resistance study for microbial isolates was performed using agar well diffusion method (Jagessar *et al.*, 2008) with increasing concentrations of Cr and Cd ranging from 50 to 300. Nutrient agar (Oxide: CMOO<sub>3</sub>) and nutrient broth media (Oxide: CM1) were used for bacterial culture. The pH of media was adjusted to a final pH 7 by using sodium hydroxide. The microorganisms were activated by inoculating a loop full of strain in 25 mL of nutrient broth medium and incubated at 37 °C on a rotatory shaker for 24 h. The overnight culture was mixed with freshly prepared nutrient agar medium (NAM) at 45 °C and was poured into the sterilised petri dishes. All petri dishes were kept at room temperature in laminar flow for solidification. In each plate, 3 wells of 5 mm diameter were made using a 1 mL of sterilised micropipette tip and sterilised needle was used for the removal of agar plug. Approximately 30 µL of each heavy metal concentration were placed in each prepared well and placed at 37 °C for 24 - 48 h. Microbial growth was determined by measuring the diameter of zone of inhibition after 24 h (Seeley *et al.*, 2001). Diameter of the clear zones (if greater than 1 mm) around each well was measured with the help of scale (Hammer *et al.*,

1999). The results of the sensitivity test were expressed as (0) for no sensitivity, \*(1 -5 mm) for low sensitivity, \*\*(>5-10 mm) for moderate sensitivity and \*\*\*(> 10-25 mm) for high sensitivity. The bacterial species having maximum resistance to these metals were selected for further study.

**Antibiogram analysis .** Sensitivity of antibiotics against test microbial strains was assessed through agar disc diffusion method (Prescott *et al.*, 1999). This method is called antibiogram assay. Sensitivity was predicted with degree of clear zone of microbial growth inhibition surrounding the disc. The potency of standard antibiotics has been indicated as Ciprofloxacin (CIP; 5 µg), Ampicillin (AMP; 10 µg), Penicillin G (P; 10 µg), Neomycin (N; 10 µg), Vancomycin (VA; 30 µg), Erythromycin (E; 15 µg), Norfloxacin (NOR; 10 µg), Tetracycline (TE; 30 µg), Gentamicin (CN; 10 µg), Oxytetracycline (OT; 30 µg), Streptomycin (S; 10 µg), Chloramphenicol (C; 30 µg), Tobramycin (TOB; 10 µg), Nalidixic acid (NA; 30 µg), Sulfamethoxazole (SXT; 25 µg) and Kanamycin (K; 30 µg). All discs of antibiotics were purchased and made by Oxoid Company.

**Bioaccumulation/biosorption study.** In this study, each isolate was cultivated anaerobically in properly labelled test tubes containing nutrient broth medium by incubating the test tubes at 37 °C in shaking incubator. Five mL of bacterial suspension (*E. luteus* , *S. aureus* and *E. coli*) having dried biomass (4, 11 and 27 mg) was mixed with 1 mL metal solution in sterilised test tubes which were then covered with aluminum foil and agitated at 150 rpm on a shaking incubator at 37 °C. Biosorption was assayed by exposing the isolates to two different metals i.e., Cr(VI) and Cd(II) concentrations. The effects of different parameters like metal ion concentration, pH and incubation time on the adsorption capacity of each isolate were studied. After incubation, the samples were then centrifuged for 5 min at 13000 rpm and supernatant was used for the estimation of metal ion concentration using double beam spectrophotometer (Shimadzu UV 1800). A control was also set containing nutrient broth medium along with metal solution keeping all other conditions same except bacterial culture. Each test was performed in triplicates and their average value was taken as result.

The following parameters on adsorption capacity of isolates were studied:

1. The effect of metal ion concentration was studied using metal concentration ranging from 50-300

µg/mL for each metal ion.

2. The effect of incubation time of bacterial culture with metal solution was also studied. The incubation time given to the solution was 24 h, 48 , 72 and 96 h.
3. The effect of pH 4, 6, 7, 8 and 10 was studied and pH was adjusted by using analytical grade sodium hydroxide and hydrochloric acid.

