Brazilian Journal of Biological Sciences, 2018, v. 5, No. 9, p. 25-32.
ISSN 2358-2731
https://doi.org/10.21472/bjbs.050903

# Biosorption of lead, chromium and cadmium in tannery effluent using indigenous microorganisms

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**Abstract**. This study investigated the biosorption of lead, chromium and cadmium in tannery effluent using indigenous microorganisms. Bacteria isolated from the tannery effluent were *Bacillus subtilis* and *B. megaterium* while fungi isolated were *Aspergillus niger* and *Penicillium* sp. The microorganisms were tested for their ability to reduce the concentration of the heavy metals in the tannery effluent using conventional methods. *B. megaterium* recorded the highest lead reduction (2.13 to 0.03 mg/L), followed by *B. subtilis* (2.13-0.04 mg/L). *A. niger* recorded the highest ability to reduce the concentration of chromium (1.38-0.08 mg/L) followed by *Penicillium* sp. (1.38-0.13 mg/L) while *B. subtilis* exhibited the highest ability to reduce the concentration of cadmium (0.4-0.03 mg/L) followed by *B. megaterium* (0.04-0.06 mg/L) after 20 days. When these values were compared to standard limits of Federal Environmental Protection Agency (FEPA), World Health Organization (WHO), National Environmental Standard and Regulations Enforcement Agency (NESREA) and Central Pollution Control Board (CPCB), the isolates recorded an acceptable reduction in the concentration of lead, chromium and cadmium in sterile and unsterile tannery effluent. The results of this showed that the isolates reduced the concentration of lead, chromium and cadmium present in the sterile and raw tannery effluent and suggest that the organisms can be used as a possible treatment of tannery effluents.

**Keywords**: Tannery effluent; Lead; Chromium; Cadmium; Biosorption; Bacteria; Fungi.

Received
March 09, 2018

Accepted
April 23, 2018

Released
April 30, 2018

Full Text Article

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## Introduction

Industrial effluents such as tannery effluent contains a high amount of metals especially chromium, copper, iron, zinc, mercury and cadmium (Malarkodi et al., 2007; Devi et al., 2011). Leather industries and tanneries

generate massive by-products, solid wastes, high amount of wastewater rich in organic wastes different load of pollutants and emissions into the air. These effluents are released onto the land as well as into the surface water, which eventually reach the level of ground water and lead to contamination due to accumulation of toxic metallic components. This results into a series of problems when consumed, because they are partially or cannot be completely degraded (Malarkodi et al., 2007). The effluents contain several types of chemical such as dispersants, levelling agents, acids, alkalis, various dyes, phenols, carbonates, alcohols, cyanides and heavy metals (Noorjahan, 2014).

Effluents from raw hide processing tanneries contain components of chromium and sulphides in most cases with a major proportion of dyes (Noorjahan, 2014). Majority of tanneries worldwide use chromium (Cr III and Cr IV), which are highly toxic and poses a serious threat to the environment upon improper disposal of their waste water. Even at low concentrations, these salts have a toxic effect on the food chain of fish and inhibits photosynthesis of aquatic plants (Bosnic et al., 2000). Bello et al. (2016) reported heavy metal accumulations in vital human organs via the consumption of crops after the discharge of tannery effluents for irrigation purpose. This results into various degrees of illnesses on acute and chronic exposure, such as cancer, kidney dysfunction, cholera and skin irritations (Mustafa et al., 2010). Heavy metals in the tannery effluent is one of the most hazardous environmental pollutants, toxic heavy metals like Cr, Cu, Zn, Pb and Cd are mostly absorbed and get accumulated in various plant part as free metals, which may adversely affect the plant growth and metabolism. Major diseases of cattle and human beings are caused by chromium and nickel and cancer (Sivakumar and Thippeswamy, 2012).

The conventional treatment of tannery effluents for the purpose of detoxification requires application of physical and chemical methods, which involve the chrome precipitation and sulphide treatment including physicochemical methods such as filtration, specific coagulation, use of activated carbon and chemical flocculation (Olukanni et al., 2006). But due to associated problems in these treatment methods such as high cost, intense experimental set-up, in complete treatment of wastewater leading to post-treatment effects (Do et al., 2002; Maier et al., 2004), other alternative treatment methods have been researched such as the use of biological methods using bacteria, fungi and algae (Srinivas and Estari, 2013).

