ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 6 No. 3 September 2020*

Isolation of Bacteria With Heavy Metal Bioremediation Potential From Tannery Effluent In Challawa Industrial Estate, Kano State, Nigeria

[1]Tagwai, J. J., [*1]Aminu , A. I. and [1]Yahaya, S.
[1]Department of Microbiology,
Faculty of Life Sciences,
Bayero University, Kano Nigeria.

Email: aishatuaminuibrahim@gmail.com

Abstract
Bioremediation using bacteria serves as an important tool in managing heavy metal pollution which already is an environmental health concern all over the world. The aim of the study was to isolate and assess Chromium (Cr) bioremediation potential of bacteria isolated from tannery effluent. The effluent samples were taken from Challawa Industrial Estate, Kano State. The physico-chemical characteristic of the effluent was determined, and the concentration of Cr was analysed using atomic absorption spectrophotometry before and after remediation. Bacteria that can utilise Cr were isolated from the effluent sample using standard microbiological methods and subjected to varying concentrations of Cr. Isolates obtained were further evaluated for bioremediation potential. The results indicated that the bacterial isolates obtained from the effluent sample were identified as *Bacillus marinus* (B_{11}) and *Bacillus badius* (B_{15}) which were inhibited at a concentration of 400mg/L by Cr. The two isolates, exhibited bioremediation potential and reduced Cr concentration from 0.2857 to 0.1429 mg/L (49.98% reduction) over a period of 48 hours by $B_{15}$and over a period of 96 hours by B_{11}. The study discloses the presence of high concentration of Cr as a heavy metal in the effluent tannery which likely contributes to environmental pollution thereby posing environmental and public health threat. The bacteria isolated in the effluent were demonstrated to possess heavy metal tolerance and was able to reduce the concentration of Cr as such can be employed as potential agents for bioremediation of tannery effluent.

Keywords: Tannery effluent, Chromium, Bacteria, Bioremediation

INTRODUCTION
Leather tanning is an age old tradition all over the world including Nigeria. The entire leather tanning process involves chemical and organic compounds with chrome tanning process now overshadowing the vegetable tanning process and accounting for 90% of global leather production as far back as 2002 (Sundar, 2002). However, effluents generated from the tanning activity have turn out to be environmentally unfriendly with devastating effects on

*Author for Correspondence

living cells including plants, animals and human beings. Currently bioremediation is one of the techniques that is use to detoxify the tannery effluents. Bioremediation is a process that is employed in order to transform toxic heavy metals into a less harmful state using microbesor its enzymes to clean-up polluted environment (Igiri *et al*., 2018).

Leather processing industries (LPI) use chromium material (chrome liqueur or chrome powder) for tanning of leather and residual chromium thus is discharged in solid or liquid effluents. However, Wionczyk *et al.* (2006) reported that only a fraction of the chromium salts used in the tanning process react with the skins, the rest of the salts remain in the tanning exhaust bath and are subsequently sent to a depuration plant where the chromium salts end up in the sludge, contributing to pollution. For example, in India, about 2000–3000 tone of chromium escapes into the environment annually from tannery industries, with chromium concentrations ranging between 2000 and 5000 mg/l in the aqueous effluent compared to the recommended permissible discharge limits of 2 mg/l (Altaf *et al.*, 2008).

Chromium exists in oxidation states of +2, +3, and +6 and the trivalent oxidation state was found to be the most stable form of chromium essential to mammals in trace concentration and relatively immobile in the aquatic system due to its low water solubility. Recent detection of significant levels of toxic Cr (VI) in surface water and groundwater around the world raises concerns on disposal of Cr-containing waste which is worsen at high pH, Cr (VI) is bio available and owing to its high mobility; posing the greatest risk of ground-water contamination. Mahdavi *et al.* (2001) earlier observed that the discharge of these tannery effluents into the environment is not only aesthetically displeasing, but also impedes light penetration, damages the quality of the receiving streams and may be toxic to the treatment processes, to food chain organisms and to aquatic life. Additionally, Bhattacharya *et al*.(2019); Tudunwada *et al*.(2007) expounded that chromium content of the effluent may pose great danger to humans in as much as it is toxic to humans from a level as low as 0.1mg/l, and, at high concentrations chromium is toxic, mutagenic, carcinogenic, and teratogenic. The hexavalent chromium is much more toxic to many plants, animals, and bacteria inhabiting aquatic environments.