Cr(VI) was analysed by diphenylcarbazide method, and Cd(II) by dithizone method. The metal removal efficiency of each strain was calculated by equation 1.

$$R. \% = (C_i - C_e) / C_i \times 100 \dots \dots \dots (1)$$

Where:

R = the removal efficiency (%),  $C_i$  = the initial metal concentration before removal (µg/mL) and  $C_e$  = the final metal concentration after removal (µg/mL) while, the amount of metal adsorption on the bacterial biomass can be calculated by equation 2.

$$q_{eq} = (C_o - C_{eq})V / M \dots \dots \dots (2)$$

Where:

$q_{eq}$  (mg/g) = the metal adsorption capacity,  $C_o$  (mg/L) = initial metal ion concentration, and  $C_{eq}$  = final metal ion concentration, respectively. V = the solution volume and M (g) = the amount of biosorbent used.

**Determination of metal concentration in the supernatant.** Atomic absorption spectrophotometer, Perkin Elmer Analyst 300 was used to determine the heavy metal concentration (chromium and cadmium). The wavelength 544 nm and 545 nm were used for cadmium and chromium. It was done by using its specific lamp for each metal and at a specific wavelength.

**Statistical analysis.** Each experiment of resistogram and antibiogram analysis was repeated in triplicates and standard deviation from absolute data was calculated (<http://easycalculation.com/statistics/standard-deviation.php>).

## Results and Discussion

**Isolation and identification of bacteria.** Three bacterial isolates such as *Enterococcus luteus*, *Staphylococcus aureus* and *Escherichia coli* were isolated from the contaminated soil samples. These strains were identified through gram's staining and biochemical tests (Table 1). *E. luteus* indicated urease and catalase positive tests

while citrate, coagulase, oxidase, indole and nitrate tests were negative. *S. aureus* is gram positive cocci and motile. *S. aureus* showed catalase, coagulase, citrate, urease, methyl red, nitrate tests positive while oxidase, Voges proskeur tests were negative. Catalase, indole, methyl red, and nitrate tests were positive for *E. coli* while citrate, coagulase, oxidase and urease tests were recorded as negative results. Glucose and lactose fermentation tests were positive for *E. coli* and *S. aureus* whereas, *E. luteus* indicated non-fermenter of carbohydrates.

**Table 1.** Identification of bacterial isolates through gram's staining and biochemical tests

Biochemical tests for bacterial identification	<i>Enterococcus luteus</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Gram staining	+ve	-ve	+ve
Colony morphology	Coccus	Rod	Coccus
Citrate test	-ve	-ve	+ve
Catalase test	+ve	+ve	+ve
Coagulase test	-ve	-ve	+ve
Indole test	-ve	+ve	-ve
Methyl red test	-ve	+ve	+ve
Oxidase test	-ve	-ve	-ve
Urease test	+ve	-ve	+ve
Motility test	-ve	+ve	+ve
Carbohydrate test (glucose and lactose)	-ve	+ve	+ve
Nitrate test	-ve	+ve	+ve
Voges Proskeur test	-ve	-ve	-ve

+ve and -ve indicate positive and negative results.

**Resistogram analysis. For chromium metal concentrations.** In resistogram analysis *E. luteus* showed resistance against Cr(IV) metal at 50 µg/mL and 100 µg/mL, but seemed to be sensitive against higher concentrations i.e., 150, 200, 250 and 300 µg/mL. Zone of inhibition was measured as 14.0 ± 0.0 mm against 150 µg/mL, 15.0 ± 0.0 mm against 200 µg/mL, 15.0 ± 0.0 mm against 250 µg/mL and 16.0 ± 0.0 mm against 300 µg/mL. *S. aureus* showed resistance against all concentrations of Cr(IV) metal whereas, *E. coli* showed high sensitivity against all concentrations of Cr(IV) metal. Zone of inhibition measured against each concentration was recorded as; 12.0 ± 0.0 mm against 50 µg/mL, 15.0 ± 0.0 mm against 100 µg/mL, 19.0 ± 0.0 mm against 150 µg/mL, 20.0 ± 0.0 mm against 200 µg/mL, 20.0 ± 0.0 mm against 250 µg/mL and 24.0 ± 0.0 mm against 300 µg/mL. In each case it was observed

that sensitivity increased with increase in metal concentrations (Table 2).

