WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 14, 643-659. Research Article ISSN 2277– 7105

A COMPARITIVE STUDY ON THE EFFICACY OF NANOPARTICLES FROM *CAULERPA SCALPELLIFORMIS* AND *CODIUM DECORTICATUM* IN TREATING THE TANNERY EFFLUENT AND THEIR ANTI MICROBIAL EFFECT

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Article Received on 23 May 2018,
Revised on 13 June 2018,
Accepted on 03 July 2018
DOI: 10.20959/wjpr201814-12575

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ABSTRACT

Tannery effluents are considered to be one of the major source for water pollution. Due to improper disposal they pose a major threat to the aquatic ecosystem, surrounding ground water quality, and also to the human health. Due to these concerns, various laws were framed to govern the toxicity levels in the outlet effluent. Due to faster remediation, chemical treatment is being deployed to treat the effluent water. However, the chemicals used too contribute to the water pollution which has turned the eyes of researchers to follow bioremediation by exploiting various aquatic plants, bacteria, algae, fungi, etc in reducing the toxicity levels. In the present study,nanoparticles synthesised from 2 green, marine algal species *Caulerpa scalpelliformis* and *Codium decorticatum* was used to test the efficiency in treating the tannery effluent. The various physico chemical parameters like pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), inorganic salts, heavy metals were analysed before and after treatment with the algal nanoparticles. The study was carried out for a period of 60 days and there was a drastic reduction in all the parameters by these algal nanoparticles. of these, nanoparticles of *Codium decorticatum* was more efficient than that of *Caulerpa scalpelliformis* nanoparticles.

KEYWORDS: Bioremediation, Marine algae, *Caulerpa scalpelliformis*, *Codium decorticatum*, Physico chemical parameters.

INTRODUCTION

Pollution means the presence in the environment of substances or contaminants that Substantially alter or impair the usefulness of the environment.[1] Water pollution is the contamination of water bodies around the world. Environmental degradation of such sorts occurs when pollutants are directly or indirectly released into water bodies without proper treatment to remove toxic compounds.[2] The sources of water pollution include direct and indirect sources. Direct sources are effluents from industries, which along with sewage waters are the largest source of water pollution.[3] Water treatment plants that give out toxic chemicals also comes under direct sources, which are released into the water bodies. Indirect sources include pollutants from ground water table and from the atmosphere via rain water.[4]

Effluentsareone of the major hazardous pollutants that when the untreatedtannery effluents are released into the natural water bodies, tend to cause environmental degradation,[5] metal toxicity to humans and aquatic life,[6] makes soil infertile,[7] and forms a nutrient medium for growth of algal blooms.Due to emerging global concerns due to these release of effluents, various laws have been framed to control the discharge of wastes into the water bodies. In the past decadenumerous treatment plants were deviced to remove nutrients by the inclusion of chemicals. At present a range of chemical methods has been followed for treating the water pollution viz. Coagulation/ flocculation, sedimentation, granular filtration, ion-exchange, membranes, sedimentation, filtration, etc.[8] But at the same time, they have harmful side effects to their credit and are not as efficient as it was thought to be and hence a surrogate method is required immediately and biological method seems to serve the purpose. Phytoremediation is one of the major biological methods, at hand currently. Phytoremediation refers to the use of plants and related soil microbes to reduce the concentrations or toxic effects of contaminants in the environment.[9] Bioremediation is proven to be cost effective, eco friendly method to treat the effluent water.[10]

Bioremediation is defined as the waste management technique which use of aquatic plants, micro or macro organisms to remove or neutralize the pollutants from the contaminated site. For this purpose, wide variety of aquatic plants like *Hydrilla verticilata*,[11] *Eicchornia crassipes*,[12] *Lemnasp*,[13] and other plants have been employed. However, In addition to plants, a number of algae are actively in use for cleaning the contaminated water. The use of micro algae or macro algae in water treatment is called as phycoremediation. Microalgae depend on carbon dioxide for its carbon source and can grow photo-autotrophically. Owing to its natural colonizing property, unicellular green algae such as Chlorella sp. And Scenedesmus sp. have been put to use in phycoremediation.[14]

In the present study, the marine macro algal species *Caulerpa scalpelliformis* and *Codium decorticatum* were used and their nanoparticleswere deployed to study their efficacy in treating the tannery effluent and thereby reducing its toxicity levels. The nanoparticles used were also tested for their antibiotic activity by well diffusion method.

