Asian Journal of Biotechnology and Bioresource Technology

3(4): 1-8, 2018; Article no.AJB2T.41884
ISSN: 2457-0125

Studies on Removal of Heavy Metals from Tannery Effluent by Native Isolates of Bacteria and Fungi

M. D. Makut[1], A. Gimba[1*] and A. Bello[1]

[1]Department of Microbiology, Nasarawa State University, Keffi, Nasarawa State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author MDM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AG and AB managed the analyses of the study. Author AG managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJB2T/2018/41884
Editor(s):
(1) Marilia Danyelle Nunes Rodrigues, Professor, Animal Genetics, Rural Federal University of Amazonia, Brazil.
Reviewers:
(1) Abdul Hameed, Govt. College of Science, Pakistan.
(2) Mohd Kashif Khan, India.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/24845

Short Research Article

Received 11th March 2018
Accepted 22nd May 2018
Published 28th May 2018

ABSTRACT

Aim: This research work is aimed at studies on removal of heavy metals from tannery effluent by species of bacteria and fungi.
Study Design: This study is designed to isolate and identify bacteria and fungi from tannery effluents, to determine the concentration of heavy metal in tannery effluent, to assess the removal of heavy metals by bacteria and fungi from tannery effluents, to assess the effect of temperature and pH on heavy metal removal.
Place and Duration of Study: Department of Microbiology, Nasarawa State University Keffi, Nasarawa State, Nigeria, between January 2017 and December 2017.
Methodology: A total of four (4) tannery effluents samples were obtained from Challawa industrial area in Kano, Nigeria. Bacteria and fungi were isolated from the effluent and identified using standard microbiological methods. The heavy metal content of the effluent was determined using Atomic Absorption Spectrometer. The heavy metal removal capacity by bacteria and fungi was also determined at different temperatures and pH.
Results: The bacteria isolated from the effluent were *Bacillus subtilis* and *Pseudomonas*

**Corresponding author: E-mail: amygimba@gmail.com;*

aeruginosa and the fungal isolates were *Penicillium notatum* and *Aspergillus niger*. The identified heavy metals were Chromium, Cadmium, Copper, Lead, Zinc and Iron. The bacterial and fungal load of the tannery effluent were within the range of; bacteria ($2.9\text{-}4.3\times10^5$ Cfu/L) and Fungi (5.0×10^4- 1.0×10^5. Cfu/L). Out of the samples of tannery effluent obtained, the occurrence of *Pseudomonas aeruginosa*, *Bacillus subtilis, Penicillium notatum* and *Aspergillus niger* were 100%. The result of the study revealed that the values for the metals were above the maximum permissible limits of both the Nigeria Standard of Drinking Water (NSDW) and World Health Organization (WHO). All isolated organisms showed an affinity for Pb, Cu, Cr and Zn.

Conclusion: From this study, it can be deduced that bacteria and fungi have a high capacity for removal of metal from tannery effluents. However, there is a need for further work to be done to validate and improve these findings.

Keywords: Bioremediation; heavy-metals; tannery effluent; Bacillus subtilis; Pseudomonas aeruginosa; Penicillium notatum; Aspergillus niger.

1. INTRODUCTION

Growth in technology and industrialization has put the burden on the environment as a result of large volumes of hazardous wastes. The buildup of heavy metals in soils and water continues to create serious universal health concerns [1]. Heavy metal contamination in the environment has increased beyond recommended limits and these metals are not easily degraded into non-toxic forms and as such poses a threat to all life forms [2]. Thus it is imperative to reduce or completely remove heavy metal contamination in order to avoid or reduce dangers of uptake into the food web. Industrial wastewaters containing aluminium, nickel, lead, copper and zinc ions are common since these metals are used in a great number of industries and industrial processes. Until recently, these heavy metals were found in the environment only in small quantities, but with industrialization and manufacturing came high technological advancement [3]. Metals are known principally and almost exclusively for their possible toxicity in the body at a certain amount though commercially they might have great benefits when used. Conservative practice for removing metals from industrial effluents comprises of chemical precipitation, coagulation, solvent extraction, electrolysis, membrane separation, ion – exchange and adsorption. Most of the approaches are expensive and have great renewal cost of the materials [4]. Therefore, there is a need for a different, advanced and cost-effective technique for the removal of toxic substances from wastewaters. Bio-sorption is a dynamic and convenient method and can be easily applied with little cost to remove heavy metals from the huge amount of industrial wastewaters. Recently, the microbial biomass of fungi, bacteria and algae have been effectively used as adsorbing agents for the elimination of heavy metals [3,4,5,6,7]. Microbial populations in metal contaminated environments adjust to toxic concentrations of heavy metals and become metal resistant [8,9].