**For Cd(II) metal concentrations.** *E. luteus* showed resistance against four Cd(II) concentrations such as 50, 100, 250, and 300 µg/mL. While against other two concentrations sensitivity was observed and the zone of inhibition was measured as 15.0 ± 0.0 mm against 150 µg/mL and 12.0 ± 0.0 mm against 200 µg/mL. *S. aureus* and *E. coli* seemed to be fully resistant against all concentrations of Cd(II) (Table 3).

**Antibiogram analysis.** In case of antibiogram analysis *E. luteus* showed sensitivity against all antibiotics. The maximum zones of inhibition were measured as 28.0 ± 0.0 mm against CIP, 13.0 ± 0.0 mm against N, 17.0 ± 0.0 mm against VA, 21.0 ± 0.0 mm against E, 19.0 ± 0.0 mm against NOR, 14.0 ± 0.0 mm against CN, 13.0 ± 0.0 mm against S, 17.0 ± 0.0 against NA, 18.0 ± 0.0 mm against C, and 12.0 ± 0.0 mm against OT

**Table 2.** Resistogram analysis of chromium concentrations against bacterial isolates

Microbes used	Chromium metal concentrations (µg/mL)						pH
	50	100	150	200	250	300	
	Zone of inhibition in mm (M±SD)						
<i>E. luteus</i>	R	R	14.0 ± 0.0	15.0 ± 0.0	15.0 ± 0.0	16.0 ± 0.0	7
<i>S. aureus</i>	R	R	R	R	R	R	7
<i>E. coli</i>	12.0 ± 0.0	15.0 ± 0.0	19.0 ± 0.0	20.0 ± 0.0	20.0 ± 0.0	24.0 ± 0.0	7
	0.0	0.0	0.0	0.0	0.0	0.0	

Growth inhibition were expressed as (0) for no sensitivity, (1- 5 mm) for low sensitivity, (>5-10 mm) for moderate sensitivity and (>10-25 mm) for high sensitivity. R for resistance; (M ± SD) Mean ± Standard deviation.

**Table 3.** Resistogram analysis of cadmium concentrations against bacterial isolates

Microbes used	Cadmium metal concentrations (µg/mL)						pH
	50	100	150	200	250	300	
	Zone of inhibition in mm (M±SD)						
<i>E. luteus</i>	R	R	15.0 ± 0.0	12.0 ± 0.0	R	R	7
<i>S. aureus</i>	R	R	R	R	R	R	7
<i>E. coli</i>	R	R	R	R	R	R	7

Growth inhibition were expressed as (0) for no sensitivity, (1- 5 mm) for low sensitivity, (>5-10 mm) for moderate sensitivity and (>10-5 mm) for high sensitivity. R for resistance; (M ± SD) Mean ± Standard deviation.

(Table 4). *S. aureus* also seemed to be sensitive against most of the antibiotics. It showed resistance against P, AMP, and SXT. Sensitivity was recorded as 20.0 ± 0.0 mm against CIP, 11.0 ± 0.0 mm against VA, 17.0 ± 0.0 mm against E, 22.0 ± 0.0 mm against NOR, 20.0 ± 0.0 mm against CS, 14.0 ± 0.0 mm against S, 13.0 ± 0.0 mm against NA, and 11.0 ± 0.0 mm against C (Table 4). *E. coli* showed resistance against P, TET, AMP, OT and SXT while, sensitivity was observed as 19.0 ± 0.0 mm against CIP, 14.0 ± 0.0 mm against N, 23.0 ± 0.0 mm against E, 23.0 ± 0.0 mm against NOR, 17.0 ± 0.0 mm against CN, 19.0 ± 0.0 mm against S, and 11.0 ± 0.0 mm against NA (Table 4).