*Caulerpscalpelliformis*has a green thallus consisting of stoloniferous prostrate axes upright frond with a height of 10cm. The axes are attached to the substratum by branches. These fronds are terete in base, closely flattened and determinate in an alternate pattern. Thallus is of coenocytic filaments.[15]

*Codium decorticatum*has dark green thallus of 38cm in height that is fixed to the substrate by a discoid base. Branches are dichotomous with flattening. It has medullary filaments which are 40 to79 µm in diameter. Utricles are individual, apices rounded and has gamatengia of 1 to 2 in nos. that are distributed over utricle, ovoid and lanceolate.[16]

MATERIALS AND METHODS

Collection of tannery effluent

The tannery effluent for the present study was collected from the Pallavaram Tannery Cluster Company Private Limited, Pallavaram, Chennai.

Collection of algae

The algae *Caulerpa scalpelliformis*and *Codium decorticatum*were collected from the deep sea of Palk Bay, Rameshwaram, Coastal region of Tamil nadu. The Taxonomical identification of the algae was done by Mr. M. Rajendra Kumar, R.K. Algae project center, Mandapam, Ramanathapuram District. The algae were washed thoroughly with sea water to remove sand. The process was repeated for 2 to 3 times. It is washed with distilled water and then shade dried. It was then stored in an air tight container.

Preparation of aqueous extract

The dried algae were finely powdered using electric mixer grinder and sieved through 1 mm^2 mesh. 10g of the powder was weighed accurately and added to 100ml of distilled water and kept in shaker for 24 hours. It is then filtered using whatman No.1 filter paper and the filtrate obtained is the aqueous extract, stored in 4°C which was used for further studies.[17]

Preparation of silver nitrate solution

Different concentrations of silver nitrate solution were prepared (1mM, 3mM and 5mM) using the formula,

Amount of silver nitrate = (Molar mass of $AgNO_3$ X Req. molarity X Req. volume) / 1000

Synthesis of silver nanoparticles

The Silver nanoparticles of the 2 alga was prepared by sunlight exposure method.[18] Various concentrations of silver nitrate solutions were prepared and optimised.

Experimental Setup

The experimental setup was designed in such a way that the control effluent of 20L was maintained from zeroth day till 60th day.4 tanks for each algae *Caulerpa scalpelliformis*, *Codium decorticatum* respectivelywere maintained (1 control and 4 tanks of tannery effluent) for a period of 60 days at an interval of 15 days from zeroth day.

The optimized nanoparticle was used for the third set of experiment where 4 tanks each were used (with tannery effluent containing algae and sprinkled nanoparticle) respectively.

Water Quality Parameter Analysis

The various physico-chemical parameters were analysed, viz. colour, odour, pH, turbidity, total dissolved solids (TDS), total suspended solids (TSS), copper, zinc, magnesium, calcium, potassium, sulphate, sodium, phosphate, biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrogen, alkalinity for the tannery effluent.

Well Diffusion Method

Antimicrobial activities of nanoparticles prepared from both the algae were evaluated using well diffusion method on Nutrient Agar. The inhibition zones were reported in millimeter (mm). Briefly, nutrient agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter=6mm) were filled with 0.5µg of the test samples and incubated at 37°C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was measured.

RESULTS AND DISCUSSION

Analysis of physico- chemical parameters in tannery effluent after treating with Silver Nanoparticles of Marine Macro Algae.

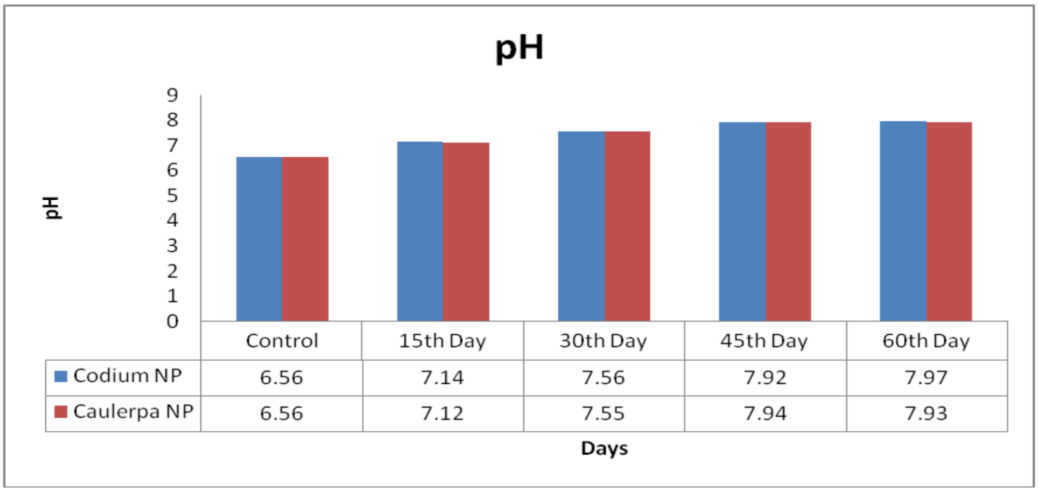


Fig. 1: Change in the pH of tannery effluent after treating with marine macro algae nanoparticles.

