



ASSESSMENT OF BIOREMEDIATION POTENTIAL OF *Spirogyra porticalis* and *Chlorella vulgaris* ON COPPER AND CHROMIUM IN TANNERY EFFLUENT FROM CHALLAWA INDUSTRIAL AREA, KANO STATE

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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 agent in comparison to conventional technologies. This research was conducted to evaluate the bioremediation potential of two algal species i.e. *Spirogyra* spp. and *Chlorella* spp. for the removal of heavy metals from two tannery industries M Tannery and GB Tannery located in Challawa Industrial area by using Atomic Absorption Spectrophotometry (AAS Unicorn 969) and also Physicochemical parameters like pH, Temperature, EC, TDS, DO and BOD were measured. In GB Tannery, *Spirogyra* spp. had the highest affinity for Cr which was 50%, 67%, 50% at 3rd, 6th and 9th weeks interval respectively. *Chlorella* spp. showed highest affinity for Cu was 60% 85% and 93%. In M tannery, *Chlorella* spp. had the highest potential to remove heavy metals from the effluent, showing high affinity for Cu which was 80%, 92%, 59% at 3rd, 6th and 9th weeks respectively. Both algal species were not effective for Cr removal. Temperature ranged between 30-31°C which might be as a result of ambient temperature, pH of both tanneries did not differ significantly and it was not within the maximum permissible limit of 6.5-8.5. EC of M Tannery was a little higher than GB Tannery (8.417±0.2627, 6.920±0.05000) which were below the permissible limit. TDS of M tannery is higher than that of GB Tannery 1919.0±68.462mg/l, 1916.0±61.944mg/l which were not within the permissible limit. DO content were within the permissible limits of 6.4mg/L. BOD of both tanneries were below the permissible limit of 4mg/L. This research shows that both species are suitable for bioremediation and are also tannery specific.

Keywords: *Chlorella* spp., Effluent, Physicochemical, *Spirogyra* spp., Tannery

INTRODUCTION

Bioremediation is a pollution control technology that uses biological systems 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 the 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 *et al.*, 2005). However, uptake of the concept has been lack-lustre, 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 *et al.*, 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 become 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 because it is marked as the centre of tanning industries in Kano. 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. It made a confluence with Kano River at Tamburawa village, about 20 km 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 from was collected.

SITE B- GB Tannery industry, located around Challawa Industrial Area, where the first effluent in which the algal species was inoculated after isolating and culturing was collected.

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

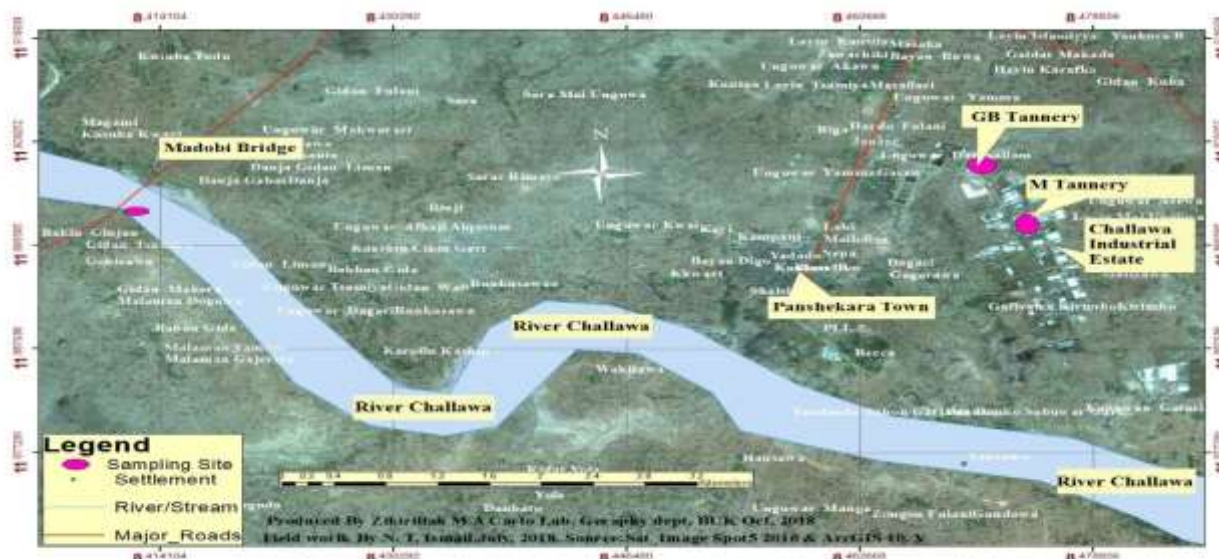


Figure 1: Map of Challawa Reservoir, Kano Showing Marked Sampling Sites.
(Source: Cartography Lab. Bayero University Kano).