2. MATERIALS AND METHODS

2.1 Sample and Sample Collection

Tannery effluent was aseptically (avoiding contamination) collected in 4 sterile containers of 2 litres each around 11:19 am from Challawa Industrial area, Kano and placed in an ice box and was transported to the microbiology laboratory for analysis.

2.2 Qualitative Analysis of Tannery Effluent

Growth on differential and selective media was used for characterization and identification of organisms while various morphological and biochemical characterization was carried out for a pure culture of identified isolates.

2.3 Isolation of Bacteria from Tannery Effluent

The standard spread plate method as described by [10] was used for the isolation of bacteria species. Serial dilutions of the sample was prepared by taking 1 ml of the effluent and adding it into a test-tube containing 9 ml of distilled water, [11] and serially diluted up to 10^{-5}; freshly prepared media (nutrient agar) was allowed to cool to 40°C and then aseptically poured into the Petri dishes to solidify then 1ml from the 10^{-5} was then pipetted into appropriately labeled Petri dishes. The Petri dishes were incubated at 37°C for 24 hours after each colony

that appeared on the plate were counted using colony forming unit (CFU/g) [12]. The colonies were sub-cultured repeatedly on nutrient agar to get a pure culture of isolates and stored in agar slant containing nutrient agar. All experiments were conducted in triplicate [13].

2.4 Isolation of Fungi from Tannery Effluent

The standard spread plate method as described by [10] was used for the isolation of the fungal species. Serial dilutions of the sample was prepared by taking 1 ml of the effluent sample and adding it into a test-tube containing 9ml of distilled water, and serially diluted up to 10^{-5}, freshly prepared media (Potato Dextrose Agar with chloramphenicol) was allowed to cool to 40℃ and then aseptically poured into the Petri dishes to solidify then 1 ml from the 10^{-5} was then pipetted into appropriately labeled Petri dishes. The Petri dishes were incubated at room temperature (28 ± 2℃) for 4 days then each distinct fungal mycelium was then been sub-cultured repeatedly on Sabouraud Dextrose Agar (with inhibitor) until a pure culture is gotten. All experiments were conducted in triplicate [13].

2.5 Determination of Heavy Metal Concentration from Tannery Effluent

The absorbance of the effluent stock solution collected was measured to determine the initial heavy metals concentration at the beginning of the experiments using Atomic Absorption Spectrophotometer (AAS) [13]. This was also measured after sterilization before each assay. The Apparatus used include a hot plate, 100ml beaker, 50 ml volumetric flask, and bumping agent. The reagent was concentrated nitric acid. 50 mls of the effluent was measured into a 100 ml beaker and 10 ml of Nitric acid was added the beaker and content was heated on hot plate to digest till white fumes of Nitric acid have escaped and it was continually heated until when content has reduced to about 10 ml it was then stored in a volumetric flask and the digest was used to determine the concentration of Cd, Cr, Pb, Zn, Cu etc with atomic absorption spectrophotometer (AAWIN PG500 Instrument) (Kapoor & Viraraghavan, 1997).

2.6 Heavy Metals Removal Studies

Aliquots of 1 ml suspension of the microbial isolates were inoculated in 50 ml Nutrient broth (for bacteria) and Sabouraud Dextrose broth (for fungi) containing the identified heavy metal from the effluent and this was done for all identified heavy metals and microbial isolates [14]. A small amount of the sample was extracted from each flask in the experimental setup every 4 days (i.e. after 0, 4, 8, 12, 16 and 20 days) and centrifuged at 4000 rpm for 25 minutes. The supernatant was digested with HNO_3 and metal concentration was determined by Atomic Absorption Spectrophotometer (AAS). All experiments were conducted in triplicate [15].

2.7 Effect of Temperature on Heavy Metal Removal

Broth medium (50 ml) containing 5 ppm of heavy metal of the identified heavy metal with a 1ml aliquot of a microbial suspension of 24 hours old in conical flasks was incubated at a temperature range between 25-37℃ With constant shaking. All experiments were conducted in triplicates. The Heavy metal concentration in the digested supernatant was measured using Atomic Absorption Spectrophotometer (AAS).