It was found that the pH was improvising and had the increasing efficiency of alkalinity for *C.scalpelliformis* nanoparticles (7.95), from the Fig 1. Optimum pH for fish is between 7.8 and 8.5 while acid water with pH below 4.4 and strong basic waters with pH greater than 8.8 generally causes gill irritation and death. The result corroborates the fndings of swayamprabha*et al*., 2013,[11] Mahmood *et al*., 2005[12], who showed the efficiency of treating *E.crassipes* with textile waste water and by Alejandro *et al*., 2009[19] with *S.obliquus*.

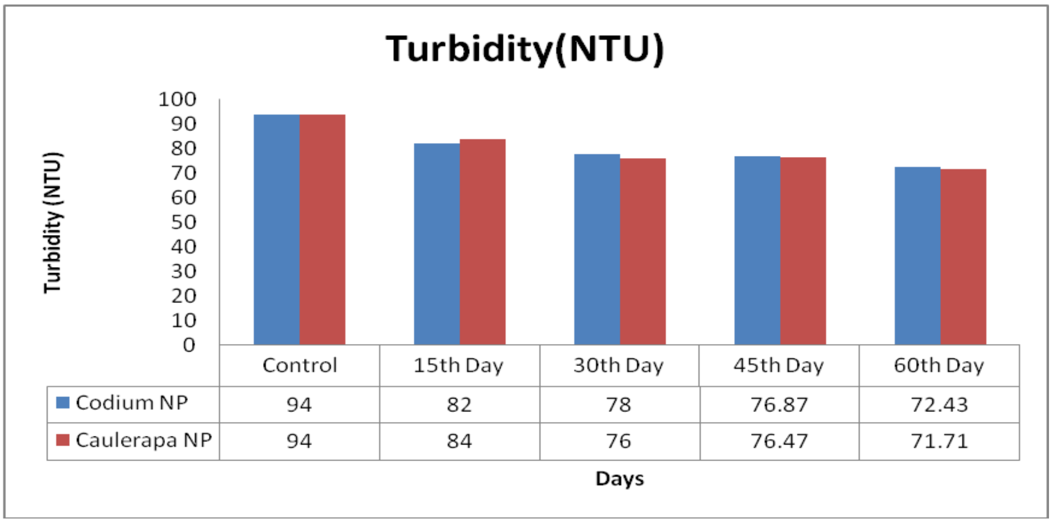


Fig. 2: Change in the Turbidity of tannery effluent after treating with marine macro algae nanoparticles.

Turbidity is the measure of relative clarity of a liquid. It makes water cloudy or opaque. High concentrations of particulate matter affect light penetration and productivity, recreational values, and habitat quality, and cause lakes to fill in faster.[20] It could be observed from Fig 2, that the turbidity of the effluent water was greatly reduced for *C.scalpelliformis* Nanoparticles (71.71 mg/l) which was similarly proved by Rawat *et al.*, 2010 using *B.braunii*.[21]