**Biosorption of Cd(II) and Cr(IV) at various pH and incubation periods.** Metal absorbance capacity of microbes is expressed in graphical way. The lines in graph show the amount of metal left in medium after microbial activity. Higher lines show much amount of metal left in medium and lower lines show less amount left while, no lines show zero % metal left behind, meaning that all the metals have been absorbed by the microbes. Different colours of lines are shown to express different concentrations of metals like dark blue colour used for 50 µg/mL, red for 100 µg/mL, green for 150 µg/mL, purple for 200 µg/mL, sky blue for 250 µg/mL and for 300 µg/mL orange.

**Cd(II) bioaccumulation by *E. luteus*.** With the decrease in pH up to acidic pH 4, *E. luteus* showed 100% absorbance of Cd(II) on all incubatory periods; 24, 48, 72, and 96 h (Fig. 1). Metal concentration seemed to had no effect on absorbance. At the pH 6, *E. luteus* showed decreased absorbance of Cd(II) after 24 h of

incubation but after 48, 72, and 96 h of incubation, *E. luteus* showed 100% absorbance capacity. Even at each concentration, absorbance capacity remained constant (Fig. 1). At pH 7, Cd(II) absorbance activity was little decreased after 24 h of incubation at 37 °C but with increase in incubatory periods i.e., after 48, 72 and 96 h the absorbance capacity of microbe decreased gradually (Fig. 1). Side by side the increase in metal concentration seemed to have negative impact on absorbance, as the absorbance decreased with increase in metal concentration. At pH 8 and 10, *E. luteus* showed absorbance which increased gradually with increase in incubation period. Maximum absorbance at pH 10 was observed after 96 h of incubation (Fig. 1).

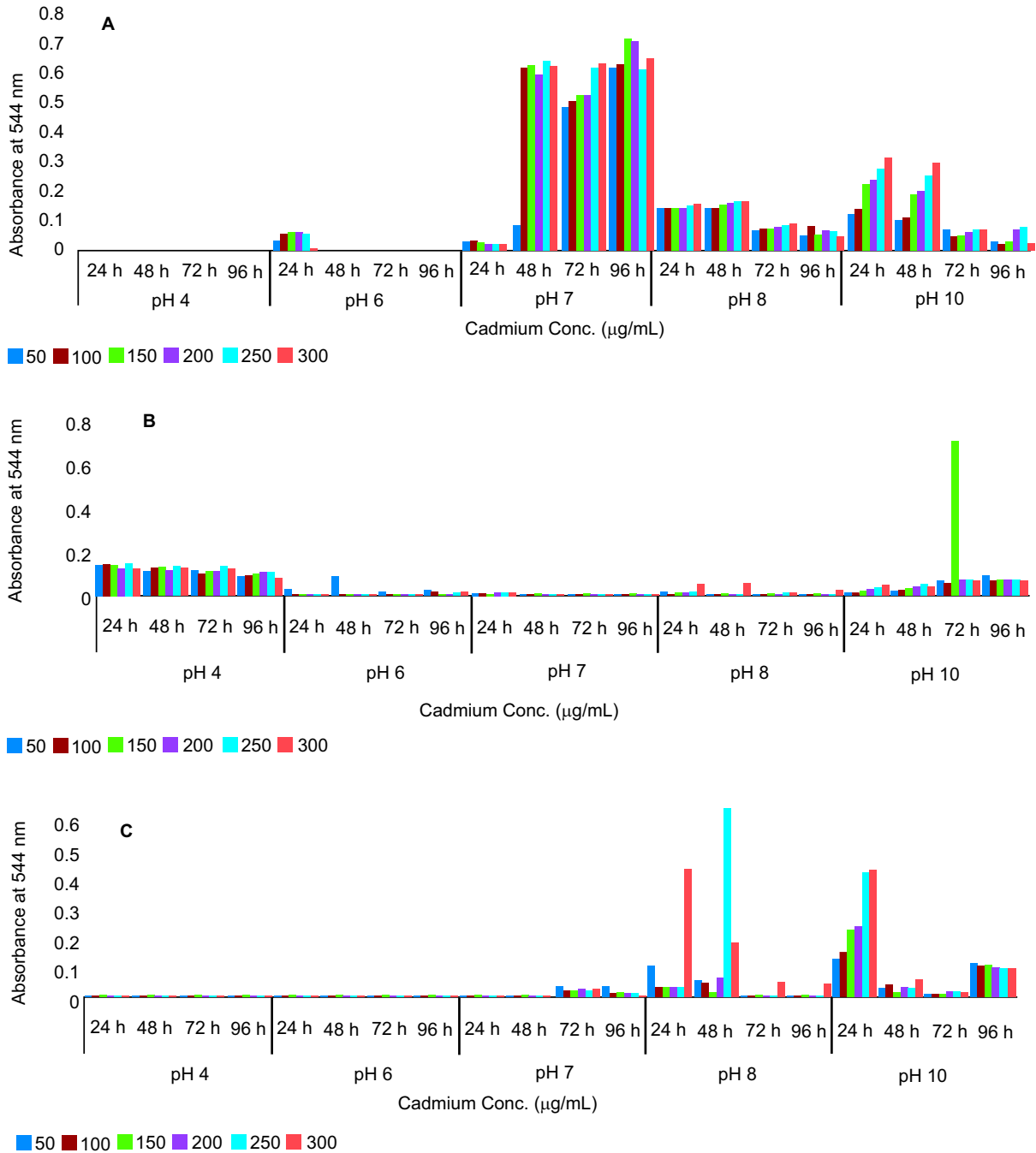
**Cd(II) bioaccumulation by *E. coli*.** At acidic pH 4 and 6, microbe *E. coli* showed very good absorbance activity. The metal was absorbed 100% at each concentration level and incubatory period. Metal concentration even did not affect the absorbance capacity of *E. coli* (Fig. 1). At pH 7 after 24 and 48 h, *E. coli* showed maximum absorbance, and even 100% at 300 µg/mL. While this activity decreased after 72 h and same results were observed after 96 h as well (Fig. 1). At pH 8, *E. coli* showed good absorbance activity after 72 and 96 h of incubation except 300 µg/mL and with increase in incubation this activity also increased. *E. coli* showed absorbance of Cd(II) at pH 10, which increased with increase in incubatory periods (Fig. 1).

