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PHYSICO-CHEMICAL PROPERTIES OF TANNERY EFFLUENTS FROM CHALLAWA INDUSTRIAL AREA IN KANO AND EVALUATION OF BIOREMEDIATION POTENTIALS OF *Spirogyra porticalis* AND *Chlorella vulgaris* ON THE EFFLUENTS

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ABSTRACT

Discharge of industrial effluent in aquatic environment is a serious threat to life due to toxic heavy metals. Algae can be used as cheap bioremediation agents in comparison to conventional technologies. The present study was conducted to evaluate the bioremediation potential of two algal species (Spirogyra porticalis and Chlorella vulgaris) for the removal of Lead from two tannery industries (M Tannery and G Tannery), located in Challawa Industrial Area. Temperature, EC, TDS, DO and BOD were measured. In G Tannery, Spirogyra porticalis had the highest affinity for Lead which was 51%, 55% and 51% at 3, 6 and 9 weeks interval respectively while Chlorella vulgaris affinity for Lead was 40% 45% and 45%. In M tannery, Chlorella vulgaris had the highest potential to remove Lead from the effluent, which was 90%, 86% and 90% while Spirogyra porticalis affinity for Lead was 10%, 41% and 10%. Temperature ranged from 30-31°C which might be as a result of ambient temperature. The pH of both tannery effluents did not differ significantly ($p < 0.5$) and it was not within the maximum permissible limit of 6.5-8.5. EC of M Tannery was a little higher than that of G Tannery (8.417 ± 0.26 , 6.920 ± 0.050) which were below the permissible limit. TDS of M tannery was higher than that of G Tannery $1919.0 \pm 68.46 \text{ mg/L}$, $1916.0 \pm 61.94 \text{ mg/l}$ which were not within the permissible limit. DO content were within the permissible limits of 6.4 mg/L . BOD of both effluent samples were below the permissible limit of 4 mg/L . This research shows that both species are suitable for bioremediation and are also tannery specific.

Keywords: Bioremediation, Effluent, Tannery, *Spirogyra porticalis*, *Chlorella vulgaris*

INTRODUCTION

Bioremediation is a pollution control technology that uses biological agents to catalyze the degradation or transformation of various toxic chemicals to less harmful forms (Kaizar and Norli, 2015).

The use of algae to remove pollutants from water, algal bioremediation, has been well studied over the past 40 years (Fu and Wang, 2011). Since 1980s, considerable research effort has been devoted to the development of algal bio-sorbents to remediate pollutants, particularly heavy metals (Hubba *et al.*, 2011). At the laboratory scale, these preparations have proven spectacularly successful at sorbing pollutants, especially heavy metals (Mehta and Gaur, 2005). However, uptake of the concept has lacked vitality, evidenced by the lack of successful commercialization. This is likely because available algal (seaweed) biomass that is produced has established markets as food and as food ingredients (Chopin and Sawhney, 2009). Furthermore, amongst the most successful preparations developed are those from brown macro algae (Davis *et al.*, 2003)

which already have particularly well established markets and command a high price. A cheaper, reliable and locally derived source of biomass is critical (Fu and Wang, 2011), and remains a bottleneck for commercial applications of algae in bioremediation.

Bioremediation is a cost effective and efficient method of decontamination that has been increasingly popular now-a-days to reduce environmental pollution. In urban and semi-urban colonies, sewage disposal has become an ecological problem (Moore, 1998). The availability of good quality water is an indispensable feature for preventing diseases and improving quality of life (Oludoro and Adewoye, 2007).

MATERIALS AND METHODS

Description of the Study Area

Challawa River was selected for the purpose of this research. The River is located within latitude 11.928128N and longitude 8.516531E and is considered as second largest river in Kano State after Kano River.

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It made a confluence with Kano River at Tamburawa village, about 20km from Kano closed settled zone and flows to the northeast joining Hadejia River (Ibrahim, 2009).

Sampling sites

Three sampling sites were selected for algal and effluent sampling.

SITE A- Challawa River, where the water sample in which algal species were isolated.

SITE B- G Tannery industry, located around Challawa Industrial Area, where the first effluent was collected and used for the culturing of algal species.

SITE C- M Tannery Industry, located around Challawa Industrial Area, where the second effluent sample was collected.

Time and frequency of sampling

The time of sampling was between 7.00am and 8.00am.

