Plant Archives Vol. 20, Supplement 2, 2020 pp. 4105-4108 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

REMOVAL OF SOME HEAVY METAL FROM AQUEOUS SOLUTIONS BY LACTIC ACID BACTERIA (WHOLE BACTERIA AND BIOFILM)

Srbeel Khalid Saheh[1] and Asmaa Sabah Ahmaed[2]

[1]Anbar Agriculture Directorate, Ministry of Agriculture, Iraq,
[2]Department of Food Sciences, College of Agricultural Engineering Sciences, University of Baghdad, Iraq

Abstract

Leuconostoc mesetroides, *Lactobacillus casei* and *Lactobacillus rhamnosus* GG a lactic acid bacteria were evaluated for its ability to remove of cadmium (Cd), lead(Pb) and cobalt(Co) from aqueous solution. The efficiency of whole bacteria was *Leuconostoc mesetroides Lactobacillus casei* and *Lactobacillus rhamnosus* GG of Cd metal was 90.848, 83.452, 71.909%, while biofilm was 40.691, 37.131, 37.033%. As for Pb, the highest removal rate for whole bacteria and biofilm for *Leuconostoc mesetroides* isolation was 91.317% and 72.313% respectively and corresponded to the Co, where the highest removal whole bacteria was by *Leuconostoc mesetroides* isolation 77.165% As for biofilm it was higher for *Lactobacillus casei* isolation was 23.359% .Experimental results reveal that all the whole bacterial isolates have potential application for remove of Cd, Pb and Co from the biofilm.

***Keywords* :** Heavy metal, Lactic acid bacteria, Biofilm, Cd, Pb, Co.

Introduction

Soil and water pollution by toxic heavy metals and organic pollutants occurs as a result of human activities and has ecome a major concern in the environment and health. Many toxic minerals such as (lead, mercury, copper, cadmium, zinc, nickel, arsenic, etc.) are released directly and indirectly from wastewater and other human activities in the environment (Gupta and Diwan, 2017). Contaminants with heavy metals are not biodegradable and able to enter the food chain through bioaccumulation. Heavy metals play a role in mineral enzymes and affect membrane stability such as ketones and affect DNA and metabolic functions (Siddiquee *et al.*, 2015, Dedykhina and Eroshin, 1991). When cadmium, lead and mercury in the environment rise, they become more toxic (Gadd, 1986). Heavy metals can be defined based on three different criteria that include density, atomic number or their chemical properties (Radogevic *et al.*, 2007). It was mentioned that heavy metals can be defined as the density of minerals (5 g / cm^3), which is five times more dense than water (Yu, 2001). Minerals are classified as elements with an atomic number greater than 20 with the exception of alkali metals, alkaline earth, lanthanides and actinides. Arora *et al.* (2008) reported that heavy metals have the ability to accumulate in different parts of the human part. Concentrations of heavy metals have the property of long-life biology and resistance to degradable processes and chemical toxicity appears in soluble water if minerals have high concentrations and enter the human body, so they absorb and bind to vital molecules such as proteins and DNA and interfere with their functions. Lead and cadmium are among the most abundant mineral elements. They are biologically non-essential and non-degradable and tend to accumulate in living organisms exposed to them. Exposure to lead causes a neurological imbalance in the blood, dysfunction of the heart, blood vessels, liver, kidneys, and reproductive disorders in a human body, especially young children(Kirillova, *et al.*,2017;Rossi.,2008). As for cadmium toxicity, it is primarily associated with renal, structural, and pulmonary disorders, hepatic, reproductive, and cardiovascular disorders (Bernard, 2008). The International Agency for Research on Cancer (IARC) has classified cadmium as a human carcinogen (Godt *et al.*, 2006). Cobalt causes negative effects on the human body by increasing its levels as it affects the respiratory system when inhaled in high concentrations as it causes asthma, pneumonia, vomiting, vision and heart problems and when dealing with long periods causes an effect on the thyroid gland (Welton *et al.*, 2007). In order to obtain safe removal of heavy metals, Bioremediation was directed to microbial removal, microorganisms have developed various techniques for bonding with minerals and thus disposa. The cells, response to microorganisms, whether they are bacteria, fungi or algae, to get rid of the trustworthy minerals of many shapes, either through the cell's wall or biofilm formation. Lactic acid bacteria a positive bacteria for the dye that is often bacillus, producing lactic acid as a major product of fermentation of carbohydrates (Axelsson, 2004). Therefore, the aim of this study was the susceptibility of some bacterial isolates producing lactic acid to produce the biofilm and studying the ability of these isolates toward Cd, Pb and Co by using whole bacterial cells and biofilm.