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 (Hallegraef, 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 Desai *et al.*, (2004) and Palmer (1980) and isolated using the Algal Culturing Techniques guide (Anderson, 2005). 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 pass 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 is called harvesting. *S. porticalis* and *C. vulgaris* were harvested by centrifugation method. OHAUS FRONTIER™ 5706 was used. The samples were centrifuged at 5000rpm for 15minutes.

Effluent Sampling/Analyses

The sample containers were prewashed with detergent, nitric acid, distilled water and then acidified at the time of collection with HNO₃ (5mL) (Philadelphia, 1985). The effluent samples were first subjected to digestion process then physicochemical analysis and heavy metals determination.

Determination of Temperature (°C), pH, Total Dissolved Solids and Electrical Conductivity

Multipara meter (HANNA MODEL HI9813-6) was used to measure the effluent temperature, pH, Total Dissolved Solids and Electrical Conductivity following manufacturer's instructions. Wastewater samples were collected in 5L container and probe digital meter terminal was dipped into the container and allowed to stabilize and the reading 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 15seconds and DO reading was recorded in mg/L.

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

Biochemical Oxygen Demand (BOD₅) was measured using(JPB607A) meter and was determined from the collected water sample after incubation for 5days in a cupboard using the

same method applied for the DO using the 5days incubated sample (DO₅). BOD₅ was determined by subtracting the DO₅ from the initially determined DO (APHA, 1985).

BOD₅ (mg/L) = Dissolved oxygen at day 1- Dissolved oxygen at day 5 (DO₁-DO₅)

Bioremediation Process

The algal species were isolated from river Challawa and the most dominant algal strain was selected which survives in the polluted water of river Challawa such as *Chlorella vulgaris* and *Spirogyra porticalis* (Kshirsagaret al., 2012). To study the role of microalgae in effluent, the following methods were 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 2mL of 25 days old culture of *C. vulgaris* and *S. porticalis* were added as initial inoculum each flask containing 200 mL of wastewater sample. The experiment was conducted under controlled conditions (Temp. 27 ± 2° C) for a total duration of 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 9weeks) for physico-chemical parameters (pH, temperature, EC, BOD, DO, TDS) using standard methods (APHA, 1998). Bioremediation potential of the algal species on the effluent was analysed using Atomic Absorption Spectrophotometer machine, (AAS Unicorn 969) as described in the

manufacturer's instructional manual 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 physiochemical parameters of the tannery effluent were shown in table 1 Temperature in both tanneries studied, ranged between 30-31°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 GB tannery and were not within the maximum permissible limits. Total dissolved solids of M Tannery are higher than that of GB 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).

For Copper concentration, *Spirogyra* spp. reduced Copper concentration by 67% at 6 weeks after inoculation so also *Chlorella* spp. decreased Copper concentration by 33% with *Spirogyra* spp. being the most effective algal specie. This result shows that both species are effective for bioremediation potential and most effective at 6 weeks after inoculation (Figure 2).

For Chromium concentration, it can be seen that none of the algal species was suitable for bioremediating concentration level of Chromium. The result shows Chromium concentration increased across the weeks (Figure 3).

Table 1. Physiochemical parameters of the tannery effluent analyzed over a period of 3, 6 and 9 Weeks

PHYSICOCHEMICAL PARAMETERS	MEAN VALUE M TANNERY	MEAN VALUE GB TANNERY	PERMISSIBLE LIMIT
Temperature(°C)	31.833±0.7024	30.300±0.7937	Ambient
Ph	12.613±0.1150	12.100±0.1000	6.5-8.5
Electrical conductivity(µS/cm)	8.417±0.2627	6.920±0.05000	1250
Total dissolved solids(mg/l)	1919.0±68.462	1916.0±61.944	500
Dissolved oxygen	4.363±0.2658	4.810±0.09539	6.4
Biochemical oxygen demand	0.6167±0.06506	0.7200±0.0458	4

Table2: Concentration of Heavy Metals in the Tannery Effluent with their Permissible limit.