2.8 Effect of pH on Heavy Metal Removal

Nutrient broth containing the metal solutions was adjusted at pH values (4 and 8). 0.1M HCl and 0.1M NaOH were used as pH regulators. All flasks were maintained at pH value range of 4 and 8 for 20 days in an incubator at a temperature of 37℃. The solution was then analyzed for the residual concentration of the metal present. [14] as previously described.

2.9 Effect of Time on Heavy Metal Removal by Bacteria and Fungi Species

Bacteria and fungi Isolates were inoculated into the medium (50 ml) containing known concentrations of heavy metals, the pH of the medium was set 7 with 37℃ incubation temperature and heavy metal concentration in the digested supernatant was analyzed using Atomic Absorption Spectrophotometer (AAS).

3. RESULTS AND DISCUSSION

3.1 Bacteria and Fungi Load Isolated from Tannery Effluent

The total viable bacteria and fungi count of species obtained from tannery effluents obtained

from challawa industrial area. Kano metropolis Nigeria is given in Table 1. The total viable count of the bacteria and fungi in the effluent were within the range of 2.9-4.3×10^5 for bacteria and 5.0×10^4 -- 1.0 ×10^5 for fungi respectively as shown in Table 1.

Table 1. Bacterial and fungi load of tannery effluents

Samples	Bacterial load (cfu/ml)	Fungi load (cfu/ml)
TE 1	4.3×10^5±2.0	6.0×10^4±0.22
TE 2	2.9×10^5±0.78	8.0×10^4±0.72
TE 3	3.6×10^5±0.42	5.0×10^4±2.71
TE 4	3.2×10^5±1.14	1.0×10^5±0.48

TE=Tannery effluent

3.2 Isolation and Identification of Fungi and Bacteria Species

The cultural, morphological and biochemical characteristics of bacterial isolates, as well as the cultural and morphological characteristics of fungi, were used to identify the isolates from Tannery effluents. The following organisms were identified *Penicillium notatum, Bacillus subtilis,* and *Pseudomonas aeroginosa* and *Aspergillus niger*.

3.3 Occurrence of Bacteria and Fungi Species

The percentage occurrence of bacteria and fungi species such as *Aspergillus niger*, and *Penicillium notatum, Bacillus subtilis,* and *Pseudomonas aeroginosa* isolated from the tannery effluents is shown in Table 2 with *Bacillus subtilis, Pseudomonas aeroginosa, Penicillum notation,* and *Aspergillus niger* all have same frequency of occurrences of hundred percent (100%) (Table 2).

3.4 Heavy Metal Concentration in Tannery Effluent

The heavy metals content of tannery effluent obtained from challawa industrial area, Kano metropolis, Nigeria is given in Table 5. They include Chromium, cadmium, Copper, Lead, Zinc and Iron. The concentration range of Chromium (Cr) was between 2.12± 0.17 to 2.33±0.16 (mg/L), Copper (Cu) 2.58± 0.99 to 2.60± 0.67 (mg/L), Lead (Pb) 6.06± 0.82 to 6.08± 0.01 (mg/L), Zinc (Zn) 7.09± 0.82 to 7.11± 0.24(mg/L) and Iron (Fe) 5.38± 0.96 to 5.41± 0.96 (mg/L) respectively as in Table 3.

3.5 Microbial Removal of Heavy Metals at Various Temperature

The result of the effect of temperature on the removal of heavy metal by species isolated from tannery effluents is shown in Table 4. The biosorption capacity at 25℃ are *Bacillus subtilis* (2.13±01.1-6.59±0.30 mg/L), *Pseudomonas aeruginosa* (0.02±0.17- 0.17±0.35 mg/L), *Aspergillus niger* (0.35±0.08-3.28±1.093 mg/L), *Penicillium notatum* (0.24±0.03- 5.22±0.57 mg/L) respectively. While at 37℃ biosorption capacity are (0.02±0.86- 1.07±0.26 mg/L), (0.95± 0.73-3.72±0.05 mg/L), (1.00±0.05- 3.20±0.63 mg/L), and (0.36±0.28- 4.47±0.15 mg/L) respectively. This is for all of the heavy metal.