Materials and Methods

Bacterial isolates

Bacterial isolates produced lactic acid were obtained *Leuconostoc mesetroides* (Lm), *Lactobacillus casei* (Lc) and *Lactobacillus rhamnosus* GG(LGG) that cultivation in MRS broth media (10ml) at 37^{o}C for 48 h.

Calculating bacterial numbers

Bacterial numbers of isolates were counted according to the Macfarland method (Vandepitte *et al.*, 2003) It used 10^8 cells/ml in subsequent experiments.

Investigation of biofilm production

The Micro titer plate method(MTP) was used to investigate the production of biofilms by bacterial isolates under study, as the accurate calibration dishes containing 96 flat bottoms and the MRS culture medium used in the study and the aqueous solution of the violet crystal dye at 0.1%

concentration and 95% ethanol for biofilm production. The bacterial isolates under revitalization were activated, with each concretion being implanted at 2% bacterial isolates under sterile conditions where (10^8 CFU). Individually, the control cells (in the control wells) add only the medium. Incubated at different times (24h, 48h, 72h and 14d) at a temperature of 37 °C. The bacteria cells that did not stick by washing to the pits are removed and three times with PBS (pH7.2). Sterilizer At room temperature 200µl of violet crystal dye (0.1%) was added to the drilling for 15 minutes and incubated at 37 °C. The drilling was washed over three periods with sterile PBS (pH7.2) to remove the unrelated dye and then dried at room temperature. 200µl ethanol (95%) was added. The ability of bacteria to produce films was measured using an ELISA reader at 630 nm (Bose *et al*., 2009; Salman and Khuder, 2015 and Wilson *et al*., 2017).

Standard metal salts solutions

Standard metallic elements solutions were prepared in the form of salts manufactured from Merk Company/ Germany from Pb $(NO_3)_2$, Cd $(NO_3)_2$, $Co(NO_3)_2$ with a 99.99% purity and concentrations of 1000 mg/l as the solutions of storage elements. Prepared solutions that required Work from them in a successive way of storing these solutions, as they were used alone or in combination.

Determination of residual concentration of heavy metals (lead, cadmium, cobalt) using the atomic absorption spectrometer

The residual concentration of heavy metals in the culture media was estimated after the development of the isolates under study as they were filtered by microscopic membrane filters with a diameter of 0.45 µm holes. The amount of the remaining minerals was estimated by the hippie atomic spectroscopy apparatus according to the following formula:

Percentage of removal = (concentration of the component before removal - concentration of the component after removal) / concentration of the component before removal * 100

Results and Discussion

The results showed the susceptibility of the lactic acid bacteria under study to the formation of biofilms and to a varying degree among them, as it was observed from Figure 1 to increase the biofilm formation of the three bacterial isolates (*Leuconostoc mesetroides*, *Lactobacillus casei*, *Lactobacillus rhamnosus* GG) Down to the incubation period of 14 days, then decreasing in 20 days. MTP is one of the most accurate quantitative tests in the field of measuring the ability of different bacterial isolates to form a biofilm (Samie and Nkgau, 2012). It depends on the ability of the bacterial cells to adhere to the walls or bottom of the MTP and it achieves its ability to form biofilms, and this method is MTP is the most sensitive, realistic and specialized method. As it was found that the highest levels of biofilm formation for bacterial isolates used for study in the bosom period are 14 days (1.214, 1.281, 1.42) for bacterial isolates (*Leuconostoc mesetroides* (Lm) '*Lactobacillus casei (Lc)' Lactobacillus rhamnosus* GG (LGG) respectively. These results agreed with (Patel and Sharma, 2010). As the formation of biofilms using the MTP method varies with different bacterial isolates.