HEAVY METALS	PERMISSIBLE LIMIT(2002)	M TANNERY	GB TANNERY
Copper	0.1	0.01	0.21
Chromium	2	1.43	1.43

In GB Tannery, after *Spirogyra* spp. and *Chlorella* spp. treatment, Cu levels of the effluent increased by 40%, 15%, 7% and 60%, 85%, 93% at 3, 6, and 9 weeks respectively. So, *Spirogyra*spp. treatment was not effective in reducing copper concentration. *Chlorella* spp.too was unable to reduce Copper concentration at the 3rd, 6th and 9th week respectively. This could be as a result of cells internal mechanism for regulating what reactions they perform as described by Baedecker, et al;(1989).

In M Tannery, after *Spirogyra* spp. and *Chlorella* spp. treatment, Cu levels of the effluent were found increase by 80%, 92%, 59% and increase by 20%, decrease by 8% and increase by 41% at 3, 6, and 9 weeks respectively. So, *Spirogyra* spp. was not suitable in reducing the level of Copper concentration while *Chlorella* spp.was most effective in the 6th week after inoculation which is also in line with Brahmhatt and Rinku, (2015).

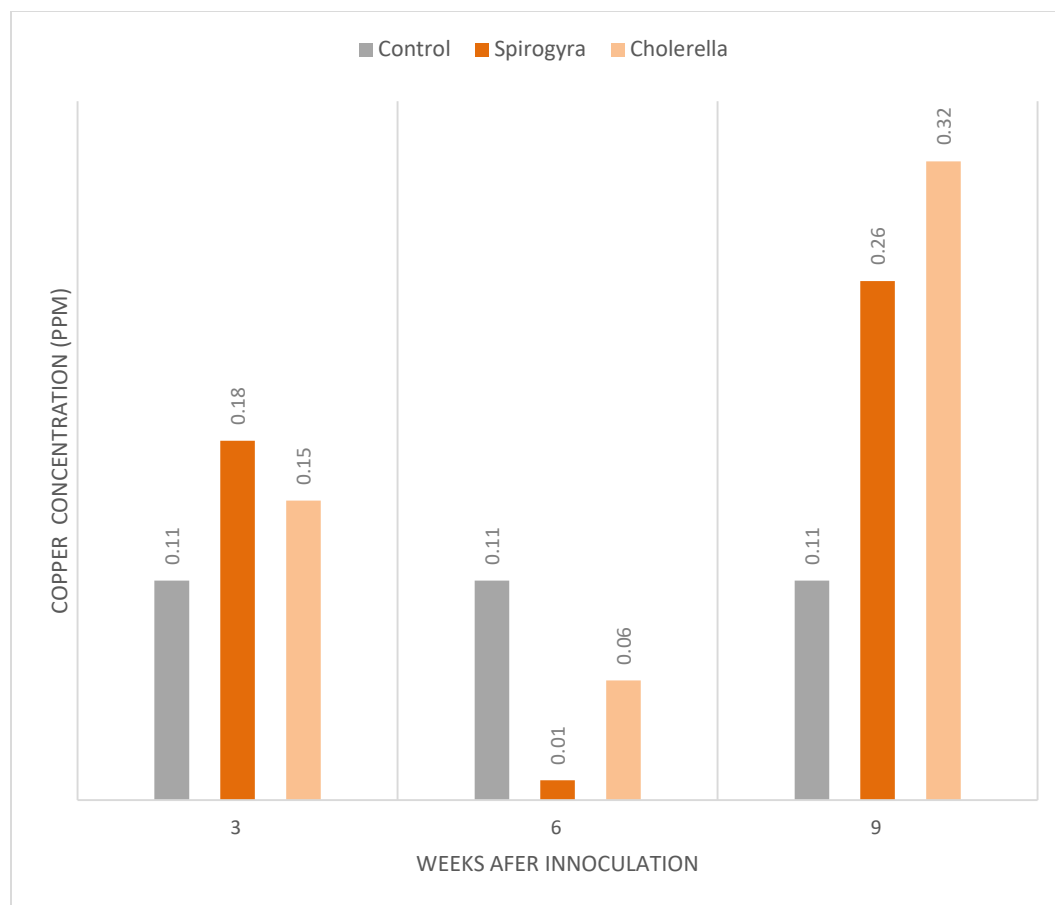


