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Effects of Some Heavy Metals on Growth, Protein Content and Pigment Production by *Streptomyces coelicolor* SM1

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Heavy metals consequently tend to accumulate in nature and in food chains causing many environmental and health problems. Heavy metals biosorption by bacteria grown in polluted environments is proved. In this study, the effects of heavy metals on bacterial growth, dry cell weights, pigment production, nitrogen content and protein synthesis were investigated. Two isolates belong to genus *Streptomyces, Streptpmyces coelicolor* SM1 and *S. anulatus* SM21 were grown in presence of different concentration of heavy metals. *Streptpmyces coelicolor* SM1 was more resistant to Cd^{++}, Cr^{+++}, Co^{++} and Cu^{++} compared to *S. anulatus* SM21, thus it was selected for more detail studies. The minimal inhibitory concentration (MIC) of each element was calculated and lower concentrations of the calculated MIC of the metals which partially limited bacterial growth was used to determine their effect for 7 days on *S. colicolor* SM1 growth, pigment production, nitrogen and protein contents and % of heavy metal removal which varied according to the nature of the metal used and time. At concentrations below the MIC, Cadmium, Cobalt and Cupper inhibited pigment production by the selected *Streptomyces* compared to control. Growth, N content and protein generally increased by time up to 7 days while they decreased significantly by the presence of the tested heavy metals. All tested metals decreased protein synthesis. It was found that removal of heavy metal increased by time. After 7 days, Cadmium (38%) and Chromium (39%) were the most adsorbed elements by *S. coelicolor* SM1 followed by Cobalt (29%) and Copper (25%). In conclusion, *Streptpmyces coelicolor* SM1 can be used significantly to remove Cadmium, Chromium, Cobalt and Copper from heavy metal contaminated areas.

Key words: Protein, growth, pigment, inhibition, *Streptomyces*, heavy metals, bio-sorption,

Heavy metals removal by microorganisms is of increasing interest and generally there is an interaction between bacteria and the present metals where bacteria have a role in conversion and transformation of metal ions in different polluted environments (Chang *et al.,* 1993). Moreover, the metal-resistant bacteria from polluted environments are used as indicators of toxicity to different forms of life (Doelman *et al.*, 1994, Hiroki, 1994). Heavy metal resistant mechanisms of baccteria may due to detoxifying of the polluted environments (Rohit and Sheela, 1994), the genetic transfer of bacteria (De Rore *et al.,* 1994; Goblenz *et al.,* 1994, Guzzo *et al.,* 1994), prevent these metals mobility and bioavailability to the edible plants (Holm *et al.,* 1995). Many possibilities are found to use these bacteria for metal removal through complex formation of metal ions and different organic acids including citric acid. Biodegradation of the complex of citric acid and metal is depending on the used microbes and nature of the metal (Brynhildsen and Rosswall, 1989). Gram-negative bacterium, *Pseudomonas aeruginosa* and Gram-positive *Bacillus thuringiensis* are resistant to many heavy metals due to intra- and extra-cellular substance productions (Lima e Silva *et al*., 1998)

In soil or waste water environments, Cu^{++}, Cr^{+++}, Cd^{++}, and Co^{++} were found and these

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metals affect bacterial biomass, growth, cell dry weight, pigment production, ammonium assimilation and protein synthesis. The objectives of this study were to determine the effect of some heavy metals on growth and metabolism of a bacterial isolate belonging to filamentous bacteria.

MATERIAL AND METHODS

The used bacterial isolates

Streptpmyces coelicolor SM1 and *S. anulatus* SM21 were isolated from shrimp shells on chitin agar medium; containing chitin as sole carbon and nitrogen sources. They were identified according to physiological, morphological, and biochemical characteristics, in addition to 16S rDNA analysis (Aly *et al*., 2011b). They were kindly obtained from Prof. M M Aly, Biology Department, Faculty of Science, KAU.