**Cd(II) bioaccumulation by *S. aureus*.** *Staphylococcus aureus* showed good and complete absorbance activity after 24 h, at pH 6, 7 and 8. While increase in concentration had no effect on absorbance capacity of

**Table 4.** Sensitivity test of selected standard antibiotics against bacterial strains

bacterial pathogens	Antibiotics															
	CIP	P	TET	N	VA	E	NOR	AMP	CN	S	NA	TOB	K	C	OT	SXT
	Zone of inhibition in mm (Mean±SD)															
<i>E. luteus</i>	28.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	13.0 ± 0.0	17.0 ± 0.0	21.0 ± 0.0	19.0 ± 0.0	5.0 ± 0.0	14.0 ± 0.0	13.0 ± 0.0	17.0 ± 0.0	2.0 ± 0.0	8.0 ± 0.0	18.0 ± 0.0	12.0 ± 0.0	10.0 ± 0.0
<i>S. aureus</i>	20.0 ± 0.0	R	4.0 ± 0.0	10.0 ± 0.0	11.0 ± 0.0	17.0 ± 0.0	22.0 ± 0.0	R	20.0 ± 0.0	14.0 ± 0.0	13.0 ± 0.0	6.0 ± 0.0	10.0 ± 0.0	11.0 ± 0.0	5.0 ± 0.0	R
<i>E. coli</i>	19.0 ± 0.0	R	R	14.0 ± 0.0	6.0 ± 0.0	23.0 ± 0.0	23.0 ± 0.0	R	17.0 ± 0.0	19.0 ± 0.0	11.0 ± 0.0	2.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	R	R