Table 2. Occurrence of bacteria and fungi species isolated from tannery effluent

Bacterial/Fungi isolate	TE 1	TE 2	TE 3	TE 4	Frequency (%)
Aspergillus niger	+	+	+	+	4(100.0)
Penicillium notatum,	+	+	+	+	4(100.0)
Bacillus subtilis	+	+	+	+	4(100.0)
Pseudomonas aeruginosa	+	+	+	+	4(100.0)

Key: TE= Tannery effluent, + = Present - = Absent

Table 3. Heavy metal concentration in tannery effluent

	Concentration (mg/L)			
Heavy metals	**TE 1**	**TE 2**	**TE 3**	**TE 4**
Chromium (Cr)	2.31± 0.03	2.12± 0.17	2.41±0.06	2.33±0.16
Copper (Cu)	2.60± 0.01	2.58± 0.99	2.60± 0.67	2.60± 0.33
Lead (Pb)	6.07± 0.50	6.06± 0.82	6.08± 0.01	6.06± 0.82
Zinc (Zn)	7.10± 0.03	7.09± 0.82	7.11± 0.24	7.10± 0.10
Iron (Fe)	5.41± 0.93	5.38± 0.96	5.41± 0.96	5.43± 0.07

Key: TE = Tannery effluent

Table 4. Microbial removal of heavy metal at various temperatures

Heavy Metal	Temperature ℃							
	Bacillus subtilis		***Pseudomonas aeroginosa***		***Penicillum notatum***		***Aspergillus niger***	
	25℃	**37℃**	**25℃**	**37℃**	**25℃**	**37℃**	**25℃**	**37℃**
Chromium (Cr)	2.13±01.1	0.55±0.15	0.02±0.17	1.86±0.64	2.02±0.19	1.97±0.10	1.19±0.26	1.14±0.34
Copper (Cu)	2.14±0.29	0.08±0.24	0.17±0.35	2.07±0.23	0.24±0.03	0.36±0.28	0.35±0.08	1.00±0.05
Lead (Pb)	4.03±0.86	0.02±0.86	0.02± 0.82	3.72±0.05	5.22±0.57	4.47±0.15	2.12±0.16	2.12±0.08
Zinc (Zn)	6.59±0.30	1.07±0.26	0.16±0.56	0.95± 0.73	4.28±0.29	4.06±0.66	3.28±1.093	3.20±0.63

Table 5. Microbial removal of heavy metal at various temperatures

Heavy Metal	Degree of Acidity and Alkaline (pH)							
	Bacillus subtilis		***Pseudomonas aeroginosa***		***Penicillium notatum***		***Aspergillus niger***	
	4	**8**	**4**	**8**	**4**	**8**	**4**	**8**
Chromium (Cr)	2.58±0.67	2.02±0.66	2.19±3.57	1.79±0.27	2.04±2.02	1.00±7.07	1.08±3.26	2.00±0.21
Copper (Cu)	2.34±0.91	2.56±1.08	1.07±2.89	1.07±0.01	2.61±1.85	2.22±0.24	1.25±4.28	1.00±0.63
Lead (Pb)	4.36±0.80	2.39±0.41	2.93±1.18	2.01±0.05	4.59±1.19	4.17±8.17	2.30±4.55	2.12±1.90
Zinc (Zn)	2.40±1.10	2.17±3.05	1.89±0.61	1.80±1.89	4.70±1.06	4.47±5.60	3.13±0.19	2.71±1.28

3.6 Microbial Removal of Heavy Metal at Various pH

The effect of pH on the rate of removal of heavy metal by species isolated from tannery effluents obtained from Challawa industrial area in Kano metropolis Nigeria is given in Table 5. pH 4 and 8 were used to determine the biosorption capacity of *Bacillus subtilis, Pseudomonas aeruginosa*, *Aspergillus niger, Penicillium notatum* which show that at pH of 4 the range for *Bacillus subtilis* is (2.34- 4.36) at pH 8 2.02-2.56*, Pseudomonas aeruginosa* at pH 4 1.07-2.93, at 8 (1.07-2.01),*Penicillium notatum* at pH 4(2.04-4.70) at 8 (1.00-4.47), while for *Aspergillus niger at* pH4 (1.08-3.13) at pH (1.0-2.71).