Bacterial growth

Bacteria were cultured in starch nitrate broth at a temperature of 30°C and 120 rpm for 7days and in 250 ml flasks containing 50 ml of Starch Nitrate broth (SNB) medium composed of (g/l): Soluble starch, 10; K_2HPO_4, 2; KNO_3, 2; $MgSO_4 \cdot 7H_2O$, 0.5; Ca $(CO_3)_2$, 2 and one ml of trace element as described by Shirling and Gottlieb (1966). Each flask was inoculated by 2 ml of the preculture ($4x10^6$ cfu/ml) which prepared in SNB medium, for 4 days at 30°C and 120 rpm. At the end of the growth period, culture filtrate was centrifuged for 10 min at 10 000 rpm and cell pellet was used for cell dry weight (µg/ml), nitrogen (µg/ 100 ml) and protein (µg/100 ml) determination.

The two bacterial isolates were grown in different concentrations of the tested heavy metals (Cd: 0.5-1.5 mM, Cr : 0.5-1.5 mM, Co: 0.1-0.5 mM and Cu: 0.5-1.5 mM). The MIC for each metal was determined as the lowest heavy metal concentration that permit the lowest bacterial growth (the lowest optical densities at 550 nm, $OD_{550\,nm}$) or that cause e" 95% count reduction in cfu/ml (Vela-Cano *et al*., 2014).

Streptpmyces coelicolor SM1 was grown in SNB medium containing lower concentration of the detected MICs of the different studied heavy metals (1mM for Cd, Cr and Cu but 0.3 mM for Co) and growth, protein, nitrogen content and % of heavy metal removal were determined after 1, 3, 5 and 7 days as described below. Control flasks without any heavy metal addition was used as control.

The used heavy metals

Heavy metal stock solutions were prepared by dissolving $Cd(NO_3)_2.4H_2O$, $CoSO_4.7H_2O$. $CrSO_4.8H_2O$ and $CuSO_4.5H_2O$ in distilled water and all the prepared solutions were filter sterilized using 0.45µm blue filter to prevent bacterial growth and all solutions were persevered until used for maximum one month at 4°C.

Protein contents

To solublize cellular protein, the collected cells were washed, dried and incubated in 5 ml of 1N NaOH for 10 min at 90°C (Jackson *et al*., 1989). Using the method of Lowry *et al*. (1951), protein content (µg/100 ml) was determined using bovine serum albumin as protein standard. All experiments were replicated three times and mean value ± Sd was calculated and compared.

Pigment production

Pigment production was determined using naked eye as +++ high pigment production, ++ moderate production, + low pigment production and – no pigment production.

Measurement for dry cell weight (µg/ml)

After bacterial growth, the cells were collected after centrifugation for 10 min at 10000 rpm and the cell pellets were washed and oven dried at 60ºC for 24 hours or until constant weight (Aly *et al*., 2011a). The experiment repeated three times and the calculated dry cell weights were expressed as µg/ml.

Nitrogen content of the cells (µg/100 ml)

Nitrogen content of the cells was measured using Kjeldahl method (Hawk *et al*., 1947). The dried cells were digested using concentrated H_2SO_4 in the presence of 1 g of a mixture of $CuSO_4.H_2O$ and K_2SO_4 as a catalyst. Distillation was carried out using NaOH and the evolved NH_3 is absorbed in standard HCl. Using Methyl Red as an indicator, the excess HCl was titrated against standard NaOH (AOAC**,** 1990)

Metal content

Heavy metal contents (Cd^{++}, Co^{++}, Cr^{+++} and Cu^{++}) of the dried and washed bacterial cell sample was determined after digestion using a mixture of $HNO_3/H_2SO_4/HClO_4$ (2/2/0.5, v/v/v), followed by atomic absorption spectrometry (Perkin Elmer 2380) as described in El-Sawi *et al*. (1994).

Statistical analysis

The obtained data were subjected to Statistical analysis using SPSS program version 16 and the data were represented by means +SD. All the data were compared to control and the difference considered significant at p d" 0.05.