Earlier studies documented that most micro-organisms are sensitive to Cr (VI) toxicity but some groups possess resistance mechanisms to tolerate high levels (Altaf *et al*., 2008). Studies by Dmytrenko *et al.* (2007) demonstrated that bacteria, which can use chromium (VI) as terminal electron acceptor during oxidation of organic compounds proves to be more economical and ecologically effective as biological process than widely known physico-chemical treatment and when use in biotechnology process of galvanic wastewater treatment allows chromium (VI) concentration to decrease in wastewater to nominal limit 0.3 mg/l.

In an earlier study Ogunwa *et al*. (2006) revealed that, in Challawa Industrial Estate, Kano, the pollution parameters of discharged effluents were observed to be higher than the acceptable limits set for tannery effluent discharge, hence pollute the Challawa River.

Thus, it is evident from above that tannery effluents remains to be ranked as the highest pollutants among industrial wastes with tendencies to public health hazards, as such there is need to exploit all avenues that could render them harmless both to the environment and humans especially with the recognition of the ability of some bacteria to reduce some metals including Cr (VI). Therefore, the aim of the study was to assess Cr bioremediation potential of bacteria isolates obtained from tannery effluent of Challawa Industrial Estate, Kano.

MATERIALS AND METHODS

Study area

Challawa industrial estate is located along Panshekara Road, bordering Zawachiki town to the West, Kumbotso town to the East, Yadanko village to the South and Panshekara town to the North, respectively (Tijjani, 2014) in Kano state. The industrial estate is occupied by several industries such as tanneries, Pulp and Paper, Beverages, Ceramics, Water bottling and Textile industries.

Sample collection

Tannery effluent was taken from Challawa industrial estate as described by Cheesebrough (2006) and American Public Health Association (1999) using dark sterile sampling bottles which were labelled, bearing name of sample, location, the date and time of collection. The waste discharge sample was taken from the point in the facility premises where the effluent was thoroughly mixed and discharged before getting to the collecting reservoir. After collection the samples were placed in a cooler (at 4^0C) brought to the Microbiology Research laboratory, Bayero University Kano for analysis.

Sample processing

i) Physicochemical analysis

Determination of pH and Electrical Conductivity (EC) of the samples

The pH and Electrical Conductivity (EC) of the samples were determined according to Tijjani (2014) where an electrometric method using Water quality meter (Model 8603) was employed. A calibrated pH meter was used to measure the pH of tannery effluent. For electrical conductivity the EC electrode was calibrated using a standard buffer and the probe was inserted into tannery effluent and allowed for 30-60 seconds to maintain a steady reading. The reading displayed was recorded as micro Siemens per centimetre (µS/cm).

Determination of Temperature, total dissolved solid and total suspended solid

Temperature, total dissolved solid (TDS) and total suspended solid (TSS) were determined using water quality meter (Model 8603) as described by Tijjani (2014) the readings were recorded as mgL^{-1}, respectively.

Determination ofdissolved oxygen (DO) and biological oxygen demand

Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD) were also determined using the water quality meter (Model 8603) as described by method of Radojavik and Bashkin (1999). The electrode of the instrument was inserted into the effluent to determine the DO at the point of collection. While the BOD was obtained after the sample was collected, diluted with sterile distilled water a hundred folds and allowed for 5 days at temperature of 25^0C. The BOD was determined by inserting the electrode of instrument into the bottle and then subtracting the initial DO from the final value to obtain BOD. The readings were recorded as milligram per litre (mg/L).