Figure 2: Effects of Algal Species on Copper in GB and M Tannery Effluents In 3rd, 6th And 9th Weeks

At three weeks after inoculation with *Spirogyra* spp. level of Chromium increased in G Tannery. In M Tannery, level of Chromium remained same.

In the sixth week after inoculation with *Spirogyra* spp. level of Chromium increased further in G Tannery by 67% while in A Tannery, after inoculation with *Chlorella* spp. level of Chromium remained same as it was in the third week.

In the ninth week after inoculation with *Spirogyra* spp. level of Chromium in G Tannery increased by 50% while level of Chromium in M Tannery increased by 100%. After inoculation with *Chlorella* spp. level of Chromium increased by 50% in G Tannery while level of Chromium in M Tannery remained same, as it was in the sixth week. This result shows that none of the algal species was suitable for reducing the concentration level of Chromium in both G and M Tanneries.

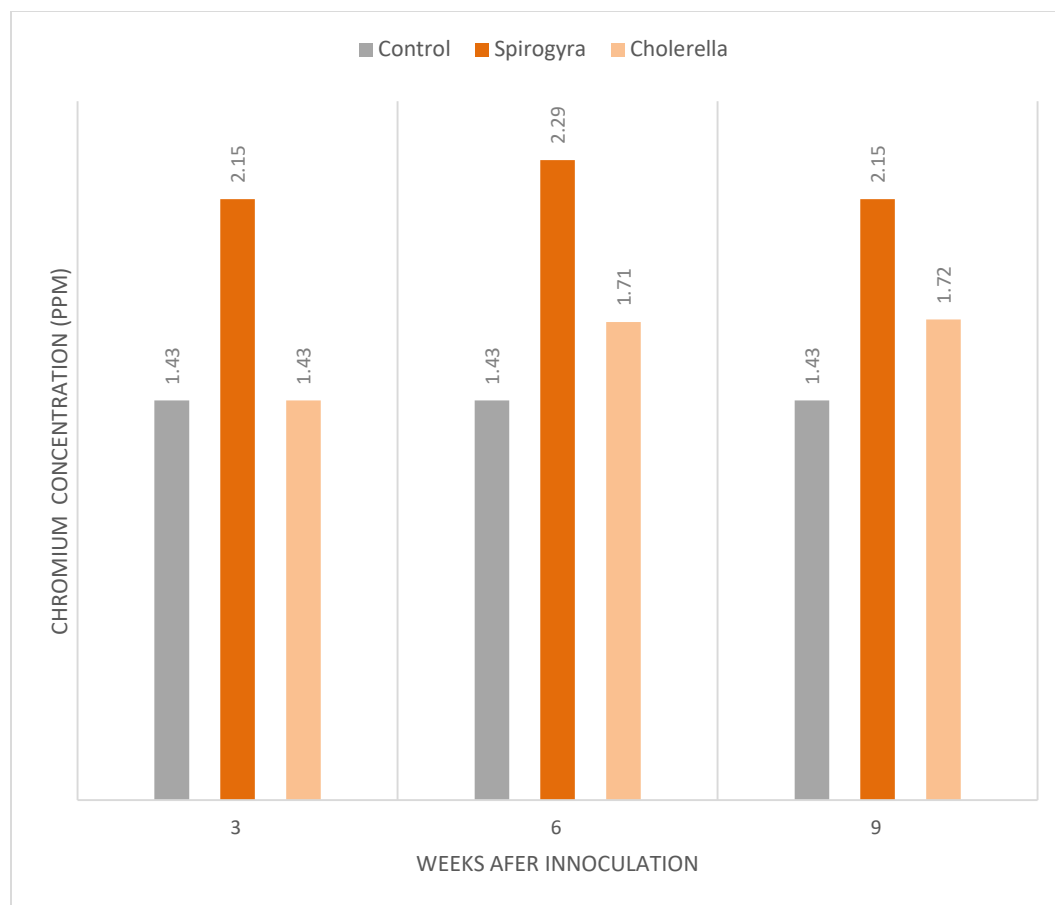


Figure 3: Effect of *Spirogyra* spp. and *Chlorella* spp. on Chromium (Cr) in 3rd, 6th and 9th Weeks