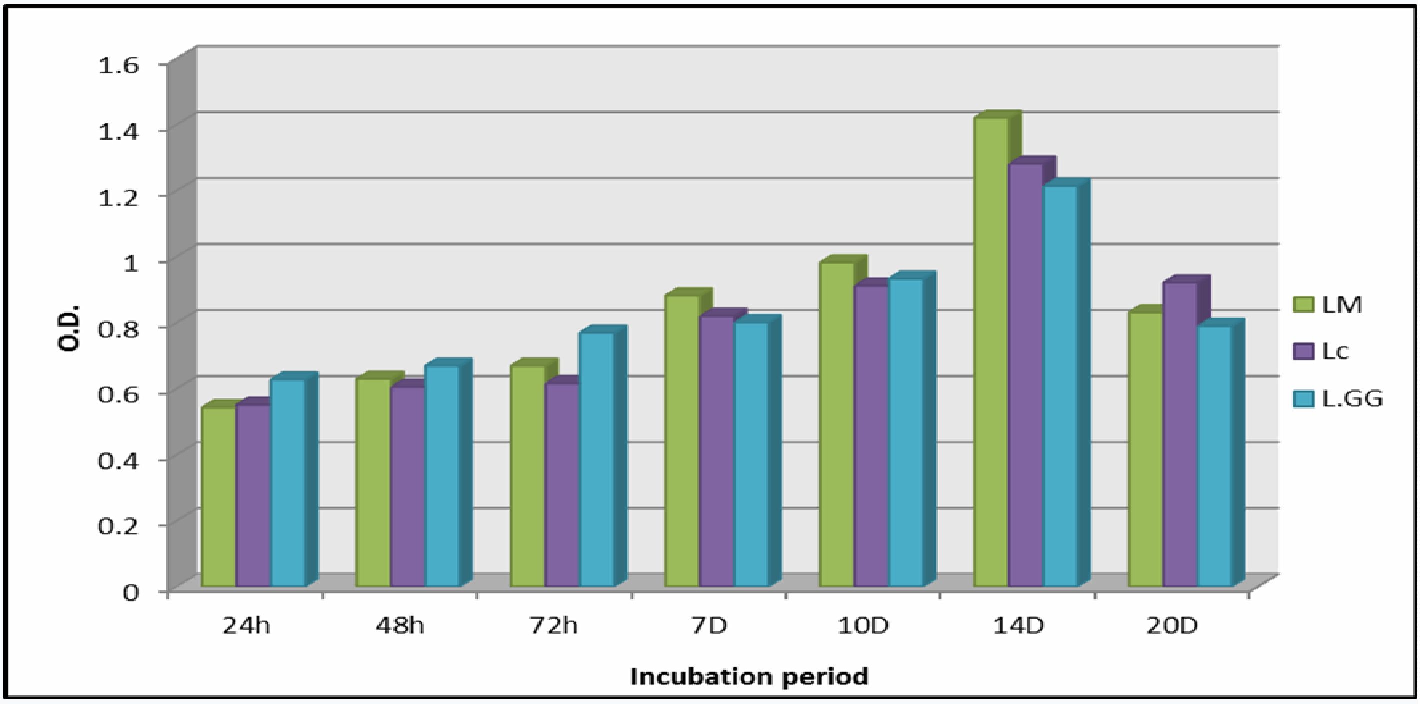

Fig. 1 : The optimum incubation period of biofilm formation.