ii) Determination of chromium concentration in the effluent sample

Determination of chromium concentration in the effluent sample was carried out according to the method described by APHA (1999) and Campos *et al*.(2005) for determining heavy metals. One hundred (100) mls of effluent sample was dispensed and 10mls concentrated HNO_3 was added. The mixture was digested using a hot plate and filtered through a filter paper (Whatman 125mm) to remove any insoluble material. The concentration of chromium concentration in the digest was determined by atomic absorption spectrophotometer (Bulk Scientific VGP 210 model).

iii) Isolation of bacteria with chromium bioremediation potential

Isolation of bacteria with chromium bioremediation potential was based on the methods described by Willey *et al.* (2008). From the tannery effluent, 1ml was serially diluted in ten folds. Then 0.1ml aliquots from the 10^{-5} tube was aseptically inoculated into already prepared plates of nutrient agar, specially formulated, containing Cr salt of known concentration (50mg/L). The plates were incubated at 37^0C for 24 hours.

After incubation, the bacterial colonies that developed were regarded as those that utilize the Cr in the medium and as such were tolerant. These isolates were two (2) and regarded as B_{11} and B_{15} and later identified using standard microbiological methods that employs cultural, morphological and standard biochemical tests as described by Bergey and Holt (2000); Cheesbrough (2006); Ogundana (1996) which include Gram staining, Starch hydrolysis (Amylase) test, Voges-Prokauer test, Catalase test, Citrate utilization test and Acid Production (Glucose).

Determination of minimum inhibitory concentration (MIC)

The identified Cr resistant bacterial isolates were further subjected to varied concentrations of Cr to determine the degree of their resistance to it and establish the minimum inhibitory concentration. The isolates were inoculated individually on nutrient agar plates containing 100, 200 up to 400mg/L Cr. These plates were incubated at 37^0C for 24 hours.

The isolates which grew in the higher concentrations of the Cr concentrations were selected, subcultured on NA slants and stored and later assessed for bioremediation potential.

Determination of the bioremediation potential of chromium tolerant bacteria

The Bioremediation potential of the Cr tolerant bacterial isolates was carried out using the tannery effluent sample. The tannery effluent was poured into a $1000cm^3$ sterile containers, closed and tyndallised by heating at 80^0C for 15 minutes and allowed to cool according to the methods described by Tijjani (2014). This procedure was carried out for three (3) days to kill all living microbes while maintaining the vital components (chemical and organic) of the effluent.

An inoculum equivalent to 0.5 McFarland Standard was prepared as described by Cheesbrough (2000) from the cultures of bacterial isolates (B_{11} and B_{15}) that have been identified with the highest tolerance to Cr.

The bioremediation set up comprised four (1000mls) flasks. First one was labelled B_{11} and the second B_{15}, the third was labelled $B_{control}$. To each of the bioremediation containers 700mls of tyndallised tannery effluent and 20mls of 1g sucrose plus 1g peptone solution were added. Then 80mls of the standardize bacterial isolate(B_{11}) was added to flask labelled B_{11} to make up to 800mls bioremediation solution. Another 20mls of standardise bacterial isolate (B_{15}) was added to flask labelled B_{15}. All the flasks including the $B_{control}$ were kept on rotary shaker at 180rpm and 37^0C for a period of 7days.

At intervals of 48 hours, 96 hours and 144 hours respectively, the bioremediation potential of the bacterial isolates was assessed and determined as described by Makut *et al.* (2018); Tijjani, (2014); Radojevic and Bashkin, (1999). A representative sample of 100ml was taken from each of the bioremediation setup (B_{11}, B_{15} and $B_{control}$) and centrifuged at 3500rpm for 15 minutes. Thereafter, 50mls of the supernatant was measured into a 250mls beaker and 10mls of nitric acid added. The mixture was heated on a hotplate. At intervals, more nitric acid was added. The mixture was heated until the content was reduced to 10mls and has digested completely, having a representative light colourless and clear solution. The content was then

filtered through a filter paper (Whatman 125mm) to remove any insoluble material. The filtrate was diluted with distilled water to 50mls. The Cr concentration of the digested sample wasthen measured using the Atomic Absorption Spectrophotometer (Bulk Scientific VGP 210 model) machine. All the results were recorded and percentage heavy metal removal was calculated as shown below:

$$\%\text{Heavy metal removal} = \frac{\text{Initial heavy metal concentration-Final heavy metal conc (mg/L)}}{\text{Initial heavy metal concentration (mg/L)}} \times 100$$