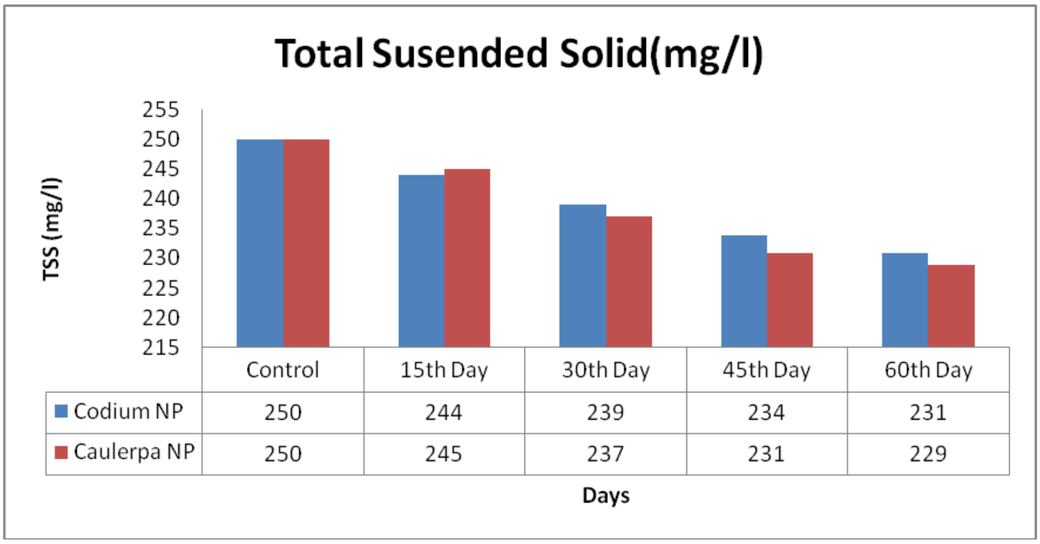


Fig. 3: Change in the TSS of tannery effluent after treating with marine macro algae nanoparticles.

Total suspended solids are very hard to remove and are considered lethal for the marine environment. In the present study it was observed that the total dissolved solids were greatly reduced for *C.scalpelliformis* nanoparticles (484 mg/l) (from Fig 3). Salts in solution affect the chemical and physical properties of the water and exert osmotic pressure. Similarly the reduction in TSS was reported using *C.vulgaris*.[22]

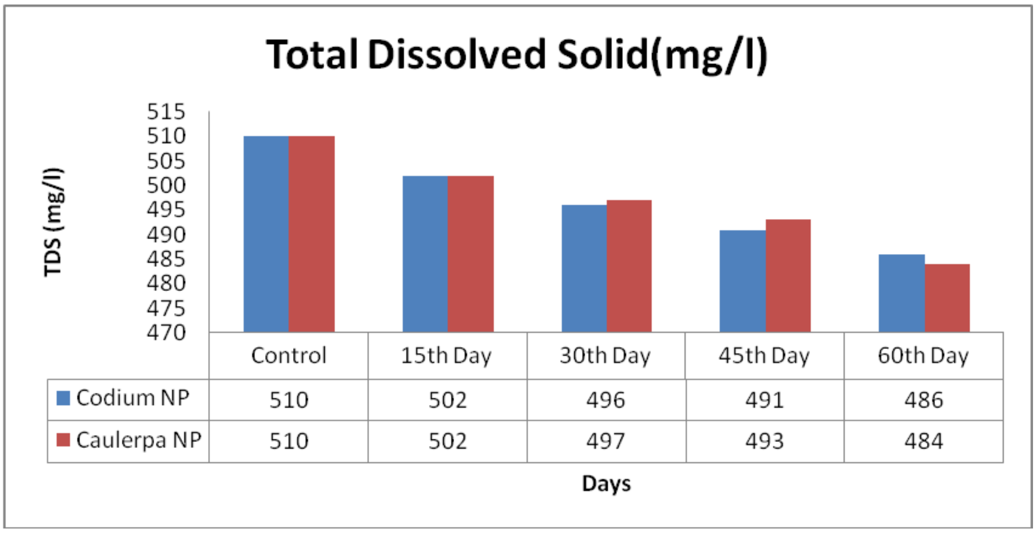


Fig. 4: Change in the TDS of tannery effluent after treating with marine macro algae nanoparticles.

Total dissolved solids in untreated effluent water(more than 1,000 mgL−1) are not permissible forirrigation as per the Indian Standard Institution(1982) and cause high osmotic pressure leading torapid deterioration of water properties. In the present study it could be observed that the total dissolved solids were greatly reduced by *C.scalpelliformis* nanoparticles (229mg/l), from Fig 4 which was reported by using *C.vulgaris.*[23]