Therefore, the aim of this study was to utilise bacteria and fungi isolated from tannery effluent for the biosorption of Cr, Pb and Cd in tannery effluent.

## Materials and methods

### Sample collection

The tannery effluent contaminated soil and tannery effluent samples were collected from the Leather Processing Industry Site in Zaria, Nigeria, in sterile different containers and were transported to the Microbiology Laboratory of the Federal University of Technology Minna, Nigeria, for microbiological and physiochemical analysis.

### Isolation of microorganisms

One gram of tannery effluent contaminated soil sample was serially diluted with 9 mL of sterile water. The 0.1 mL of the diluted soil sample was plated on Nutrient Agar (for bacteria) and Sabouraud Dextrose Agar (for fungi) using pour plate method of isolation. The plates were incubated at 37 $^{o}$C for 24 h (for bacteria) while for fungi, the plates were incubated at 28 ± 2 $^{o}$C for 4 days.

**Identification of bacterial isolates**

Each colony that differ in size, shape and colour were subcultured to obtain pure isolates. The bacteria isolated were identified based on the colonial, microscopic and biochemical characteristics. The following biochemical tests were used: Gram staining**,** motility, indole production, methyl red, Voges Proskauer, citrate utilization, production of oxidase, catalase, coagulase, and urease, sugar fermentation test, spore staining, nitrate reduction test and starch hydrolysis (Cheesbrough, 2006). The bacterial isolates were identified by comparing their characteristics with those of known taxa (Holts et al., 1994).

**Identification of fungi isolates**

Fungi identification was based on the morphological and microscopic examination of each isolate. Morphological examination included colonial and colour while microscopic examination involved staining with lactophenol cotton blue and viewed using x10 and x40 objective lens. The fungal isolates were identified by comparing their characteristics with those of known taxa (Domsch and Gams, 1970).

**Preparation of heavy metal solutions**

The stock solution of chromium was prepared by dissolving 0.002 g of potassium dichromate in 500 mL of distilled water. To obtain 1.5 ppm, the solution was shaken for 15 minutes and allowed to stand for 24 h. The stock solution of lead was prepared by dissolving 0.0015 g of lead acetate in 500 mL of distilled water to obtain 1.5 ppm. The stock solutions of cadmium was prepared by dissolving 0.0017 g of cadmium sulphate in 500 mL of distilled water to obtain 1.5 ppm. To obtain 1.0 ppm, 167 mL from each stock solutions was measured into 250 mL volumetric flask, and then distilled water was added to make it to 250 mL, while the concentration of 0.5 ppm, 50 mL from 250 mL (1.0 ppm concentration) was measured into 100 mL volumetric flask and distilled water was added to make I to 100 mL (Knopka and Zakharova, 1999).

**Screening of the isolates for the potential to utilise heavy metals**

The different concentrations (1.5 ppm, 1.0 ppm and 0.5 ppm in 500 mL, 250 mL, and 100 mL) of each heavy metal (chromium, lead and cadmium) were prepared using the agar dilution method, with nutrient agar for bacteria and Sabouraud dextrose agar for fungi (Jayanthi et al., 2014). The media were sterilised using autoclave at 121 $^{o}$C for 15 min. After sterilisation, each was poured into Petri dish. The test organisms were inoculated using streak plate technique and incubated at 37 $^{o}$C (bacteria) and 28 ± 2 $^{o}$C (fungi) for 72 h. Development of bacterial and fungal colonies indicate the ability of the isolates to tolerate the heavy metal while absence of visible colonies indicates that the test organisms cannot tolerate the heavy metals.

**Determination of Pb, Cr and Cd in tannery effluent**

The 1 L of potatoes dextrose broth was prepared by boiling 300 g of fresh potatoes at 100$^{o}$C in 500 mL of tannery effluent in a beaker. The potatoes were removed and the beaker was filled with tannery effluent to make up 1 L. The 20 g of glucose and 0.4 g of chloramphenicol were added (for fungi). For the preparation of nutrient broth medium, 15 g of nutrient broth was dissolved in 1 L of tannery effluent (for bacteria).