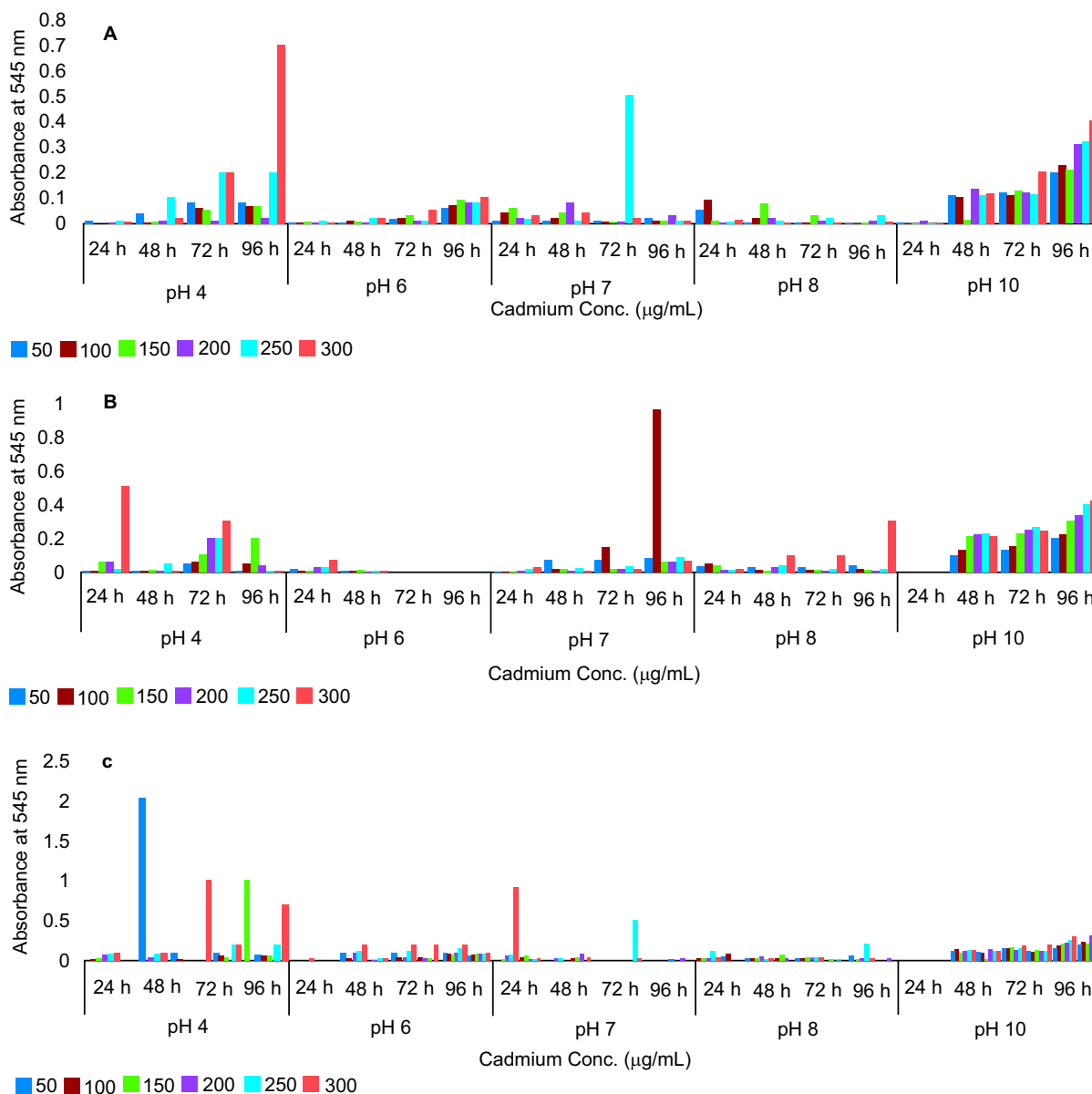
The results of the sensitivity tests were expressed as (0) for no sensitivity, (1- 10 mm) for low sensitivity, (11-19 mm) for moderate sensitivity and (20-35 mm) for high sensitivity. R indicates resistance. CIP (ciproflaxin); P =pencillin G; TE =tetracycline; N =neomycin; VA =vancomycin; E =erythromycin; NOR =norflaxin; AMP =ampicillin; CN getamycin; S =streptomycin; NA =nalidixic acid; TOB =tobramycin, K =kanamycin; C =chloramphenicol; OT =oxytetracyclin, SXT =sulfmethoxyzole.