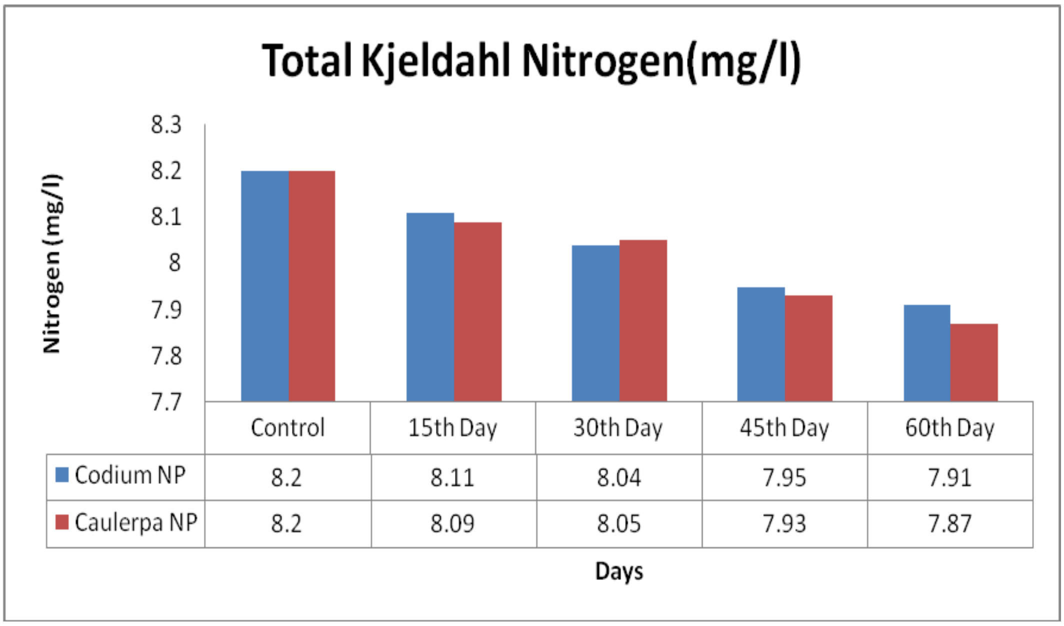
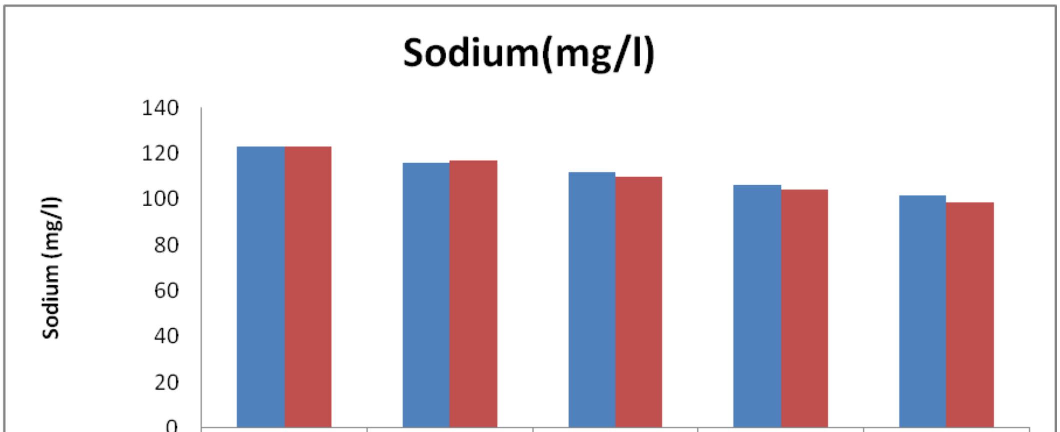


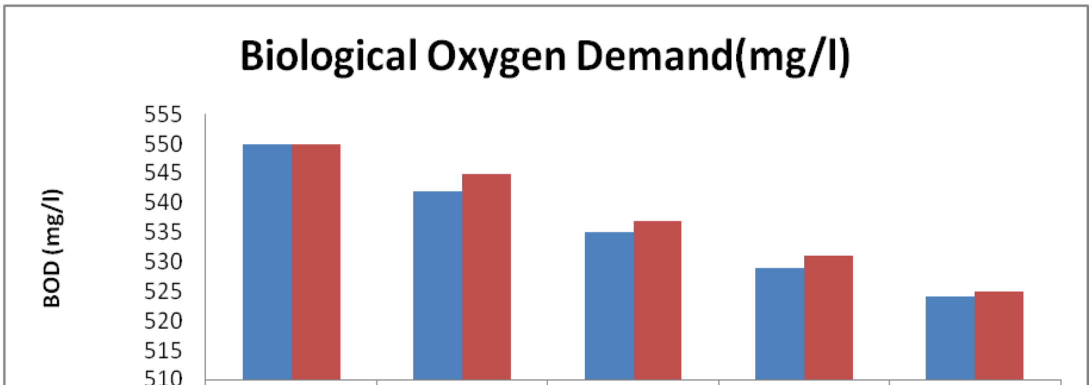
Fig. 5: Change in the Nitrogen of tannery effluent after treating with marine macro algae nanoparticles.