The 50 mL of each medium was dispensed into 56 of 100 mL conical flask. The 24 of the flasks were sterilised while the other 24 were not sterilised and 8 of the conical flasks were used as control (no microorganism). The 1 mL of 3 h old culture of each isolate (in

triplicate) was inoculated into the 24 flask.

The inoculated flasks were incubated aerobically in an incubator shaker at 37°C, while the fungi were incubated at 28±2°C (Deivasgamani and Das, 2011) for 20 days. Samples were removed at 5 days interval and centrifuged at 1200 rpm for 15 min to separate the residue from supernatant. The supernatant was dispensed into clean sterile container and analysed to determine the level of Pb, Cr and Cd using AAS.

### Statistical analysis

Analysis of variance was used to determine significant difference between means, using SPSS version 16.0 and one tailed paired student's t-test was used to determine statistical significance between the untreated and treated parameters.

## Results

The bacteria isolated from the tannery effluents were identified as *Bacillus subtilis, B. megaterium* while the fungi isolates were identified as *Aspergillus niger and Penicillium* sp. (Table 1). There was a significant reduction in lead concentration in sterile effluents by the isolates when compared with day 0 throughout the period of 20 days. *A. niger* that showed a significant increase in lead concentration at day 5. *B. megaterium* recorded the highest lead reduction (2.13 to 0.03 mg/L), followed by *B. subtilis* (2.13-0.04 mg/L), *A. niger* (2.13-0.1 mg/L) and *Penicillium* sp. (2.13-0.14 mg/L) after 20 days (Table 1). *A. niger* recorded the highest ability to reduce the concentration of chromium (1.38–0.08 mg/L), followed by *Penicillium* sp. (1.38-0.13 mg/L), *B. megaterium* (1.38-0.20 mg/L) and the least was *B. subtilis* (1.38-0.23 mg/L) (Table 2). *B. subtilis* exhibited the highest ability to reduce the concentration of cadmium (0.4-0.03 mg/L) followed by *B. megaterium* (0.04-0.06 mg/L), *A. niger* (0.4-0.08 mg/L) and *Penicillium* had the least (0.4-0.1 mg/L) (Table 3).

**Table 1**. Microorganisms isolated from the effluent and their ability to uptake Pb in sterile tannery effluent.

| Time (days) | *Bacillus subtilis* | *B. megaterium* | *Aspergillus niger* | *Penicillium* sp. |
|---|---|---|---|---|
| 0 | 2.13 ± 0.10$^{a}$ | 2.13 ± 0.10$^{a}$ | 2.13 ± 0.04$^{a}$ | 2.13 ± 0.04$^{a}$ |
| 5 | 2.00 ± 0.00$^{b}$ | 1.30 ± 0.005$^{b}$ | 2.41 ± 0.02$^{a}$ | 1.56 ± 0.04$^{b}$ |
| 10 | 1.32 ± 0.005$^{c}$ | 1.17 ± 0.01$^{c}$ | 1.37 ± 0.03$^{b}$ | 1.24 ± 0.01$^{c}$ |
| 15 | 0.09 ± 0.00$^{d}$ | 0.06 ± 0.051$^{d}$ | 0.47 ± 0.02$^{c}$ | 0.47 ± 0.02$^{d}$ |
| 20 | 0.04 ± 0.01$^{c}$ | 0.03 ± 0.005$^{e}$ | 0.01 ± 0.003$^{d}$ | 0.14 ± 0.001$^{e}$ |

Values are mean ± standard error of mean of triplicate determination. Values with different letters down the row are significantly different ($p<0.05$). Concentration (mg/mL).

**Table 2**. Microorganisms isolated from the effluent and their ability to uptake Cr in sterile tannery effluent.

| Time (Days) | *Bacillus subtilis* | *B. megaterium* | *Aspergillus niger* | *Penicillium* sp. |
|---|---|---|---|---|
| 0 | 1.38 ± 0.00$^{a}$ | 1.38 ± 0.00$^{a}$ | 1.38 ± 0.00$^{a}$ | 1.38 ± 0.00$^{a}$ |
| 5 | 1.16 ± 0.015$^{b}$ | 1.25 ± 0.003$^{b}$ | 1.06 ± 0.002$^{b}$ | 1.07 ± 0.006$^{b}$ |
| 10 | 1.03 ± 0.005$^{c}$ | 1.02 ± 0.01$^{c}$ | 0.91 ± 0.01$^{c}$ | 0.86 ± 0.07$^{c}$ |
| 15 | 0.84 ± 0.01$^{d}$ | 0.86 ± 0.005$^{d}$ | 0.38 ± 0.006$^{d}$ | 0.44 ± 0.004$^{d}$ |
| 20 | 0.23 ± 0.005$^{e}$ | 0.20 ± 0.005$^{e}$ | 0.08 ± 0.003$^{e}$ | 0.13 ± 0.005$^{e}$ |