DISCUSSION

Physico-chemical parameters of the effluent from the sampling sites.

Temperature in both tanneries studied, ranged between 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 (O and Co gases). Dissolved oxygen is slightly lesser during winter (Ahmed and Krishnamurthy, 1990).

Algal species from the sites (*Chlorella vulgaris*. and *Spirogyra porticalis*.) were the dominant species found in this study. Though other species such as *Anabaena* and *Neochloris* were also seen but the dominant ones (*Chlorella vulgaris*. and

Spirogyra porticalis.) were used to carry out the research because previous studies have shown they have bioremediation potential.

Many studies have demonstrated successful wastewater treatment with microalgae (Ambast, 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.

Aron, 1949 has also reported biosorption of heavy metals from aqueous solution by freshwater filamentous algae (*Spirogyra hatillensis*). Limited efforts have been made to use algal biomass for removing toxic heavy metals such as Cr and Cd from aqueous solutions. Out of thousand algal species, only few have an ability to remove toxic heavy metals (Cr, Cd) from wastewaters (Hellawell, 1986). The commonly used species for removing Cr and Cd from wastewater are fresh water green algae like *Chlorella vulgaris* and *Spirogyra porticalis*.

Atomic Absorption Spectrophotometer machine, was used to determine the Presence and concentration levels of heavy metals (Cu and Cr) in the effluent.

CONCLUSION

The physico chemical parameters of the effluent were within the permissible limit of WHO (2002) except electrical conductivity and dissolved oxygen which were significantly high.

The algae *Spirogyra porticalis* and *Chlorella vulgaris* were collected, identified, isolated and cultured using the standard operating procedure of (Desai *et al.*, 2004, Palmer 1980, and Anderson, 2005) which were used to achieve the bioremediation potential.

Copper was present and the concentration level in the effluent (before and after inoculation) was taken which shows high remediation potential by algae.

In both the tannery effluent GB and M Tannery located around Challawa Industrial area, there was high bioremediation potential of Copper by *Spirogyra porticalis* and *Chlorella vulgaris* in GB Tannery at 3, 6 and 9 weeks interval. While in M Tannery, *Chlorella Vulgaris* had high bioremediation potential on Copper at 3, 6 and 9 weeks interval. Both algal species have less affinity for Chromium removal. The result shows that bioremediation took place across 9 weeks after inoculation of the algae into the tannery effluent and bioremediation being at its peak at 6 weeks in all the tannery effluent.