RESULTS AND DISCUSSION

Actually, the existing heavy metals in polluted environments are of great interest and couse serious problems. The undesirable elements, Cadmium, Chromium, Cobalt and Copper strongly inhibited the bacterial growth of *Streptomyces coelicolor* SM1 and *S. anulatus* SM21 (Table 1 and 2). *Streptpmyces coelicolor* SM1 was more resistant to Cd^{++}, Cr^{+++}, Co^{++} and Cu^{++} compared to *S. anulatus* SM21 (Table 1 and 2) and the inhibition depends on the nature of the metal used and its concentration, and the bacterial isolate examined. Similarly, Lima e Silva *et al.* (2012) recorded that cell resistant to heavy metals was affected by type of cell, time of exposure and metal type and concentration. Resistant of bacteria to heavy metals may due to biofilms formation (Xu *et al*., 1998), secretion of detoxifying enzymes or adsorption of metals by living or dead cells (Hassett *et al.,* 1999). In control medium, growth, pigment production, N content and protein generally increased by time up to 7 days (Table 3) while they were decreased significantly by the presence of the tested heavy metal**.** The minimal inhibitory concentration (MIC) of each element was calculated as described by Hassen *et al*., (1998). Lower concentrations of the calculated MIC of each metal was used to determine their effect for 7 days on *S. colicolor* SM1 growth, pigment production, nitrogen content, proteins and heavy metal removal compared to control (Table 4, 5, 6 and 7). At concentrations below the MIC, Cadmium, Cobalt and Cupper inhibited pigment production by the selected *Streptomyces* compared to control while the effect of Chromium ions on pigment was not detected. Pigment production by *Pseudomonas aeruginosa* was affected by the presence of heavy metals (Lima e Silva *et al*., 2012).

Heavy metals decreased the organic matter formation and accumulation (Vela-Cano *et al*., 2014) in bacteria which played an important role in waste water and soil cleaning from heavy metals due to bacterial trapping and sequestration. Kathiravan *et al*., (2010) found that growth, morphology and metabolism of microorganisms present in soil and biological waste water treatment were affected by metals and increasing metal concentration increased lag time and decreased or inhibited growth rate. Decreasing bacterial growth may be due to inhibition of biodegradation processes of organic compound (Sandrin and Maier, 2003). In our study, all the tested metals decreased cell nitrogen and protein content. As well known, bacteria have the ability to produce acids, gases, and intermediate products, toxins, agglutinins, and many other protein complexes during metabolisms and all are affected by the presence of heavy metals (Lima e Silva *et al*., 2012, Mustapha and Halimoon, 2015). Metal ions may act as cofactor for many enzymes and are essential for normal bacterial cells while their excess undergo a Fenton reaction which produced reactive oxygen species (ROS) and Thiols, leading to damage of the living cells (Nies,. 1999, Lemire *et al*., 2013) or cell toxicity (Workentine *et al*., 2008). The harmful effect of heavy metal may due to inhibition of DNA transcription or release intracellular Fe ions into the cytoplasm leading to ROS formation (Xu *et al*., 2013). In addition, Cr ions inhibit uptake of sulphate which is essential element (Nies and Silver,1995, Holland *et al*., 2010) while lipid peroxidation was noticed by copper and cadmium uptake (Hong *et al*., 2012) Copper ions cause cell lysis due to destruction of extracellular DNA (Dibrov *et al*., 2002; Warnes *et al*., 2012) and cations can be pumped out of the cell (Harrison *et al*., 2004). It was found that removal of heavy metal increased by time. After 7 days, Cadmium (38%) and chromium (39%) were the most adsorbed elements by *Streptpmyces coelicolor* SM1 followed by Cobalt (29%) and Copper (25%). *Pseudomonas earuginosa* removed Cr ions up to 35-55% and lower removal activity was recorded by *Bacillus thuringiesis* (Hassen *et al*., 1998). Ozturk (2016) reported that *S. coelicolor* A3(2) can tolerate higher concentrations of heavy metals due to the property of vancomycin which act as a zinc chelator and *Streptomyces* isolates were heavy metal resistant and had heavy metal binding capacity (Schmidt *et al*., 2009, Alvarez *et al*., 2013, Daboor *et al*., 2014). In conclusion, *S. coelicolor* SM1can be used significantly to remove