RESULTS

The physico-chemical analysis result indicated that the raw tannery effluent had lower temperature of 32^0C compared to the tyndallised effluent which had 37^0C(Table 1). Also, the pH of the tyndallised tannery was higher compared to that of the raw tannery effluent (Table 1). However, the values of other parameters such as Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Electrical Conductivity (EC) and Biological Oxygen Demand (BOD) were higher in the raw tannery effluent compared to the tyndallised tannery effluent(Table 1). More so, colour and odour in both raw and tyndallised tannery effluent were the same having the characteristic peach colour and pungent odour, respectively. The concentration of Cr was higher in the raw tannery effluent when compared to the tyndallised tannery effluent (Table 1).

Table 1: Physico-chemical Analysis of Raw and Tyndallised Tannery Effluent

Physico-chemicalParameters	Tannery Effluent	
	Raw	Tyndallised
Temperature (^{0}C)	32	37
pH	5.1	5.6
Colour	Peach	Peach
Odour	Pungent	Pungent
TDS (mg/L)	4882	4564
TSS (mg/L)	3930	3518
EC (µS/cm)	7629	7131
BOD_5 (mg/L)	432	424
Cr (mg/L)	0.5714	0.2857

Two bacterial isolates (B_{11} and B_{15}) were isolated from the tannery effluent and their Grams reaction and morphological characteristics revealed that all the isolates were gram positive spore forming rods, bearing a central spore and based on biochemical tests B_{11} was identified as *Bacillus marinus* and B_{15} *Bacillus badius*. Table 2 reveals that both bacterial isolates ($B_{11}$and B_{15}) were inhibited at Cr concentration of400mg/L.

Table 2: Minimum Inhibitory Concentration (MIC) Results for Bacterial Isolates

Isolates	Minimum Inhibitory Concentration (MIC) of Chromium			
	100mg/L	200mg/L	300mg/L	400mg/L
B_{11} (*Bacillus marinus*)	+	+	+	-
B_{15} (*Bacillus badius*)	+	+	+	-

Key: += Growth (No inhibition);- = Inhibition (Growth)

The results of the study further revealed that the two isolates, B_{11} and B_{15} exhibited bioremediation potential and that Cr concentration was lowered from 0.2857 to 0.1429 mg/l (49.98% reduction) over a period of 96 hours by B_{11} and over a period of 48 hours by B_{15} (Table 3).

Table 3: % Removal of Chromium by Bacterial Isolates from Tannery Effluent

Isolates	Time (hrs)	Heavy metal Concentration and % removal	
		Cr (mg/L)	% reduction
***Bacillus marinus* (B_{11})**	48	0.2857	-
	96	0.1429	49.98
	144	0.2857	-
***Bacillusbadius* (B_{15})**	48	0.1429	49.98
	96	0.2857	-
	144	0.1429	49.98

Note: Initial concentration of Chromium before remediation at 0 hours = 0.2857mg/L; Negative (-) = No %reduction recorded; hrs = hours; Cr = Chromium

DISCUSSION

The findings of this study are consistent with earlier studies that tannery effluents are a major source of environmental pollution posing public health issues. This is evidenced by the physico-chemical parameters of the tannery effluent analysed in this study which revealed higher concentrations of TSS, TDS, BOD amongst others. Also, the characteristic pungent odour and peach colour of the tannery effluent did not meet the standard described by the National Environmental Standards and Regulation Agency (NESREA) (2009). This is not surprising as Challawa Industrial Estate in Kano which is famous with tanning activities is impacted with foul odour which causes serious discomfort and also contributes to the pollution problems witnessed in the area. Earlier studies explained that the tannery plants emit ammonia, hydrogen sulphide, volatile hydrocarbons, amines and aldehydes as effluents and that volatile organic compounds (VOC) emissions during the various tanning process, if not adequately controlled, could pose a threat to the atmosphere (Dixit *et al.*, 2015). The findings of this study indicated that heat (tyndallisation) may have an effect on the concentration of heavy metals which were shown to reduce after heating as revealed by the difference in Cr concentration of the raw and tyndallised tannery effluent.