Water Sample Collection

The sample was collected towards the midstream and upstream so as to avoid a bias sample. The bottles were rinsed with ambient water three times, discarding the rinsed water downstream. The bottles were gripped on one hand around the base and the lid was removed with the other hand. The container was inverted and submerged to a depth of 0.2m below the surface. The mouth of the bottle was turned upwards and towards the current. When the bottle became full, it was removed from the water rapidly and the lid was replaced. Care was taken to keep fingers clear of the lid liner and neck of the bottle (Hallegraeff, 2003).

Preparation of Culture Medium

In preparing the medium, distilled water and quality chemicals were used and concentrated stock solutions were made before making the medium. The culture medium used was BG-11 medium (Stanier *et al.*, 1971).

Isolation and Identification of Microalgae

Before isolating the cells, the algal species were identified using identification guide of Palmer (1980) and Desai and Dona, (2004) and isolated using the Algal Culturing Techniques guide (Hallegraeff *et al.*, 2003). The narrow end of the capillary tube was heated with a Bunsen burner turned low pulling it out to obtain bristle-like thinness. After viewing the cells under the microscope, the end of the capillary tube was dipped into the drop of water sample on the glass slide and brought close to the cell, which passes into the narrow end of the capillary tube by capillary action. The captured cells were then blown into the medium contained in a flask (Bischoff and Bold, 1963).

Spirogyra porticalis and *Chlorella vulgaris* were isolated from the water sample by pipetting method. The individual cells were picked up, washed and inoculated into the medium. The process was carried out using a microscope (Hund WETZLER H600), whereby a drop of the water sample was placed on a clean glass slide and viewed under the microscope.

Culturing of Microalgae

Spirogyra porticalis and *Chlorella vulgaris* were cultured using BG-11 medium under controlled condition at room temperature and 16/8-hour light/dark cycle (Venkataramen, 2010).

Harvesting of biomass

Recovery of microalgae biomass from the medium was conducted. The isolates were harvested by centrifugation method with the aid of OHAUS FRONTIER™ 5706 centrifuge machine. The samples were centrifuged at 5000rpm for 15 minutes.

Effluent sampling

All sample containers were pre-washed with detergent, nitric acid and distilled water and samples were acidified at the time of collection with HNO₃ (5ml/L) (Philadelphia, 1985).

Effluent Analyses

The effluent samples were first subjected to digestion process then physicochemical analysis and heavy metals determination.

Digestion

The effluents were acidified at the time of collection with concentrated 5mls of nitric acid. The samples were well mixed and 100mls of the well mixed samples were transferred to a 100ml beaker. The effluents were heated using a steam bath until the volume was reduced to 15-20mls. [It was made certain the sample did not boil]. Afterwards, the sample was filtered to remove any insoluble material and the digested sample was adjusted to pH 4 by dropwise addition of 5.0N Sodium hydroxide standard solution. The effluents were mixed thoroughly and pH checked after each addition. The effluents were quantitatively transferred to a 100ml volumetric flask and diluted to volume with deionized water. A reagent blank was also carried through the digestion and measurement procedures (USEPA, 2010).

Determination of temperature (°C)

HANNA MODEL HI9813-6 meter was used to measure the effluent temperature following manufacturer's instructions. Wastewater samples were collected in 5Litre container and digital thermometer terminal was dipped into the container and allowed to stabilize and the temperature reading was taken immediately.

Determination of pH

HANNA MODEL HI 9813-6 meter was used to measure the effluent pH following manufacturer's instructions. Wastewater samples were collected in 5 Litre container and digital thermometer terminal was dipped into the container and allowed to stabilize and the pH was taken immediately.

Determination of Dissolved Oxygen (mg/L)

This was measured using DO meter (JPB607A) in which the meter was inserted into the wastewater for about 15 seconds and DO reading was recorded in mg/L (Mustapha, 2009).

Determination of Biochemical Oxygen Demand (BOD₅) (mg/L)

Biochemical Oxygen Demand (BOD₅) was measured using meter (JPB607A) and was determined from the collected water samples after incubation for 5 days in a cupboard using the same method applied for the DO using the 5 days incubated sample (DO₅). BOD₅ was determined by subtracting the DO₅ from the initially determined DO (APHA, 1985).

$BOD_5 \text{ (mg/L)} = \text{Dissolved oxygen at day 1} - \text{Dissolved oxygen at day 5 (DO}_1 - \text{DO}_5)$.

Determination of Total Dissolved Solids (TDS) (mg/L)

HANNA MODEL HI 9813-6 was used to measure the effluent total dissolved solids following manufacturer's instructions. Wastewater samples were collected in 5 Litre container and digital thermometer terminal was dipped into the container and allowed to stabilize and the total dissolved was taken immediately.