Table 1. Effect of different concentrations of Cd^{++} and Cr^{+++} on bacterial growth (dry weight, µg/ml) after growing in starch nitrate broth medium for 7 days at 30°C and 120 rpm

Tested isolate	Control	Cd^{++} (mM)				Cr^{+++} (mM)			
		0.5	1.00	1.25	1.5	0.5	1.00	1.25	1.5
S. coelicolor SM1	44.0±5.1	53.9±0.1	20.54±4.1*	17.1±0.0 *	11.0±0.0*	35.3±0.3	21.2±1.1 *	9.16±3.25*	8.14±2.03*
S. anulatus SM21	41.1±0.2	31.0±0.1	14.4±1.14*	11.0± 2.0 *	9. 4±0.0 *	41.1±0.5	12.0±0.1 *	6.22±1.28 *	4.2±1.11*

*: Significant result compared to control at p≤0.05, S: Streptomyces

Table 2. Effect of different concentrations of Co^{++} and Cu^{++} on bacterial growth after growth (dry weight, µg/ml) after growing in starch nitrate broth medium for 7 days at 30°C and 120 rpm

Tested isolate	Control	Co^{++} (mM)				Cu^{++} (mM)			
		0.1	0.3	0.4	0.5	0.5	1.00	1.25	1.5
S. coelicolor SM1	44.0±5.1	51.0±3.11	29±0.11*	20.0±3.3*	16.0±5.1*	50.0±8.22	31.0±9.1*	15.2±1.1*	9. 4±4.1*
S. anulatus SM21	41.9 ± 3.2	31.0±1.1*	25.8±0.4*	20.8±1.2*	14.3±2.9*	33.0±0.52	21.0±0.1*	12.4±5.2*	4. 2±0.9*

*: significant results compared to control at p ≤ 0.05, *S: Streptomyces*

Table 3. Growth, pigment and protein production of *Streptomyces coelicolor* SM1 under normal condition (Control) for 7 days at 30°C, pH7 and 120 rpm

Tested character	Control Time (days)				
	0	1	3	5	7
Pigment production	-	+	+	++	++
Dry weight (µg/ml)	0.008±0.003	13.3±0.1	23.7±0.07	49.9±4.22	44.2±0.33
N (µg/100 ml)	1.33±0.33	3.7±1.2	4.5±0.73	5.1±1.20	6.1±1.23
Protein (µg/100 ml)	7.00±4.23	23.0±6.24	33.0±3.25	39.0±4.43	44.2±2.83

- : No pigment, +: Low pigment production, +++: High pigment production

Table 4. Growth, pigment and protein production and heavy metal removal of *Streptomyces coelicolor* SM1 under the presence of 1 mM of Cadmium for 7 days at 30°C, pH7 and 120 rpm in starch nitrate broth

Tested character	Cd^{++} (1 mM)				
	0	1	3	5	7
Pigment production	-	+	+	+	+
Dry weight (µg/ml)	0.008±0.003	11.0±1.0	22.3±2.43	32.7±3.5	35.77±5.5
N (µg/100 ml)	1.33±0.23	1.17±0.2	1.52±0.33	2.11±0.6	3.11±0.4
Protein (µg/100 ml)	7.0±4.23	13.2±1.2	13.5±1.22	19.5±1.33	24±3.03
Metal adsorbed (µg/ mg of DW)	ND	1.4±0.1	3.5±0.11	7.4±0.0	9.98±0.01
% Percentage of removal	ND	1.1±1.2	12.4±0.14	21. 4±0.0	38.04±0.00

- : No pigment, +: Low pigment production, ND: Not detected

Table 5. Growth, pigment and protein production and heavy metal removal of *Streptomyces coelicolor* SM1 under the presence of 1 mM of Cromium for 7 days at 30?C, pH7 and 120 rpm in starch nitrate broth