Bacillus marinus and *Bacillus badius* were isolated from this study and were demonstrated to exhibit Cr tolerance as such considered potential agents for bioremediation. Both bacteria were inhibited at a concentration of 400mg/L by Cr. Earlier studies by Alzahrani and Ahamed (2015) indicated that two strains of *Bacillus subtilis* (Strain OSTAM2 and Strain OSTAM1) had MIC of 1000mg/L to Pb. Similar works by Makut *et al.* (2018) and Tijjani (2014) also identify *Bacillus* species (*Bacillus subtilis* and *Bacillus cereus*) among their bacterial isolates. Another study by Marzan *et al.* (2017) revealed that the test bacteria *Gemella sp.* had a MIC of between 1900µg/ml against Pb and 360µg/ml against Cr, respectively, while *Micrococcus* sp. had a MIC of 1800µg/ml against Pb and 345µg/ml against Cr.

The findings of this study are in agreement with previous documented works that revealed that the bacteria Bacillus (Firmicutes) are implicated in several bioremediation of pollutants and according to Sorkhoh *et al.* (1993) 368 isolates belonging to the genus Bacillus were isolated from desert samples. Both bacterial isolates in this study were able to reduce Crconcentration in the effluent after a period of time. Percentage heavy metal (Cr) removal by *Bacillus badius* after 48 hours was 49.99% (from initial concentration of 0.2857mg/Lto 0.1429mg/L) while *Bacillus marinus* reduced Cr concentration by 49.99% over a period of 96

hours. Compared with the findings of this study, Tijjani (2014) reported a 20.8% Cr uptake by *Bacillus cereus* and a 25.3% Cr uptake by *Pseudomonas aeruginosa*, respectively, after 120 hours of bioremediation. Godheja *et al.* (2017) reported that *Bacillus cereus* showed resistance against some antibiotics, tolerance to some heavy metals and degraded selected hydrocarbons. Singh *et al*. (2010) also reported multiple resistances against heavy metals and antibiotics by *Bacillus cereus* isolates. Earlier studies have shown that bacteria exhibit different mechanism for tolerance to and bioremediation of heavy metals which may be plasmid mediated (Silver and Phung, 1996) or chromosomal mediated (Virender *et al*., 2010). Studies by Sanjay *et al.* (2018) on Cr reducing bacteria revealed *Klebsiella pneumonia* and *Mangrovibacter yixingensis* as possessing chromium reductase gene which explained their tolerance to Cr (VI), upto 80mg/L and 100mg/L, respectively. In another study González *et al*. (2014) demonstrated that *Serratia* sp. could reduce 80% of 20mg/L Cr (VI), while Kabir *et al*. (2018) isolated five novel Cr (VI) reducing bacteria from tannery effluents and solid wastes identified as *Kosakonia cowanii* MKPF2, *Klebsiella pneumonia* MKPF5, *Acinetobacter gerneri* MKPF7, *Klebsiella variicola* MKPF8 and *Serratia marcescens* MKPF12.

It is interesting to note that in some instances for example, for *Bacillus badius* (B_{15}) the 49.98% reduction of Cr after 48hours of bioremediation reversed (desorbed) after 96hours and at 144 hours was reduced again. Gupta *et al*. (2016) reported that the process of heavy metal ion binding to bacterial cell wall can be metabolism dependant (active) or independent (passive) and that the first step is passive biosorption which proceeds rapidly by any of the following metal binding mechanisms; coordination, complexation, ion exchange physical adsorption or inorganic micro-precipitation. They further explained that the biosorption is a dynamic equilibrium of reversible adsorption-desorption and that metal ions bound on the surface can be eluted by other ions, chelating agents or acids and this could explain the fluctuations in Cr removal ability of the bacteria observed in the present study. The passive uptake of heavy metals takes place immediately and are thought to be as a result of physical adsorption or ion exchange at the cell surface, reaching the equilibrium within 30-40 minutes.