3.7 Microbial Removal of Heavy Metal on Different Days

The removal of Heavy Metals by bacteria and fungi remove heavy metals from tannery effluents over a period of 20 days was taken at an interval of 0, 4, 8, 12, 16 and 20 days respectively. For effluents treated with *Bacillus subtilis Pseudomonas aeruginosa*, *Aspergillus niger, Penicillium notatum*. The range of removal for *Bacillus subtilis* is Cr (0.06±0.11-2.31±0.61), Cu (0.08±0.98-2.09±0.64) Pb (0.02±0.10-5.61±0.99), Zn (1.07±0.70-6.59±1.00), Fe (0.33±0.07-5.36±0.62) Table 6. While for tannery effluent treated *Pseudomonas aeruginosa* was within the range of Cr (0.04±5.89-2.08±9.58), Cu (0.17±5.06-2.15±6.61) Pb (0.02±0.67-6.40±2.25), Zn (0.16±6.84-6.40±2.25), Fe (0.05±0.96-5.75±2.40) Table 7. In Table 8, it shows tannery effluent treated *Aspergillus niger* which is in the range of Cr (1.19±0.12-2.20±0.27), Cu (0.35±0.91-2.36±0.47) Pb (0.16±0.44- 5.32±0.38), Zn (0.16±6.84-6.43±1.61), Fe (0.03±0.08-6.39±0.43).

Table 6. Effect of heavy metal concentration (mg/L) in tannery effluent treated with *Bacillus subtilis*

Sample	Cr	Cu	Pb	Zn	Fe
Bac D 0	2.24±0.37	2.09±0.64	5.61±0.99	6.59±1.00	5.36±0.62
Bac D 4	2.08±0.61	1.93±0.02	4.03±0.14	5.37±0.49	4.32±0.91
Bac D 8	1.25±0.47	0.67±0.90	2.68±0.66	3.42±0.67	2.59±0.67
Bac D 12	0.92±0.86	0.55±0.97	0.85±0.13	2.38±0.43	1.27±0.88
Bac D 16	0.13±0.79	0.32±0.12	0.17±0.93	1.26±0.44	0.83±0.02
Bac D 20	0.06±0.11	0.08±0.98	0.02±0.10	1.07±0.70	0.33±0.07

Key: Bac = Bacillus Subtilis; D=Day

Table 7. Effect of heavy metal concentration (mg/L) in tannery effluent treated with *Pseudomonas aeruginosa*

Sample	Cr	Cu	Pb	Zn	Fe
Pseu D 0	2.08±9.58	2.15±6.61	5.43±1.36	6.40±2.25	5.75±2.40
Pseu D 4	1.86±9.35	2.07±0.27	3.72±2.01	5.43±0.68	4.49±6.56
Pseu D 8	1.37±0.68	1.94±0.65	2.19±0.22	2.81±2.68	2.73±5.89
Pseu 12	0.89±3.07	0.82±5.47	1.06±0.67	1.73±1.40	1.64±7.64
Pseu D 16	0.04±5.89	0.28±0.75	0.09±5.16	0.42±6.03	0.12±6.08
Pseu D 20	0.02±1.45	0.17±5.06	0.02±0.67	0.16±6.84	0.05±0.96

Key: Pseu = Pseudomonas aeruginosa; D= Day

Table 8. Effect of heavy metal concentration (mg/L) in tannery effluent treated with *Aspergillus niger*

Sample	Cr	Cu	Pb	Zn	Fe
Ani D 0	2.20±0.27	2.36±0.47	5.32±0.38	6.43±1.61	6.39±0.43
Ani D 4	2.01±0.37	2.12±0.47	4.58±0.47	5.14±0.58	5.41±0.03
Ani D 8	1.85±0.64	1.58±0.69	3.26±0.57	4.77±0.01	3.28±0.51
Ani D 12	1.43±1.34	1.17±1.30	2.12±0.60	3.24±0.37	1.45±0.59
Ani D 16	1.21±2.10	1.04±1.03	1.09±0.65	1.20±0.36	0.25±0.11
Ani D 20	1.19±0.12	0.35±0.91	0.16±0.44	1.06±0.04	0.03±0.08

Key: Ani = Aspergillus niger; D= Day

Table 9. Effect of heavy metal concentration (mg/L) in tannery effluent treated with *Penicillium notatum*