Tested character	Cr^{+++} (1.0 mM)				
	0	1	3	5	7
Pigment production	-	+	+	++	++
Dry weight (µg/ml)	0.008±0.003	23.13±0.13	35.28±0.04	39.49±0.12	38.0±0.13
N (µg/100 ml)	1.33±0.23	1.77±0.4	2.55±0.22	3.11±0.11	5.11±1.67
Protein (µg/100 ml)	7.0±4.23	13.6±1.24	13.3±3.25	19.4±4.43	34.5±2.83
Metal adsorbed (µg/ mg of DW)	ND	0.14±0.24	0.4±0.13	0.54±0.03	0.68±0.21
% Percentage of removal	ND	6.11±0.03	26.44±0.03	28.04±0.0	39.04±0.00

- : No pigment, +: Low pigment production, ++: moderate pigment production, ND: Not detected

Table 6. Growth, pigment and protein production and heavy metal removal of *Streptomyces coelicolo*r SM1 under the presence of 0.3 mM of Cobalt for 7 days at 30°C, pH7 and 120 rpm in starch nitrate broth

Tested character	Co^{++} (0.3 mM)				
	0	1	3	5	7
Pigment production	-	+	+	+	+
Dry weight (mg/l)	0.008±0.003	22.03±0.12	37.22±0.04	39.39±0.03	39.50±0.111
N (µg/100 ml)	1.33±0.23	1.55±0.13	1.35±0.21	1.11±0.53	1.21±0.44
Protein (µg/100 ml)	7.0±4.23	4.9±1.24	6.23±3.25	9.24±4.43	14.5±2.83
Metal adsorbed (µg/ mg of)	ND	0.130±0.24	0.114±0.014	0.204±0.03	0.49±0.021
% Percentage of removal	ND	1.11±0.43	4.44±0.223	8.04±0.6	29.64±0.60

- : No pigment, +: Low pigment production, ND: Not detected

Table 7. Growth, pigment and protein production and heavy metal removal of *Streptomyces coelicolor SM1* under the presence of 1 mM of Copper for 7 days at 30°C, pH7 and 120 rpm in starch nitrate broth

Tested character	Cu^{++} (1.0 mM)				
	0	1	3	5	7
Pigment production	-	-	-	-	+
Dry weight (mg/l)	0.008±0.003	12.1±4.10	17.18±2.04	24.4 ±3.22	29.0±2.12
N (µg/100 ml)	1.33±0.23	1.07±0.13	1.15±0.29	1.14±0.49	1.31±0.39
Protein (µg/100 ml)	7.0±4.23	5.6±1.77	11.3±1.2	10.9±1.4	14.5±2.83
Metal adsorbed (µg/ mg of DW)	ND	0.04±0.24	0.14±0.13	0.22±0.03	0.42±0.21
% Percentage of Removal	ND	1.11±0.09	8.00±1.13	12.04±1.10	25.04±0.20

-: No pigment, +: Low pigment production, ND: Not detected

Cadmium, Chromium, Cobalt and Copper from contaminated areas.