Values are mean ± standard error of mean of triplicate determination. Values with different letters down the row are significantly different ($p<0.05$). Concentration (mg/mL).

**Table 3**. Microorganisms isolated from the effluent and their ability to uptake Cd in sterile tannery effluent.

| Time (Days) | *Bacillus subtilis* | *B. megaterium* | *Aspergillus niger* | *Penicillium* sp. |
|---|---|---|---|---|
| 0 | 0.40 ± 0.00[a] | 0.40 ± 0.00[a] | 0.40 ± 0.00[a] | 0.40 ± 0.00[a] |
| 5 | 0.24 ± 0.02[b] | 0.24 ± 0.02[b] | 0.31 ± 0.02[b] | 0.44 ± 0.01[b] |
| 10 | 0.21 ± 0.00[c] | 0.18 ± 0.005[c] | 0.28 ± 0.005[c] | 0.36 ± 0.004[d] |
| 15 | 0.095 ± 0.01[d] | 0.12 ± 0.005[d] | 0.18 ± 0.001[d] | 0.27 ± 0.002[c] |
| 20 | 0.03 ± 0.005[e] | 0.06 ± 0.00[e] | 0.08 ± 0.01[e] | 0.10 ± 0.02[b] |

Values are mean ± standard error of mean of triplicate determination. Values with different letters down the row are significantly different ($p < 0.05$).

When these values were compared to standard limits of Federal Environmental Protection Agency, World Health Organization, National Environmental Standard and Regulations Enforcement Agency and Central Pollution Control Board, the isolates recorded an acceptable reduction in the concentration of lead, chromium and cadmium in sterile (Table 4) and unsterile tannery effluent (Table 5) after 20 days.

**Table 4**. The presence of heavy metals in sterile tannery effluents treated with the isolates compared with standard limits after 20 days.

| Concentration (mg/mL) | Initial value | *B. subtilis* | *B. megaterium* | *A. niger* | *Penicillium* sp. | FEPA Limits (mg/mL) | Who limits (mg/mL) | NESREA Limits (mg/mL) | CPCB Limits (mg/mL) |
|---|---|---|---|---|---|---|---|---|---|
| Pb | 2.13 | 0.04 | 0.03 | 0.10 | 0.14 | < 1.00 | 1.00 | 0.1 | 0.1 |
| Cd | 0.44 | 0.03 | 0.06 | 0.08 | 0.10 | - | - | 0.1-0.4 | 2.0 |
| Cr | 1.38 | 0.23 | 0.20 | 0.08 | 0.13 | < 1.00 | 1.00 | 0.1-0.5 | 0.1 |

FEPA: Federal Environmental Protection Agency, WHO: World Health Organization, NESREA: National Environmental Standard and Regulations Enforcement Agency, CPCB: Central Pollution Control Board, -: No fixed standard.

**Table 5**. The presence of heavy metals in sterile tannery effluents treated with the isolates compared with standard limits after 20 days.

| Concentration (mg/mL) | Initial value | *B. subtilis* | *B. megaterium* | *A. niger* | *Penicillium* sp. | FEPA Limits (mg/mL) | Who limits (mg/mL) | NESREA Limits (mg/mL) | CPCB Limits (mg/mL) |
|---|---|---|---|---|---|---|---|---|---|
| Pb | 1.38 | 1.12 | 0.21 | 0.50 | 0.42 | < 1.00 | 1.00 | 0.1 | 0.1 |
| Cd | 0.33 | 0.18 | 0.15 | 0.16 | 0.20 | - | - | 0.1– 0.4 | 2.0 |
| Cr | 1.65 | 0.65 | 0.91 | 0.22 | 0.22 | < 1.00 | 1.00 | 0.1 – 0.5 | 0.1 |

FEPA: Federal Environmental Protection Agency, WHO: World Health Organization, NESREA: National Environmental Standard and Regulations Enforcement Agency, CPCB: Central Pollution Control Board, -: No fixed standard.