**Fig. 1.** Bioaccumulation of cadmium concentrations by bacterial isolates at various pH and incubation periods. A) *E. luteus*, B) *S. aureus*, C) *E. coli*.

microbes (Fig. 1). *S. aureus* showed average results for absorbance of Cd(II) at pH 4, but the absorbance capacity increased with increase in incubatory period. Basic pH 10 and acidic pH 4 seemed to be very ineffective for the *S. aureus* to absorb Cd(II) metal (Fig. 1).

**Cr(IV) bioaccumulation by *E. luteus*.** At pH 4 *E. luteus* showed almost 100% absorbance capacity for Cr(IV) for all metal concentrations after 24 h of incubation but the absorbance ability was decreased gradually along with increase in incubation after each



**Fig. 2.** Bioaccumulation of chromium concentrations by bacterial isolates at various pH and incubation periods. A) *E. luteus*, A) *S. aureus*, C) *E. coli*.

24 h and finally the capacity decreased much after 96 h (Fig. 2). At pH 6, *E. luteus* showed gradual decrease in absorbance capacity for Cr(IV) metal concentrations with increase in incubatory period i.e., after each 24 h of incubation. At pH 7, metal concentration in media showed a little decrease after 48 h but after 72 h *E. luteus* showed high absorbance capacity showing decrease in metal concentration but after 96 h capacity again decreased. At basic pH 8, *E. luteus* showed good absorbance capacity after each 24 h of incubation. At

high basic pH 10 microbe showed decrease in absorbance capacity after each 24 h of incubation. After 24 h *E. luteus* absorbed 100% of each metal concentration except 50 and 200  $\mu\text{g/mL}$ .

**Cr(IV) bioaccumulation by *E. coli*.** *Escherichia coli* absorbance activity for Cr(IV) metal concentration showed good activity after 24 h of incubation at pH 4. At acidic pH 6, *E. coli* showed near to 100% absorbance at 24 h incubation but after that with increase in incubation the absorbance capacity of microbe decreased

gradually. At pH 7, *E. coli* showed good absorbance even after 24 h of incubation and this capacity increased with increase in incubatory period. After 96 h of incubation the capacity remained 100% while at others like 48 and 72 h the capacity remained close to 100%. Another effect seen was that the absorbance capacity was decreased with increase in concentration of metal i.e., from 50 to 250  $\mu\text{g/mL}$ . At highest concentration i.e., 300  $\mu\text{g/mL}$  the microbe showed maximum absorbance that remained close to 100% at last two incubatory periods (Fig. 2). At pH 8, microbe showed good absorbance capacity after 24 h of incubation but this capacity was decreased gradually with increase in incubation. At high basic pH 10, *E. coli* showed 100% absorbance after 24 h of incubation. But after further incubation, the capacity of metal absorbance decreased gradually.

**Cr(IV) bioaccumulation by *S. aureus*.** *Staphylococcus aureus* at acidic pH 4 showed best absorbance capacity after 48 h of incubation (Fig. 2). After some incubations metal concentration showed negative impact on absorbance e.g., after 24, 72 and 96 h of incubation. At neutral pH *S. aureus* showed maximum capacity to absorb Cr(IV) metal upto 72 of incubations but decline absorbance was recorded after 96 h. Metal concentration had no clear effect on absorbance. At pH 6, *S. aureus* showed good absorbance activity after 24 h of incubation. *S. aureus* showed near to 100% absorbance capacity for the concentrations from 50 to 200  $\mu\text{g/mL}$  but for 250  $\mu\text{g/mL}$  and 300  $\mu\text{g/mL}$  the capacity was decreased. After further incubation of 48, 72 and 96 h, the capacity of metal absorbance of *S. aureus* decreased gradually (Fig. 2). At basic pH 8 after 24 h incubation the estimated capacity of absorbance was good but maximum absorbance capacity was seen after 48 h incubation. The maximum capacity of absorbance of *S. aureus* at basic pH 10 was seen after 24 h of incubation. After that the capacity decreased with increase in incubation i.e., 48, 72 and 96 h of incubation (Fig. 2). Increase in metal concentration gradually decreased the absorbance of metal.