REFERENCES

1. Alvarez A., Catalano S. A. and Amoroso M. J., Heavy metal resistant strains are widespread along Streptomyces phylogeny. *Mol Phylogenet Evol* 2013; **66**: 1083-1088.
2. Aly M. M., Tork S., Al-Garni S. M. and Kabli S. A., Chitinolytic enzyme production and genetic improvement of a new isolate belonging to *Streptomyces anulatus*. *Annal. Microbiol.* 2011b; **61**(3): 453-461.
3. Aly M.M., Kabely S. and Garny S., Production of biodegradable plastic by filamentous bacteria isolated from Saudi Arabia. *Journal of Food, Agriculture & Environment,* 2011a; **9**(1): 751-756.
4. AOAC. Association of Official Analytical Chemists. Official Methods of Analysis (Volume 1),15th Edition. http://creativecommons.org/publicdomain/zero/1.0/,1990
5. Brynhildsen L. and Rosswall T., Effects of cadmium, copper, magnesium and zinc on the decomposition of citrate by *Klebsiella* sp. *Appl. Environ. Microbiol.,* 1989; **55**: 1375-1379
6. Chang, J.S., Hong, J., Ogunseitan, O.A. and Olson, H.B., Interaction of mercuric ions with the bacterial growth medium and its effects in enzymatic reduction of mercury. *Biotechnol Prog* 1993; **9**: 526-532.
7. Daboor SM, Haroon AM, Esmael NAE. and Hanona SI., Heavy metal adsorption of *Streptomyces chromofuscus*. *Journal of Coastal Life Medicine* 2014; **2**: 431-437.
8. De Rore H., Top E., Houwen F. Mergeay M. and Verstraete W., Evolution of heavy metal resistant transconjugants in a soil environment with a concomitant selective pressure. *FEMS Microbial. Ecol*.,1994; **14**: 263-273.
9. Dibrov P., Dzioba J., Gosink K. K. and Hase C. C., Chemiosmotic mechanism of antimicrobial activity of Ag^{+} in *Vibrio cholerae*. *Antimicrob. Agents Chemother*., 2002; **46**:2668-2670.
10. Doelman P., Jansen E., Michels M. and van Til M., Effects of heavy metals in soil on microbial diversity and activity as shown by the sensitivity-resistance index, an ecologically relevant parameter. *Bioi. Fertil. Soils,* 1994; **17**: 177-184
11. El-Sawi N.M., El-Maghraby O.M.O., Mohran H.S. and Abo-Gharbia M.A., Abnormal contamination of cottage cheese in Egypt. *J Appl. Animal. Res*., 1994; **6**: 81-90.
12. Goblenz A., Wolf K. and Bauda P., The role of glutathione biosynthesis in heavy metal resistance in the fission yeast *Schizosaccharmyces pombe*. Metals and microorganisms: relationships and applications. *FEMS Microbial. Revi.,* 1994; **14**: 303-308.
13. Guzzo A., Du Bow M. and Bauda P., Identification and characterization of genetically programmed responses to toxic metal exposure in *Escherichia coli.* Metals and microorganisms: relationships and applications. *FEMS Microbial. Revi.,* 1994; **14**: 369-374.
14. Harrison JJ1, Ceri H, Stremick CA, Turner RJ., Biofilm susceptibility to metal toxicity. *Environ Microbiol.;* 2004;**6**(12): 1220-7.
15. Hassen A, Saidi N, Cherifh M and Boudabous A, Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus Thuringiensis*, *Bioresource Technology* 1998; **65**: 73-82.
16. Hassett D. J., Ma J. F., Elkins J. G., McDermott T. R., Ochsner U. A., West S. E., Huang C. T., Fredericks J., Burnett S., Stewart P. S., McFeters G., Passador L. and Iglewski B. H., Quorum sensing in *Pseudomonas aeruginosa* controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to

hydrogen peroxide. *Mol. Microbiol*., 1999; **34**:1082-1093.