Other factors that are responsible for the heavy metal desorption are pH and biomass (Das, 2008). Galun *et al*. (1987); Friis and Keith (1986) identified the pH as the most important parameter in the biosorption process as it affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of the metallic ions. Das (2008) noted that biomass concentration in the solution seems to influence the specific uptake; for lower values of biomass concentration leads to interference between the binding sites. In a review Igiri *et al*. (2018) explained that microbial-metal interactions is primarily focused on metals removal, i.e., remediation and depollution and that several factors which influences and limit bioremediation efficiency include temperature, pH, redox potential, nutritional status, moisture, and chemical composition of heavy metals.

CONCLUSION

The study concluded that the tannery effluent was highly polluted and contains high concentration of Cr that poses environmental and public health threat. The study isolated two bacteria (*Bacillus marinus* and *Bacillus badius)* that exhibited Cr tolerance. Both bacteria demonstrated bioremediation potential with *Bacillus badius* reducing 49.98% Cr over a period of 48 hours and *Bacillus marinus* over a period of 96 hours. The study identifies the bacterial isolates as potential bioremediation agents which if fully harnessed and optimised could contribute significantly to environmental friendly approach in management of heavy metal pollution from tannery effluent.

REFERENCES

Altaf, M. M., Masood, F. and Malik, A. (2008). Impact of Long-Term Application of Treated Tannery Effluents on the Emergence of Resistance Traits in Rhizobium Sp. Isolated from *Trifolium alexandrinum*. *Turkish Journal of Biology*, **32**: 1-8.https://dergipark.org.tr/en/download/article-file/121353.

Alzahrani, O. M. and Ahamed, N. T. (2015). Isolation and Characterization of Heavy Metal Resistant *Bacillus subtilis* spp. Collected from Water Sources of Taif Province of Saudi Arabia. *International Journal of Current Microbiology and Applied Sciences*, **4** (6): 350-357. http://www.ijcmas.com

American Public Health Association (APHA) (1999). *Standard Methods for the Examination of Water and Wastewater.*20th edition. American Public Health Association, American Water Works Association, Water Environment Federation.

Bergey, D. H. and Holt, J. G. (2000). *Bergey's Manual of Determinative Bacteriology.* 9th ed. Philadelphia: Lippincott Williams & Wilkins.

Bhattacharya, A., Gupta, A., Kaur, A. and Malik, D. (2019). Alleviation of Hexavalent Chromium by using Microorganisms: Insight into the Strategies and Complications. *Water Science & Technology*, **79** (3): 411-423.doi: 10.2166/wst.2019.060.

Campos, V. L., Moraga, R., Ya´nez, J., Zaror, C.A. and Mondaca, M. A. (2005). Chromate Reduction by *Serratiamarcescens* Isolated from Tannery Effluent. *Bulletin of Environmental Contamination Toxicol*ogy, **75**:400-406. DOI:10.1007/s00128-005-0767-z

Cheesebrough, M. (2006).*District laboratory practice in tropical countries.* Part 2.Low Price Edition. Cambridge University Press, London, pp28-90.

Das, N.,Vimala, R. and Karthika, P.(2008).Biosorption of Heavy Metals – An Overview. *Indian Journal of Biotechnology,* **7** (2) : 159-169. https://www.researchgate.net/publication/228614323_Biosorption_of_Heavy_Metals_an_Overview.