When metals are dissolved in huge volumes at relatively low concentrations, metal removing technologies such as chemical precipitation, ion exchange, evaporation floatation and filtration become generally ineffective (e.g., less than 100 mg/L) (Patterson, 1985). The research is for efficient and particularly cost-effective remedies (Blöcher *et al.*, 2003; Volesky, 2001). Results of this study demonstrated that the biomass concentration

strongly affected the amount of metal removed from aqueous solution. Moreover, as the biomass concentration rises, the maximum biosorption capacity drops, indicating poorer biomass utilisation. It shows that the sorption of metals is more correctly described by more than one model. In recent research, the same effect was observed in most of the results of metal absorbance, that the increase in metal concentration caused decrease in absorbance of metal, such as Cd(II) absorbance by *E. luteus* at pH 7, 8, 10, by *E. coli* at pH 8, and by *S. aureus* at pH 4, 8, 10 and for Cr(IV), *S. aureus* at pH 4, 8, 10, *E. coli* at pH 4, 6, 10, and *E. luteus* at pH 4, 6, and 10. In general, *E. luteus* seemed to be effective biosorbent at acidic pH 6 for Cr(IV) metal, while at basic pH 10, showed minimum absorbance. *E. coli* also absorbed significant quantity of Cr(IV) metal in acidic pH, while at basic pH, the absorbance capacity decreased, at pH 7, the microbe remained active absorbent. In case of *S. aureus* pH 6, 7 and 8 was favourable to absorb Cr(IV).

From bioaccumulation assay it was revealed that maximum biosorption was recorded after 24 h. It means that exponential phase of microbial growth is very crucial for bioaccumulation of heavy metals. At this phase maximum absorbance could be possible due to less biomass of microbes. Our findings are consistent with Abdel Aty *et al.* (2013), who reported that biosorption of metals was rapid in the first 20 min then was gradually increased till the equilibrium attained at 60 and 90 min for Cd and Pb, respectively and the biosorption became almost constant thereafter. It was observed that when heavy metal concentration was more used, *E. luteus* showed sensitivity whereas resistant at 50  $\mu\text{g/mL}$ . *E. luteus* showed biosorption of Cr(IV) when concentration is increased upto 200 -300  $\mu\text{g/mL}$  at pH 10 but greater absorption was measured at pH 6, 7 and 8. It shows that both dead and live biomass was used for the Cr(IV) biosorption. Similar results were recorded for Cd(II) and Pb(II) onto *A. sphaerica* biomass. It may be carried out chemically *via* involving valence forces through sharing or exchange of electrons between sorbent and sorbate (Smith, 1981). Kefala *et al.* (1999) examined two specific strains of gram-positive *Actinomycetes*, living, non-living bacterial biomass for Cd(II) removal. They revealed that non-living biomass exhibited higher metal uptake. Our results are consistent with them that *S. aureus* and *E. coli* showed maximum absorption of Cd(II) as dead biomass. It is concluded that bacteria have the ability to absorb the metals within



the cell body or on their cell wall and cell membrane as well.

Various types of biomasses such as bacteria, fungi, mushrooms, plants as well as chemically modified biosorbents were used for the removal of heavy metals (Kumar, 2014; Ghaima *et al.*, 2013; Singh *et al.*, 2012; Volesky, 1986). The attraction or affiliation of removal is dependent upon the chemical composition or structure of the organisms. The results show that for Cd(II) and Cr(IV) removal both dead and live biomass could be used. Chemical composition play vital role in heavy metal affiliation for removal from waste materials e.g., the overexpression of metallothioneins result in enhanced metal accumulation which provide an excellent strategy for the development of microbial based biosorbents for the remediation of metal contamination (Pazirandeh *et al.*, 1995). Similarly, the expression of proteins on the surface of bacterial cell provides an inexpensive and affinity adsorbents.

### Conclusion

It was concluded that optimisation of exponential phase of microbial growth, pH of media, incubation period, attraction between sorbent and sorbate and biomass (dead or alive) are important for biosorption process. More research is needed to clarify the impact of heavy metals on cellular structure and which part or organelle is efficient absorber for heavy metals.

### Acknowledgement

Authors are thankful to Department of Zoology, University of Azad Jammu and Kashmir, Pakistan for financial support to carry out this research work.

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