17. Hawk P, Oser B. and Summerson W., Practical Physiological Chemistry. 12th ed. Toronto, Canada: Blakiston, 1947.
18. Hiroki M., Populations of Cd-tolerant microorganisms in soil polluted with heavy metals. *Soil Sci. Plant. Nutr*., 1994; **40**: 515-24.
19. Holland S. L., Ghosh E. and Avery S. V., Chromate-induced sulfur starvation and mRNA mistranslation in yeast are linked in a common mechanism of Cr toxicity. *Toxicol. in. Vitro.* 2010; **24**: 1764-1767.
20. Holm P. E., Christensen T. H., Tjell J. C. and McGrath S. P., Heavy metals in the environment. Speciation of cadmium and zinc with application to soil solutions. *J. Environ. Qual*., 1995; **24**: 183-190.
21. Hong R., Kang T. Y., Michels C. A. and Gadura N., Membrane lipid peroxidation in copper alloy-mediated contact killing of *Escherichia coli*. *Appl. Environ. Microbiol*. 2012;**78**: 1776-1784.
22. Jackson M. A., Slininger P. J. and Bothast R. J., Effect of zinc, iron, cobalt and manganese on *Fusarium monilifotrne* NRRL 13616 growth and fusarin C biosynthesis in submerged cultures. *Appl. Environ. Microbial*., 1989; **55**: 649-655.
23. Kathiravan M. N., Karthiga Rani, R., Karthick, R., and Muthukumar, K., Mass transfer studies on the reduction of Cr(VI) using calcium alginate immobilized *Bacillus* sp. In packed bed reactor. *Bioresource Technology,* 2010; **101**(3): 853-858
24. Lemire J. A., Harrison J. J. and Turner R. J., Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nat Rev Micro.,* 2013; **11**: 371-384.
25. Lima de Silva AA , de Carvalho MA, de Souza SA, Dias PM, da Silva Filho RG, de Meirelles Saramago CS, de Melo Bento CA, Hofer E., Heavy metal tolerance (Cr, Ag AND Hg) in bacteria isolated from sewage. *Braz J Microbiol.,* 2012; **43**(4):1620-31.
26. Lima e Silva A A, Carvalho M A., Souza S A, Dias P M. , Silva F R , Saramago C S. , Bento C A. and Hofer E., Heavy metal tolerance (Cr, Ag and Hg) in bacteria isolated from sewage. *Brazilian Journal of Microbiology,* 2012; **9**: 1620-1631
27. Lima e Silva, S. M. M., Duarte, M. A. V. & Guimarães, G., A correlation function for thermal properties estimation applied to a large thickness sample with a single surface sensor, *Review of Scientific Instruments,* 1998; **69**(9): 3290-3297.
28. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem*., 1951; **193**: 265-275.
29. Mustapha MU, Halimoon N., Microorganisms and Biosorption of Heavy Metals in the Environment: A Review Paper. *J Microb Biochem Technol* 2015; **7**:253-256.
30. Nies D. H.., Microbial heavy-metal resistance. *Appl. Microbiol. Biotechnol*., 1999; **51**:730-50.
31. Nies D. H. and S. Silver., Ion efflux systems involved in bacterial metal resistances. *J. Ind. Microbiol.*, 1995; **14**:186-199.
32. Ozturk A., The use of *Streptomyces coelicolor* in the removal of heavy metals. *Adv Tech Biol Med* 2016; **4**: 168-172.
33. Rohit, M. and Sheela, S., Uptake of zinc in *Pseudomonas* sp. strain UDG26. *Appl. Environ. Microbial.*, 1994; **60**: 2367-2370.
34. Sandrin, T. R., and Maier, R. M., Impact of metals on the biodegradation of organic pollutants. *Environmental Health Perspectives*, 2003; **111**(8): 1093-1101.
35. Schmidt A, Haferburg G, Schmidt A, Lischke U, Metren D., Heavy metal resistance to the extreme: *Streptomyces* strains from a former uranium mining area. *Chemie der Erde*, 2009; **69**: 35-44.
36. Shirling E.B and Gottlieb D., Methods for characterization of *Streptomyces* species *Int J. Syst. Bacteriol.*, 1966; **16**: 313-340
37. Vela-Cano, M., Castellano-Hinojosa, A., Vivas, A.F. and Toledo, M.V.M., Effect of heavy metals on the growth of bacteria isolated from sewage sludge compost tea. *Advances in Microbiology,* 2014; **4**: 644-655.
38. Warnes S. L., Highmore C. J. and C. W. Keevil., Horizontal transfer of antibiotic resistance genes on abiotic touch surfaces: Implications for Public Health. *mBio*., 2012; **3**(6):e00489-12
39. Workentine M. L., Harrison J. J., Stenroos P. U., Ceri H. and Turner R. J., *Pseudomonas fluorescens'* view of the periodic table. *Environmental Microbiology*, 2008; **10** (1): 238-250.
40. Xu H., Qu F., Xu H., Lai W., Andrew Wang Y., Aguilar Z. P. and Wei H., Role of reactive oxygen species in the antibacterial mechanism of silver nanoparticles on *Escherichia coli* O157:H7. *Biometals*., 2013; **25**:45-53.
41. Xu K. D., Stewart P. S., Xia F., Huang C. T., and McFeters G. A., Spatial physiological heterogeneity in *Pseudomonas aeruginosa* biofilm is determined by oxygen availability. *Appl. Environ. Microbiol*., 1998; **64**:4035-4039.