Percentage of removal using biomass and biofilm

The efficiency of the biomass of the three isolates in removing cadmium, lead and cobalt is more than removing the three elements by the biofilm formed by these isolates. It is noted from Figure (2) that the efficiency of isolation L.M is more if the removal of cadmium metal (90.848, 83.452, 71.909) %respectively for biomass, while L.GG isolation was the highest in removal (40.691, 37.131, 37.033)% respectively for the biofilm.

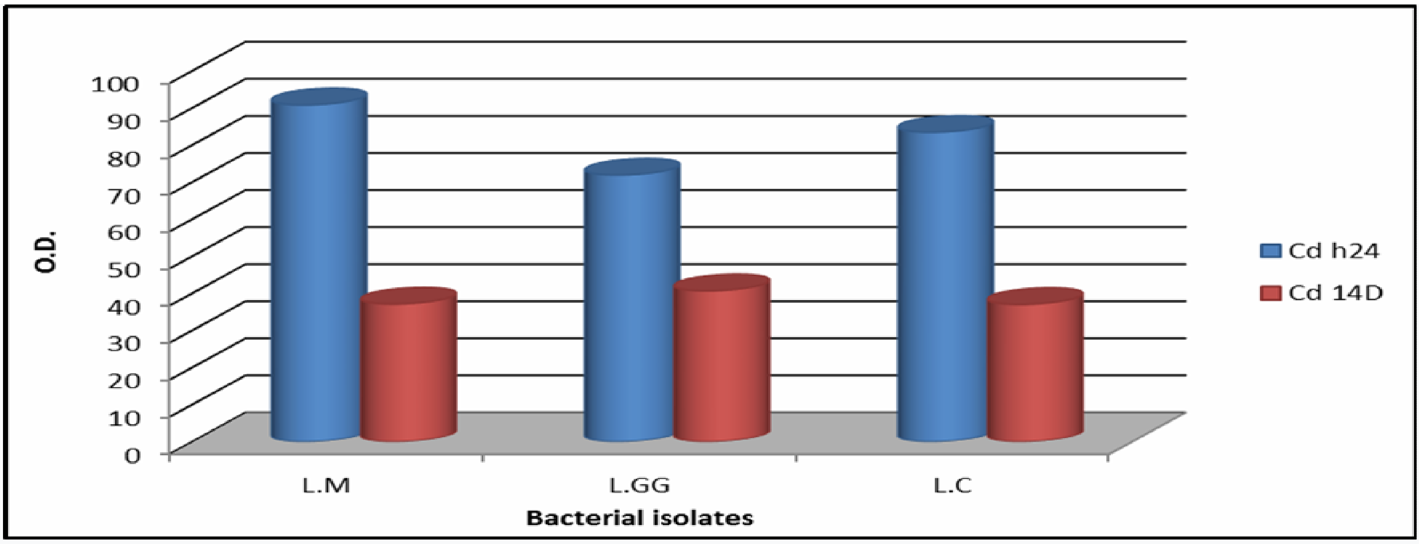

Fig. 2 : Percentage of Cadmium removal by three bacterial isolates.

This was similar to the cobalt element which was the removal (77.165, 55.946, 37.598)% respectively for the biomass of the three isolates (*Leuconostoc mesetroides*, *Lactobacillus casei*, *Lactobacillus rhamnosus* GG). While L.GG isolate was the highest in removal (23.359, 14.584, 13.906)% biofilm (Figure 3)