Dixit, S., Yadav, A., Dwivedi, P. D. and Das, M. (2015). Toxic Hazards of Leather Industry and Technologies to Combat Threat: A Review. *Journal of Cleaner Production,* **87**: 39-49.https://www.academia.edu/10356294/Toxic_hazards_of_leather_industry_and_technologies_to_combat_threat_a_review

Dmytrenko, G.M, Ereshko, T.V., and Konovalova, V.V. (2007).Reduction of Chromium (VI) by Bacteria Collection Strains of Different Physiological Groups.In: Heipie, H. J. (eds) Bioremediation of Soils Contaminated with Aromatic Compounds.NATO Science Series, 76. Springer Publisher, Netherlands, pp125-130. https://doi.org/10.1007/978-1-4020-5693-2_11.

Friis, M. and Keith, M. (1986). Biosorption of Uranium and Lead by *Streptomyces logwoodensis*. *Biotechnology and Bioengineering*, **28** (1): 21-28.doi: 10.1002/bit.260280105.

Galun, M., Galun, E., Siegel, B. Z., Keller, P., Lehr, H. and Siegel, S.M. (1987). Removal of metal ions from aqueous solution by *Penicillium* biomass: Kinetic and uptake parameters. *Water, Air and Soil Pollution*, **33**:359-371.https://doi.org/10.1007/BF00294204.

González, P.S., Ambrosio, L.F., Paisio, C.E., Talano, M.A., Medina, M.I. and Agostini, E. (2014). Chromium (VI) Remediation by a Native Strain: Effect of Environmental Conditions and Removal Mechanisms Involved. *Environmental Science Pollution and Research*,**21**(23):13551-9. doi: 10.1007/s11356-014-3311-z.

Godheja, J., Shekhar, S.K., Satyanarayan, G.N.V., Singh, S.P. and Modi, D.R. (2017). Antibiotic and Heavy Metal Tolerance of some Indigeneous bacteria Isolated from Petroleum Contaminated Soil Sediments with a Study of their Aromatic hydrocarbon Degradation Potential. *International Journal of Current Microbiology and Applied Sciences*, **6** (3): 194-211.https://doi.org/10.20546/ijcmas.2017.603.021

Gupta, A., Joia, J., Sood, A., Sood, R., Sidhu, C. and Kaur, G. (2016). Microbes as Potential Tool for Remediation of Heavy Metals: A Review. *Journal of Microbial and Biochemical Technology*,**8**: 364-372. doi:10.4172/1948-5948.1000310

Igiri, B.E.,Okoduwa, S.R.I., Idoko, G.O., Akabuogu, E.P., Adeyi, A.O. and Ejiogu, I.K. (2018). Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review.*Journal of Toxicology*, **2018** (Article ID 2568038):1-16. https://doi.org/10.1155/2018/2568038

Kabir, M.M., Fakhruddin, A.N.M., Chowdhury , M.A.Z., Pramanik, M.K. and Fardous, Z. (2018). Isolation and Characterization of Chromium(VI)-reducing Bacteria from Tannery Effluents and Solid Wastes. *World Journal of Microbiology and Biotechnology,* **34** (9) : 126. DOI: 10.1007/s11274-018-2510-z

Mahdavi, T. A., Donnelly, T., and Anderson, G. K. (2001). Color Removal from a Simulated Dye Wastewater using A Two-Phase Anaerobic Packed Bed Reactor. *Water Resources*, **35**(2): 425-432.doi: 10.1016/s0043-1354(00)00280-3.

Makut, M.D., Gimba A. and Bello A. (2018). Studies on Removal of Heavy Metal from Tannery Effluent by Native Isolates of Bacteria and Fungi. *Asian Journal of Biotechnology and Bioresource Technology*, **3** (4): 1-8.

Marzan L.W., Hossain M., Mina S.A., Akter Y., Chowdhury, A.M.M. A. (2017). Isolation and Biochemical Characterization of Heavy-Metal Resistant Bacteria from Tannery Effluent in Chittagong City, Bangladesh: Bioremediation Viewpoint. National Institute of Oceanography and Fisheries. Elsevier. *Egyptian Journal of Aquatic Research,* **43:**65-74.DOI:10.1016/j.ejar.2016.11.002.