## Discussion

The soil is a suitable habitat for microorganism and most bacteria and fungi are indigenous to soil, but only four isolates were obtained from the tannery effluent and the soil sample. This indicates that it is only these organisms that are able to utilize the effluent for their growth and metabolism. The presence of microorganisms in tannery effluent soil may be as a result of their

ability to utilize the compounds in the effluent samples or its soil. Their abundance and diversity may be attributed to high tanning activities and destabilization of soil ecological balance arising from the contaminations due to the discharge of the tannery waste water (Rabah and Ibrahim, 2010).

Generally, bacteria and fungi have developed various processed such as transport across to cell membrane, biosorption to cell was, entrapments in extracellular capsules, precipitation, complexation and oxidation-reduction reactions They have proven to take up heavy metals from aqueous solutions, especially when the metal concentrations in the effluent range from less than 1 mg/L to about 20 mg/L (Salman et al., 2014).

According to Meenambigail et al. (2016), microorganisms a play a vital role in bioremediation of heavy metals from contaminated soil and wastewater, but when microorganisms are exposed to higher concentration of heavy metals, it may have a deleterious effects on their growth and activities. Bacteria isolated included *Bacillus subtilis* and *B. megaterium* (Table 1). This is similar to the report of Bello et al. (2016) who reported the presence of Bacillus species in soil contaminated with industrial wastewater due to their ability to survive in extreme conditions such as heat, chemical and desiccation due to the spores. In addition, Bacillus sp. Have the ability to secrete hydrolytic enzymes capable of degrading heavy metals. Abioye et al. (2015) reported that the presence of techoic acid in *B. subtilis* (a Gram positive bacterium) may also be an added advantage because tit serves as a source of carboxyl groups that are the main agents in heavy metal uptake. *B. subtilis*. The presence of iron chelating siderophores enable them to be used in metal pollution (León et al., 2009). Similarly, *A. niger* and *Penicillium* sp. were isolated among the fungus. Hakeem and Bhatnagar (2010) argued that these organisms are widespread in nature and have been associated with industrial wastewater.

Although the four isolates showed the ability to tolerate and reduce lead from textile tannery effluent (Table1), the 2 bacteria performed better than the 2 fungi. Verma et al. (2009 reported that Bacillus species shows differences in resistance and removal of heavy metals such as lead, which may be due to the presence or absence of a metal ion transporter of a particular specificity in the bacterial species and the physiology of the cell. This is similar to how some microorganisms exhibit resistance to antimicrobial agents, which may be related to the process of their survival and their environmental adaptation. The two fungi however, were able to utilize the chromium better than the bacteria. Santhi and Guru (2014) stated that exposure of *Aspergillus* sp. to toxic heavy metals might have led to physiological adaptation and increased metal tolerance ability, which led to an increased metal biosorption capacity. Fungi have the ability to secrete a wide range of extracellular enzymes in their growth environment or growth medium. This may lead to the capability to grow on a wide range of carbon source (Bello et al., 2016). Jayanth et al. (2014) also argued that filamentous fungi are have a high ion exchange capacity within their cell walls. These biding sites have a high covalent affinity towards toxic transition metal ions such as cadmium, lead, chromium and zinc (Akar and Tunali, 2006). Akar and Tunali (2006) also reported that fungi may have affinity for cadmium, lead, chromium, carboxyl, phosphate and hydroxyl groups that are involved in the binding of heavy metals.

When compared to different standards (Table 4 and Table 5), the isolates have a great potentials for the removal of heavy metals in tannery wastes. As stated by Ezzouhri et al. (2009) and Shazai et al. (2013), the variation in the metal tolerance may be due to the presence of one or more strategies of tolerance or resistant

mechanisms exhibited by microorganisms and the level of resistance depended on the isolate.

## Conclusions

The bacterial isolates (*Bacillus subtilis* and *B. megaterium*) and fungal isolates (*A. niger* and *Penicillium* sp.) reduced the concentration of lead, chromium and cadmium present in the sterile and raw tannery effluent.

## Conflict of interest statement

Authors declare that they have no conflict of interests.

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