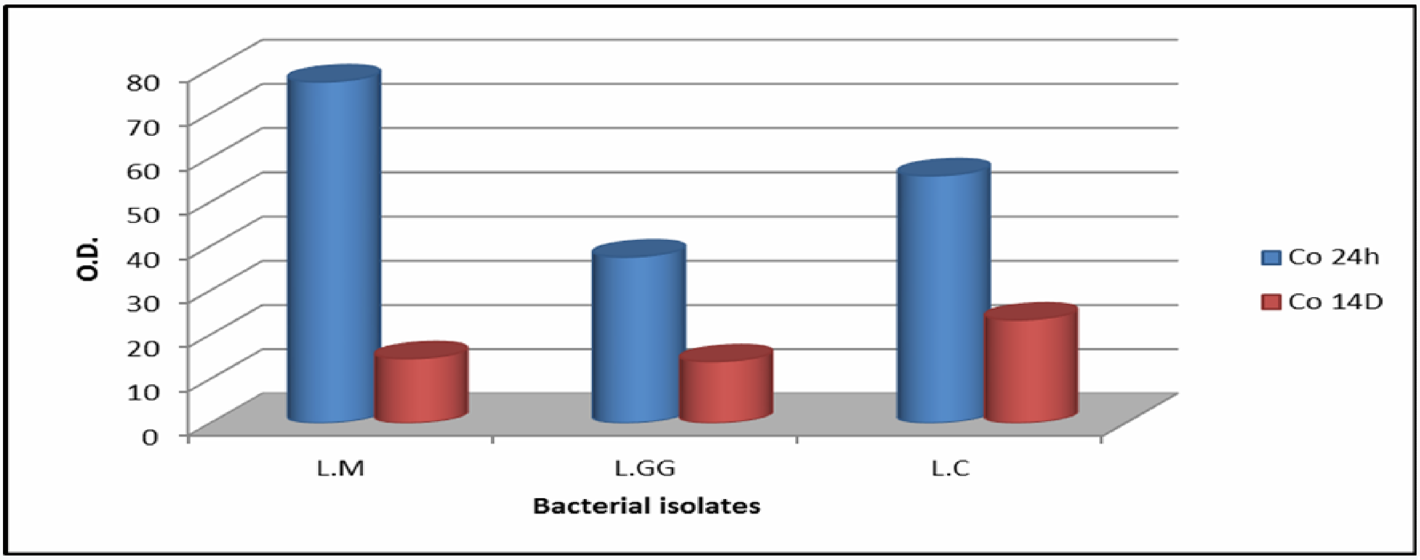

Fig. 3 : Percentage of Cobalt removal by three bacterial isolates

The highest removal was for lead and both biomass and the biofilm for isolation L.M (91.317, 72.313)% While the other two isolates were decreased by L.GG and L.C (78.31 ,85.42)% and(48.515, 17.302)% (Figure 4)