National Environmental Standards and Regulations Enforcement Agency (NESREA) (2009). National Environment (Textile, Wearing Apparel, Leather and Footwear Industry) Regulations 2009, Schedule 1b: Effluent Limitation Standard for Tanning and Leather Finishing. *Federal Republic of Nigeria Official Gazette*, **96** (62): 1253-1273.https://www.nesrea.gov.ng/wp-Wearing%20Apparel_Leather_and_Footwear_Industry_ Regulations%202009. pdf

Ogundana S.K. (1996). Introduction to Microbiology: *A Laboratory Manual*. Lagos Nigeria: Vikas Publishers.

Ogunwa, U.B., Ogabiela, E.E., Lawal, F.A. and Owoeye, L.D. (2006). Analysis of Tannery Effluents from the Challawa Industrial Estate in Kano, Nigeria. *Global Journal of Pure and Applied Sciences*, **12**: 69-72.**DOI:** 10.4314/gjpas.v12i1.16568

Radojavik, M. and Bashkin, V. N. (1999). *Practical Environmental Analysis*. Cambridge. The Royal Society of Chemisty, Cambridge, pp. 189-203.

Silver, S. andPhung, L.T. (1996). Bacterial Heavy Metal Resistance: New Surprises. *Annual Revision Microbiology*, **50**: 753-789.https://doi.org/10.1146/annurev.micro.50.1.753.

Singh, S.K., Tripathi, V.R., Jain, R.K., Vikram, S. and Garg, S.K. (2010). An Antibiotic, Heavy Metal Resistant and Halotolerant *Bacillus cereus* SIU1 and its Thermoalkaline Protease. *Microbial Cell Factories,* **9**:9-59. doi:10.1186/1475-2859-9-59.

Sorkhoh, N.A., Al-Hasan, R.H., Khanafer, M. and Radwan, S.S. (1995). Establishment of Oil-Degrading Bacteria Associated with Cyanobacteria in Oil-Polluted Soil. *Journal of Applied Bacteriology,* **78**: 194-199.https://doi.org/10.1111/j.1365-2672.1995.tb02842.x

Sundar V. J., Rao J. R. and Muralidharan C. (2002). Cleaner Chrome Tanning-Emerging Options.*Journal of cleaner production,* **10:** 69-74.

Sanjay, M.S., Sudarsanam, D., Raj, G.A. and Baskar, K. (2018). Isolation and Identification of Chromium Reducing Bacteria from Tannery Effluent. *Journal of King Saud University Science.* **32** (1): 265-271. DOI: 10.1016/j.jksus.2018.05.001.

Tijjani, M.B. (2014). Impact of Tannery Effluents on the Quality of Environmental Samples and Bioremediation Potentials of Microbial Isolates. A Dissertation Submitted to the

School of Postgraduate Studies, Ahmadu Bello University, Zaria, Nigeria. http://hdl.handle.net/123456789/5932.

Tudunwada, I. Y., Essiet, E.U. and Muhammad, S. G. (2007). The effects of tannery sludge on heavy metals concentration in cereals on small-holder farms in Kano, Nigeria. *Journal of Environmental Control,* **35** (2): 65-69.DOI:10.15835/nbha352214.

Virender, S., Chauhan, P.K., Kanta, R., Tejpal, D. and Vinod, K. (2010). Isolation and Characterisation of Pseudomonas Resistant to Heavy Metals Contaminants. *International Journal of Pharmaceutical Science Revision Resistance,* **3**(2): 164-167. www.globalresearchonline.net.

Willey, J.M, Sherwood, L.M. and Woolverton C.J. (2008). *Prescott, Harley, and Klein's Microbiology*. 7th edition. McGraw-Hill Companies New York, pp 114-115.

Wionczyk B., Apostoluk, W. and Charewicz, W. A. (2006). Solvent Extraction of Chromium (III) from Spent Tanning Liquors with Aliquat 336. *Journal of Hydrometallurgy*, **82** (1-2): 83-92. https://doi.org/10.1016/j.hydromet.2006.03.055