	Control	15th Day	30th Day	45th Day	60th Day
Codium NP	123	116.17	111.95	106.28	101.63
Caulerpa NP	123	117.33	110.12	104.16	98.91

Fig. 6: Reduction in the Sodium of tannery effluent after treating with marine macro algae nanoparticles.

Organic nitrogenis not pathologically significant but is sometimes considered as an indication of pollution. While high ratio of sodium to calcium plus magnesium is deleterious to soil structure. It was found that the nitrogen and sodium content in the effluent water was greatly reduced for *C.scalpelliformis* nanoparticles when compared with *C.decorticatum* nanoparticles from (Fig5, 6). This study was similar to work reported by Alejandro *et al*., 2009.[19]



	Control	15th Day	30th Day	45th Day	60th Day
Codium NP	550	542	535	529	524
Caulerpa NP	550	545	537	531	525

Fig. 7: Change in the BOD of tannery effluent after treating with marine macro algae nanoparticles.

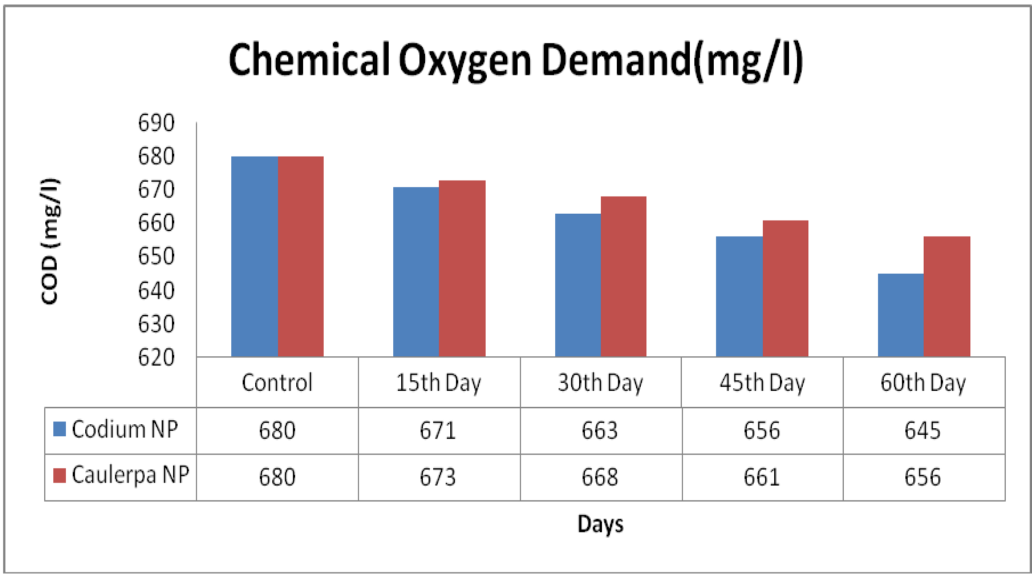


Fig. 8: Change in the COD of tannery effluent after treating with marine macro algae nanoparticles.

BOD and CODdetermines the relative oxygen required for biological and chemical oxidation of wastewaters, effluents, and polluted waters. It is the used to calculate the amount of oxygen required by bacteria and chemical oxidants while stabilizing decomposable organic matter. It was observed that the BOD and COD levels were significantly reduced equally for both the algae; *C.scalpelliformis* nanoparticlesand *C.decorticatum* nanoparticles (Fig 7,8) as reported similarly by *B.braunii*[21] and *Chlorella sp.*[24]