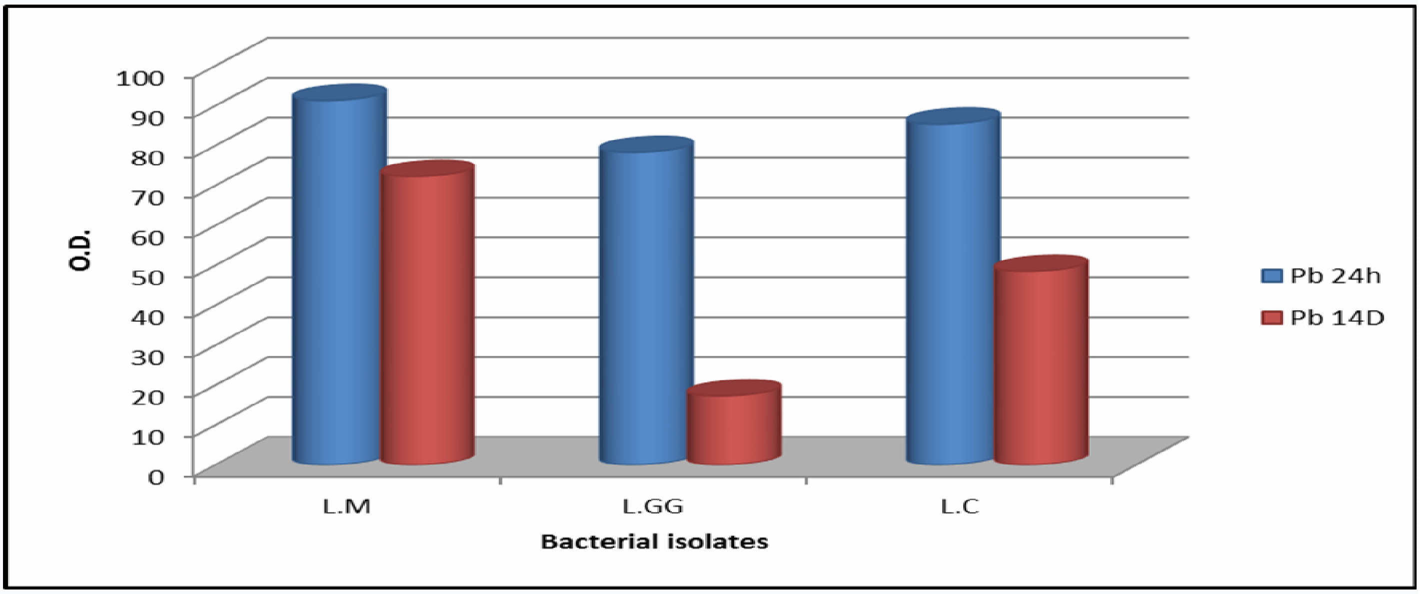

Fig. 4 : Percentage of lead removal by three bacterial isolates

Researcher (Samarth *et al*., 2012) confirmed that the peptidoglycan layer with Teichuronic acid, Teichoic acid that have the main role in the process of binding bacteria (Especially that bacteria are positive for the gram stain) of metals in its wall. While researcher (Avall-jaaskelainen and Palva, 2005) found that lactic acid bacteria produce surface layer proteins (S layer proteins) not covalently bound and their size is from 25-50 Microdalton. It is very basic and this layer may be associated with minerals and help in the process of removing them because lactic acid bacteria when forming the biological membrane so the effective sites in the wall are occupied with the formation of external sugars and are very complex which results in less removal of minerals. (Halttuen *et al*., 2007) found the cell wall of Lactia acid bacteria contains a large number of negative charge functional groups such as carboxyl, hydroxyl, and phosphates and therefore has a large number of different potential sites that are able to bind to positive ions such as cadmium and lead and that the increase in biomass increases from metal removal. As for biofilms to develop and form biofilms, it may take a few days to months, depending on the concentration of the bulk membership. Biofilms had a degree of complexity and may be insoluble or insoluble (cellulose, mitan, amiodoid) or structural (vesicles, nanotubes, capillaries) and the outer space is defined by the components of the biofilm matrix and the outer space may consist of bacterial structures produced from the surfaces of the bacteria cells to Cellular Outside Ocean (PILI, TUBES) (Balaguer *et al*., 2016).

References

Arora, M.; Kiran, B.; Rani, S.; Rani, A. and Kaur, B. (2008). Heavy Metals accumulation in vegetables irrigated with water from different sources. Food Chem. 111: 811-815.

Åvall-Jääskelainen, S. and Palva, A. (2005). *Lactobacillus* surface layers and their applications. FEMS Microbiol Rev., 29: 511-529.

Axelsson, L. (2004). Lactic acid bacteria: Calssification and physiology. In: Lactic acid bacteria, microbiological and functional aspects, 3rd edition. Editors:Salminen, S., von Wright, A. and Ouwehand, A. pp. 1-67, New York, Marcel Deccer Inc

Balaguer, M.D.; Helena, G. and Anna, M.R. (2016). Aquatic biofilms: ecology, water quality and wastewater treatment .Caister Academic Press.

Bernard, A. (2008). Cadmium and its adverse effects on human health, Indian Journal of Medical Research, 128(4): 557–564.

Bose, S.; Khodke, M.; Basak, S. and Mallick, S.K. (2009). Detection of biofilm producing *Staphylococci* :need of the hour. J. Clin. Diag. Res., 3: 1915-1920.