Sample	Cr	Cu	Pb	Zn	Fe
Pen D 0	2.10±0.08	1.88±0.25	5.79±0.48	5.43±0.71	6.36±0.50
Pen D 4	2.02±0.58	1.25±0.82	5.22±0.01	4.28±0.44	5.39±0.79
Pen D 8	1.97±0.71	0.76±0.49	4.47±0.83	4.06±0.41	4.84±0.34
Pen D 12	1.43±0.88	0.24±0.88	3.56±1.00	2.30±0.44	4.67±0.78
Pen D 16	0.20±0.79	0.08±0.59	2.68±0.10	1.25±0.81	3.73±0.73
Pen D 20	0.07±0.27	0.03±0.38	1.15±0.90	0.76±0.76	2.35±1.80

Key: Pen= Penicillium notatum; D= Day

Lastly, Table 9 shows the range value of tannery effluent treated *Penicillium notatum.* Cr (0.07±0.27-2.10±0.08), Cu (0.03±0.38-1.88±0.25) Pb (1.15±0.90- 5.32±0.38), Zn (0.76±0.76-5.43±0.71), Fe (2.35±1.80-6.36±0.50).

4. CONCLUSION

Bacteria and fungi species ability to remove heavy metal have been shown to be efficient and low cost as compared to another method of heavy metal removal from solution. The process of removal of heavy metal has become very attractive as it allows for removal of metals ion over a relatively broad range of pH and temperature. Many researchers have studied removal of heavy metal (biosorption) by different microorganisms which provide enough arguments for the use of microbial species for heavy metal removal from solutions. Consequently, through further research and studies, microbial removal of heavy metal (biosorption) would be the most conventional, cost effective, environmental friendly means of removal of heavy metal not only from tannery effluents but from all form of liquid waste containing heavy metal.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chatterjee SK, Bhattacharjee I, Chandra G. Biosorption of heavy metals from industrial waste water by *Geobacillus thermodenitrificans.* Journal of Hazardous Material. 2010;175:117-125.
2. Iqbal M, Edyvean RGJ. Loofa sponge immobilized fungal biosorbent: A robust system for cadmium and other dissolved metal removal from aqueous solution. Chemosphere Journal of Chemistry. 2005;61:510–518.
3. Sardrood BP, Goltapeh EM, Varma A. An introduction to bioremediation fungi as bioremediation. Springer Journal. 2013;3-27.
4. Wang J, Chen C. Biosorbents for heavy metals removal and their future. Journal of Biotechnology Advancement. 2009;27:195-226.
5. Munoz R, Guieysse B. Algal-bacterial processes for the treatment of hazardous contaminants: A review. Journal of Water Resources. 2006;40:2799–2815.
6. Lone MI, He ZL, Stoffella PJ, Yang XE. Phytoremediation of heavy metal polluted soils and water: Progress and perspectives. J Zhejiang University Science Biology. 2008;9:210-220.
7. Cho DH, Kim EY, Hung YT. Heavy metal removal by microbial biosorbents. Journal of Environmental Bioengineering Springer. 2010;11:375-402.
8. Prasenjit B, Sumathi S. Uptake of chromium by *Aspergillus foetidus.* Journal of Material Cycles Waste Management. 2005;7:88-92.
9. Shankar C, Sridevi D, Joonhong P, Michael D, Kaliannan T. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. Journal of Hazardous Materials. 2007;146: 270–277.
10. Fawole M, Oso B. Laboratory manual of microbiology. Spectrum Books Limited, Ibadan, Nigeria. 2007;1:15-33.
11. Adekunle AS, Eniola ITK. Impact of industrial effluent on quality of segment of Asariver within an industrial estate in Ilorin, Nigeria. New York Science Journal. 2008;1(1):17-21.
12. Nazir R, Bhat GA. Characteristics of liquid and solid waste generated at four major hospitals of Srinagar city, Kashmir. Journal

of Himalayan Ecology and Sustainable Development. 2012;7:64-69.
13. Kapoor A, Viraraghavan T. Heavy metal biosorption sites in *Aspergillus niger.* Journal of Bio Resources Technology. 1997;61:221–227.
14. Homaidan A, Hadeel J, Hazzari A, Elgaaly G, Moubayed N. Biosorption of copper ions from aqueous solutions by *Spirulina platensis* biomass. Arabian Journal of Chemistry. 2014;7(1):57-62.
15. Zafar S, Aqil F, Ahmad I. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Journal of Biology Technology. 2006;98:2557-2561.

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/24845