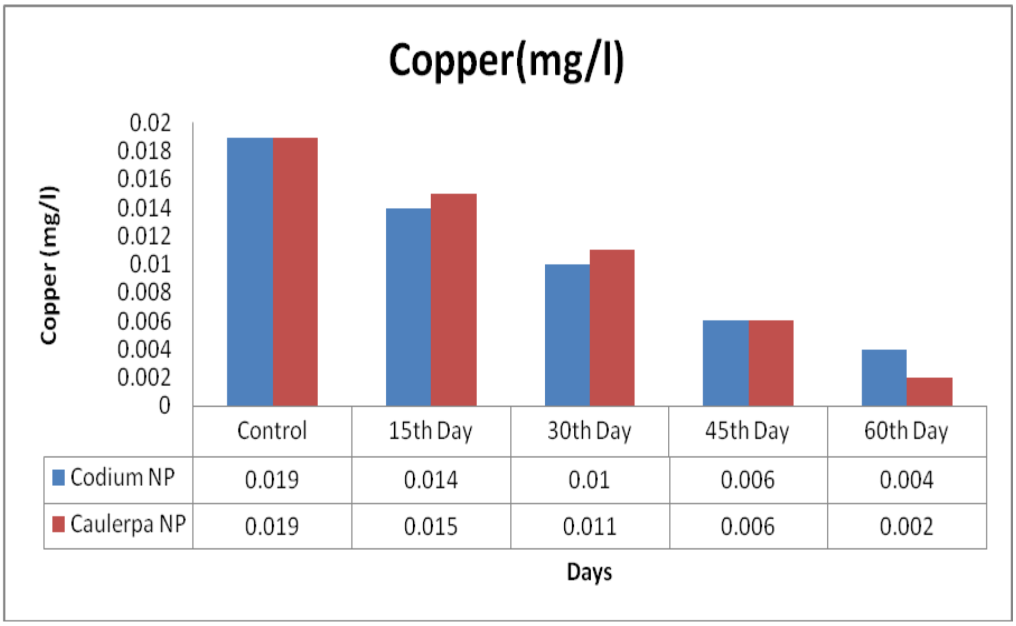


Fig. 9: Change in the Copper of tannery effluent after treating with marine macro algae nanoparticles.

Copper is said to impart disagreeable metallic taste to water. The presence of copper in more than trace amounts can usually be attributed to corrosive action to use the copper salts as a control for algae and other aquatic growths. Considering the potential environmental risk factor it was revealed from the present study that the copper level in the effluent water was narrowed down by greater levels by the algae *C.scalpelliformis* nanoparticles (0.002mg/l), (Fig 9). Similar study was reported with*Padina sp.*[25]

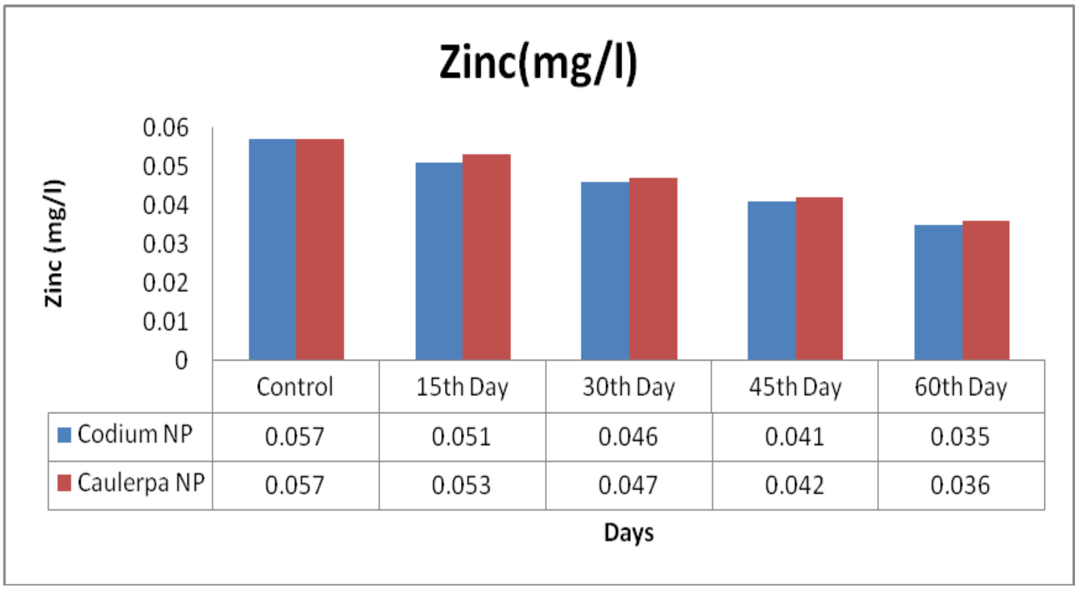


Fig. 10: Reduction in the Zinc content of tannery effluent after treating with marine macro algae nanoparticles.