Dedyukhina, E.G. and Eroshin, V.K. (1991). Essential metal ions in the control ofmicrobial metabolism. Proc Biochem., 26: 31-37.

Gadd, G.M. (1986). Fungal responses towards heavy metals. In: Herbert R.A ,Codd GA, Microbes in Extreme Environments. Academic Press, London.

Godt, J.; Scheidig, F. and Grosse-Siestrup, C. (2006). The toxicity of cadmium and resulting hazards for human health, Journal of Occupational Medicine and Toxicology, 1(1): article 22.

Gupta, P. and Diwan, B. (2017). Bacterial Exopolysaccharide mediated heavy metal removal: A Review on biosynthesis, mechanism and remediation strategies. Biotechnology Reports, 13 : 58–71.

Halttuen, T. (2007). Removal of Cadmium, Lead and Arsenic from Water by Lactic Acid Bacteria. Functional Food Forum, Department of Biochemistry and Food Chemistry. University of Turku.

Kirillova, A.V.; Danilushkina, A.A.; Irisov, D.S.; Bruslik, N.L.; Fakhrullin, R.F.; Zakharov, Y,A.; Bukhmin, V.S. and Yarullina, D.R. (2017). Assessment of Resistance and Bioremediation Ability of *Lactobacillus* Strains to Lead and Cadmium. International Journal of Microbiology, P.p7.

Patel, J. and Sharma, M. (2010). Differences in attachment of Salmonella enteric serovars to cabbage and lettuce leaves. International journal of food microbiology, 139(1-2): 41-47.

Radojevic, M.; Abdullah, M.H. and Aris, A.Z. (2007). Analisis Air. Scholar Press, Selangor.

Rossi, E. (2008). Low level environmental lead exposure—a continuing challenge," The Clinical Biochemist Reviews, 29: 63–70.

Salman, J. and Khuder, A. (2015). Antibacterial And Antibiofilm Effect of Biosurfactant Produced from *Leuconostoc mesenteroides* ssp. *Cremris* against Bacteria from Catheters, World J. of Pharmaceutical Research, 4(10): 320-333.

Samarth, D.; Chandekar, C.J. and Bhadekar, R.K. (2012). Biosorption of Heavy Metal from Aqueous Solution using *Bacillus* Kicheniformis Int, J. Pure Appl. Sci. Technol., 10(2): 12-19.

Samie, A. and Nkgau, T.F. (2012). Biofilm production and antibiotic susceptibility profile of *Escherichia coli* isolates from HIV and AIDS patients in the Limpopo province. Afr. J. Biotechnol., 11(34): 8560-8570.

Siddiquee, S.H.; Rovina, K.; Azad, S.; Naher, L.; Suryani, S. and Chaikaew, P. (2015). Heavy Metal Contaminants Removal from Wastewater Using the potential Filamentous Fungi Biomass: A Review, J. Microb. Biochem, 7: 6.

Vandepitte, J.; Verhaegen, J.; Engbeck, K.; Rohnerr and Euck, C.L. (2003). Basic laboratory procedure in clinical bacter-iology.2nd ed., World Geneva. Health Organization.

Welton, A.J.; Dietrich, A.M.; Burlingame, G.A.; Schechs, M. and Duncan, S.E. (2007). Minerals in drinking water: impacts on test and importance to Consumer health.Water Technol., 55: 283-291.

Wilson, C.; Lukowicz, R.; Merchant, S.; Valquier-Flynn, H.; Caballero, J.; Sandoval, J.; Okuom, M.; Huber, C.; Brooks, T.D.; Wilson, E.; Clement, B.; Wentworth, C.D. and Holmes, A.H. (2017). Quantitative and qualitative assessment methods for biofilm growth:Amini-review.Res.Rev.J.Eng.Technol, 6(4):1-25.

Yu, M.H. (2001). Environment Toxicology: Impacts of Environmental Toxicantson Living Systems. (3rd Edn)CRS Press LLC, Florida