Determination of Electrical Conductivity (µS/cm)

HANNA MODEL HI 9813-6 meter was used to measure the effluent electrical conductivity following manufacturer's instructions. Wastewater samples were collected in 5 Litre container and digital thermometer terminal was dipped into the container and allowed to stabilize and the electrical conductivity was taken immediately.

Bioremediation Process

The algal species used in this study were isolated from water, from river Challawa. Firstly, most dominant algal strain selected which survives in the polluted water of river Challawa such as *Chlorella vulgaris* and *Spirogyra porticalis* (Kshirsagar and Gunale, 2011).

To study the role of microalgae in effluent treatment, the following method was employed

(i) Effluent was treated with culture of *C. vulgaris* and *S. porticalis*, separately and (ii) Effluent was treated without culture of *C. vulgaris* and *S. porticalis* separately (Control). Experiments were conducted in five replicates.

Uniform suspension containing 2mls of 25 days old culture of *C. vulgaris* and *S. porticalis* were added as initial inoculum in each flask containing 200 ml of wastewater sample. The experiment was conducted under controlled conditions (Temp. $27 \pm 2^\circ \text{C}$) for 6 months. Samples were periodically analyzed at 3 weeks' interval for physico-chemical parameters (pH, Temperature, EC, DO, BOD, and TDS) using standard methods (APHA, 1998) for a period of 9 weeks.

Determination of Bioremediation potential

The digested effluent containing the inoculated algal species was periodically analyzed (every 3 weeks for a period of 9 weeks) for physico-chemical parameters (pH, temperature, EC, BOD, DO, TDS) using standard methods (APHA, 1998). Bioremediation potential of the algal species on the effluent using Atomic Absorption Spectrophotometer machine (AAS Unicorn 969) as described in the manufacturer's instructional manual was used to determine the concentration levels of the heavy metals in the effluent. This process was used to determine the bioaccumulation or biodegradation potential of these organisms (APHA, 1998).

RESULTS

Results on physicochemical parameters of the tannery effluents are shown in Table 1.0. Temperature in both tanneries studied, ranged from $30-31^\circ \text{C}$ which might be as a result of ambient temperature as set by International Standards WHO (2002).

The effluent pH of both tanneries did not differ significantly and were not within the maximum permissible limits of pH 6.5-8.5. Electrical conductivity of M Tannery is a little higher than that of G tannery and were not within the maximum permissible limits. Total dissolved solids of M Tannery are higher than that of G Tannery and are both not within the maximum permissible limits of 500mg/L. DO content in both tanneries were within the safety limits for maintenance of aquatic life of 5.00mgL^{-1} (WHO, 2002). Lead concentration of Tannery effluent from M and G Tannery exceeded the permissible limit as set by WHO (2002).

Table 1: Means of Physicochemical Parameters of the Tannery Effluents Analyzed Over a Period of Nine Weeks

PHYSICOCHEMICAL PARAMETERS	MEAN VALUE M TANNERY	MEAN VALUE GB TANNERY	PERMISSIBLE LIMIT (WHO, 2002)
Temperature(°C)	31.83±0.70	30.30±0.79	Ambient
pH	12.61±0.12	12.10±0.10	6.5-8.5
Electrical conductivity(μS/cm)	8.42±0.26	6.92±0.050	1250
Total dissolved solids(mg/l)	1919.0±68.46	1916.0±61.94	500
Dissolved oxygen(mg/L)	4.36±0.27	4.81±0.095	6.4
Biochemical oxygen demand(mg/L)	0.62±0.07	0.72±0.05	4
Lead	0.83	0.67	0.1