REFERENCES

- Ambast, R.S. (1990). *Environment and Pollution (An Ecological Approach)*. 1st (Eds.), Students Friends Publications, Lanka Varanasi, India.
- APHA (1985): *Standard Methods of Examination of Waters and Wastewaters*. 16th Ed. American Public Health Authority. Washington D.C. 2112.
- APHA (1998). *Standard Methods for the Examination of Water and Wastewater*. 18th Edition. American Public Health Association, Washington, DC Pp 45-60
- Bischoff, H.W. and Bold, H.C. (1963). *Phycological Studies in Some Soil Algae from Enchanted Rock and Related Algal Species*, University of Texas publications **6318**:1-95.
- Bosnic, M., Buljan, J., and Daniels, R.P. (2000). *Regional program for pollution control in the Tanning industry US/RAS/92?120 in South-East Asia*, pp:1-14.
- Brahmbhatt N.H., and Rinku V. Patel (2012). *Bioremediation potential of Spirogyra, toxicity and biosorption studies on Lead (Pb)*. V.P. and R.P.T.P. Science College, Vallabh Vidyanagar, S.P. University, Gujarat, India.
- Chopin, T., Sawhney, M. (2009). *Seaweeds and their mariculture*. In: Steele JH, Thorpe SA, Turekian KK, eds. *The Encyclopedia of Ocean Sciences*. Oxford: Elsevier. pp 4477–4487
- Davis, T.A., Volesky, B., Mucci, A. (2003). *A review of the Biochemistry of Heavy Metal Biosorption by Brown Algae*. Water Research, **37**: 4311–4330.
- Desai, X.N. and Dona, P. (2004), *Phytoplankton Identification Manual*, **1 (1)**: 40-110p.
- FEPA (2015), Federal Environment Protection Agency.
- Fu, F. and Wang, Q. (2011). *Removal of Heavy Metal Ions from Waste*. *Bioremoval of heavy metals by the use of microalgae*. Biotechnology Advances. (Issue 4ewaters: A review. *Journal of Environmental Management*, **92**: 407–418.
- Hallegraeff, G.M., Anderson, D.M., Cambella, A.D. (eds) 2003. *Manual on Harmful Marine Microalgae* UNESCO Publishing, Paris.
- Hellawell, J.M. (1986). *Biological Indicators of Freshwater Pollution and Environmental Management*. Elsevier Applied Science Publishers Ltd., London, pp: 546.
- Hubba, M.A., Hasan, S.H., Ducoste, J.J. (2011). *Cellulosic substrates for removal of pollutants from aqueous systems: A review*. I. Metals Bioresources **6**: 2161- U2914. 6.
- Ibrahim, S. (2009): A survey of zooplankton Diversity of Challawa River, Kano and Evaluation of some of its Physico-Chemical condition. Department of Biological Sciences, Bayero University, Bayero Journal of Pure and Applied Sciences, **2(1)**: 19-26.
- Kaizar Hossain and Norli Ismail (2015). Bioremediation and Detoxification of Pulp and Paper Mill Effluent. A Review. *Research Journal of Environmental Toxicology*. **9**:113-134.
- Kamble, S.M., Kamble, A.H. and Narke, S.V. (2009). *Study of physico-chemical parameters of Ruti dam*, Tq: Ashti, dist, Beed, Maharashtra. *J. Aqua. Biol.* **24(2)**:86-89.
- Masood Ahmed and Krishnamurthy R., (1990): *Hydrobiological studies of Wohar reservoir Aurangabad (Maharashtra state) India*. *J. Environ. Biol.*, **11(3)**: 355-343.
- Mehta, S.K., Gaur, J.P. (2005). *Use of Algae for Removing Heavy Metal Ions From Wastewater: Progress and prospects*. *Critical Reviews in Biotechnology* (**25**): 113–152.
- Moore, P.D. (1998). *Essential Elements From Waste*.
- Mustapha, M.K. (2009). *Zooplankton Assemblage of Oyun Reservoir*, Offa, Nigeria *Rev. Biol. Trop.*, (57), (Issue 4): 1027-1047.
- Nkwocha, A.C., Ekeke, I.C., Kamen, F.I., and Oghome, P.I. *Quality assessment of effluent discharges from vegetable oil plants*. Nov, 2013.
- Oluduro AO, Adewoye BI (2007). *Efficiency of moringa oleifera seed extract on the microflora of surface and ground water* *J. PLANT SCI.* **6**: 453-438.
- Palmer, M.C. (1980). *Algae and Water Pollution*. Castle House Publications Ltd, England. 68-71p.
- Philadelphia, PA. (1985). Annual Book of ASTM Standards, Vol. 11.01; "Standard Specification for Reagent Water"; ASTM: D1193-77.

Pringsham, E.G.(1946). Cited by: Venkataraman, G.S. 2010. *Removal of cadmium, mercury and lead from aqueous solution using marine macroalgae as low cost adsorbents. J. Pure and Applied Science.*

Stanier RY, Kunisawa R, Mandel M and Cohen Bazire G (1971) *Purification and Properties of Unicellular blue-green algae (order Chroococcales). Bacteriol. Rev., 35: 171-205.*

WHO (2002), *Water pollutants: Biological agents, dissolved chemicals, Non dissolved chemicals, sediments, Heat*, WHO CHEA, Amman, Jordan. 22626-81228-1-PB



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