Effect of Algal Species on Lead in Tannery Effluents

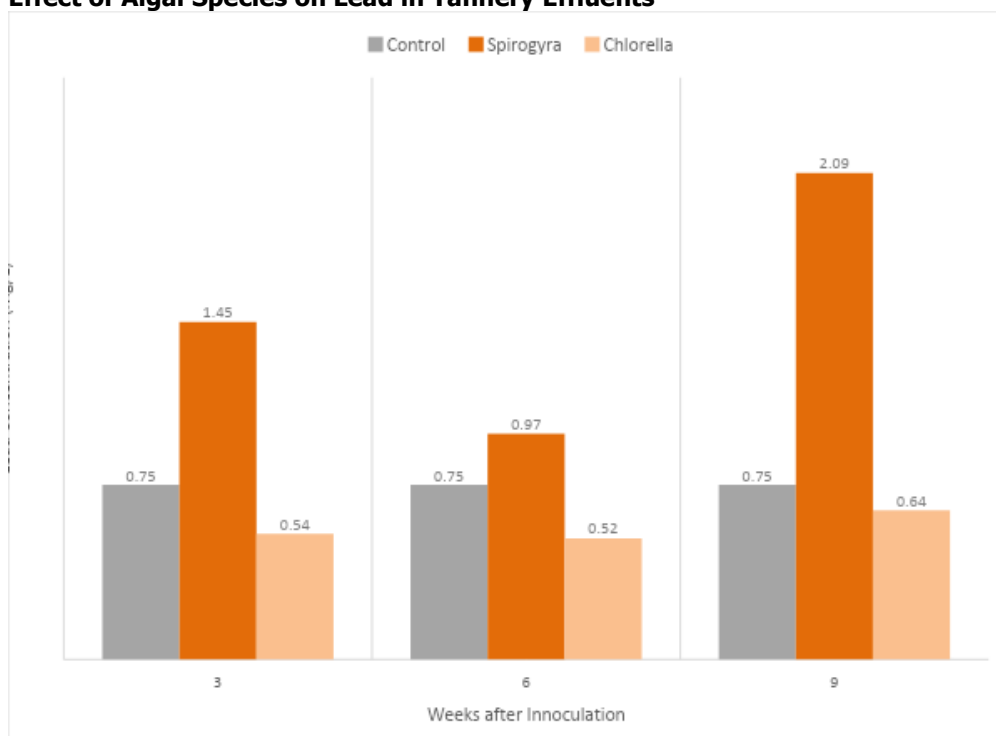


Figure 1: Effect of *Spirogyra* spp. and *Chlorella* spp. on Lead (Pb)

DISCUSSION

Physico-chemical parameters of the effluent from the sampling sites

Temperature in both tanneries studied, ranged from 30-31°C which might be as a result of ambient temperature as set by WHO (2002).

From the result of this study, pH, EC, TDS and BOD were found to exceed the limit set by international standards. Temperature depends on the temperature of the environment. However, these findings are comparable to the study of Nkwocha *et al*, (2013). Dissolved Oxygen values obtained were found to be lower than that set by FEPA (Federal Environment Protection Agency, 2015).

Factors like air, temperature bring about changes in pH of water. The reduced rates of photosynthetic activities reduce the assimilation of carbon dioxide and bicarbonates which are ultimately responsible for increase in pH. Higher pH favours higher metal removal (Kamble *et al*, 2009). Low Dissolved Oxygen in summer is due to decrease in temperature and duration of less sunlight has influence on the % of soluble gases (O₂ and CO₂ gases). Dissolved oxygen is slightly lesser during winter (Ahmed and Krishnamurthy, 1990).

In most cases, species of the family of *Chlorella*, such as *Chlorella vulgaris* and *Spirogyra porticalis* (Bosnic *et al*, 2000) have been employed for wastewater treatment.

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Lead level in both tanneries were beyond the standard limit while which is also in line with the findings of Ahmad and Krishnamurthy, 1990 Jahan *et al.* (2014).

Lead Concentration

After *Spirogyra porticalis* and *Chlorella vulgaris* treatment, (Table 1) Lead levels of the effluent decreased by 51%, 55%, 55% and 40%, 45%, 45% at 3, 6, and 9 weeks respectively. So, *Spirogyra* spp. treatment was most effective compared to *Chlorella* spp. between 3-6 weeks after inoculation which is also in line with the report of Brahmabhatt and Rinku, (2015).

Similarly, after *Spirogyra porticalis* and *Chlorella vulgaris* treatment, (Table 1) Pb levels of the effluent increased by 90%, 86% and 90% and decreased by 10%, increased by 41% and decreased again by 10% at 3, 6, and 9 weeks respectively. So, *Spirogyra porticalis* was not effective in reducing Pb level in M Tannery while *Chlorella vulgaris* was most effective in reducing concentration level of Lead in M Tannery in the

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3rd and 9th week which is also in line with Brahmabhatt and Rinku (2015).

CONCLUSION

Spirogyra porticalis and *Chlorella vulgaris* were the most predominantly found algal species and were isolated and cultured for bioremediation of heavy metals in G and M. Tannery effluent located around Challawa Industrial area. The findings from this research work shows that in G Tannery, *Spirogyra porticalis* had the highest affinity for Pb which was 51%, 55% and 51% at 3, 6 and 9 weeks respectively. In M tannery, *Chlorella vulgaris* had the highest potential to remove Lead from the effluent, which was 90%, 86% and 90% at 3rd, 6th and 9th weeks respectively.

The result also shows that bioremediation took place across the nine weeks after inoculation of algal species in the tannery effluent and bioremediation being at its peak at the sixth week.

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