Zinc salts give water an unpleasant astringent taste and form a greasy film on boiling water. Small quantities of zinc are toxic to various aquatic animals and plants. With that note it was observed that the levels of zinc were significantly reduced for both the alga. There is a slight difference in the level of zinc reduced by *C.decorticatum* nanoparticles (0.035mg/l) compared to *C.scalpelliformis* nanoparticles (0.036mg/l), (Fig 10). The result was similar to where *Chlorella sp.* were able to reduce the levels of zinc in the waste water.[24]

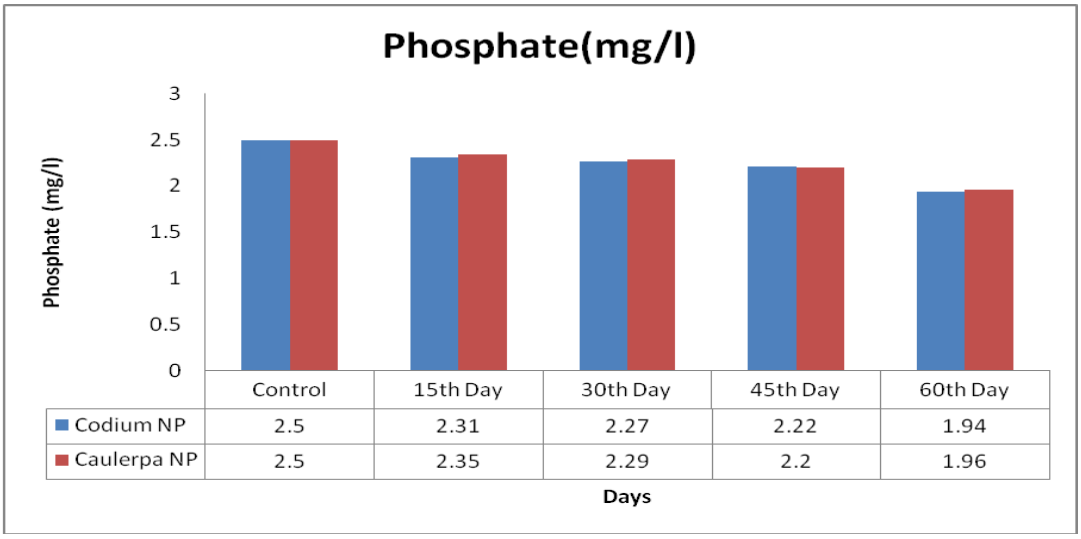


Fig. 11: Reduction in the phosphates in tannery effluent after treating with marine macro algae nanoparticles.

From Fig 11, it could be found that the phosphate levels were reduced effectively by *C.decorticatum* nanoparticles (1.94 mg/l). This demonstrates the use of algal nanoparticles as an alternative source in reducing the toxic substances from the effluent water. The findings have seen to corroborate with the work of Jing *et al*., 2007.[26]

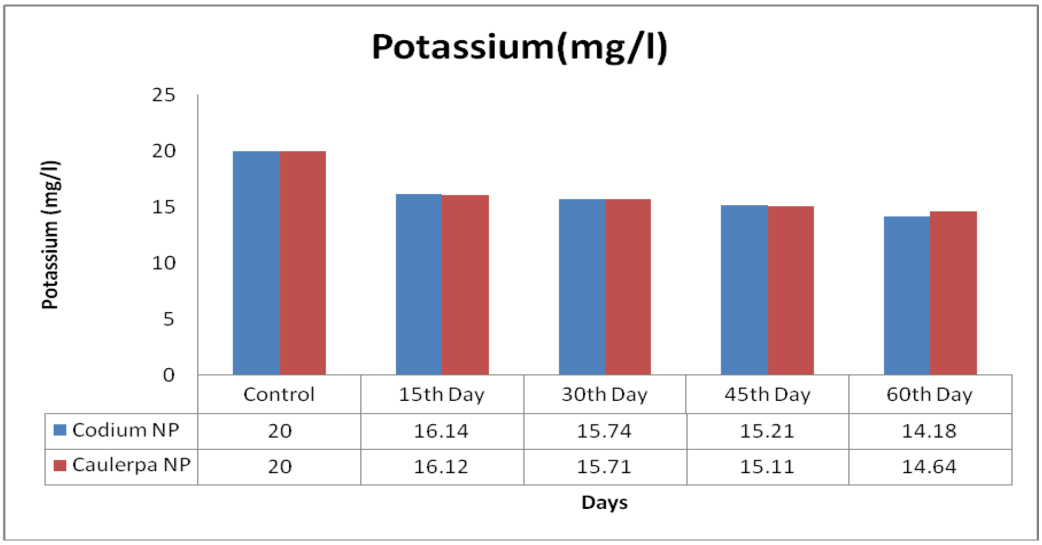


Fig. 12: Reduction in the Potassium of tannery effluent after treating with marine macro algae nanoparticles.

Potassium in water is reported to cause foaming. It stimulates plankton growth which is considered to be toxic for fish and shell fish. From the above figure, the amount of potassium was reduced maximum by *C.decorticatum* (14.18 mg/l) than the other algae which was similarly with the study carried by Liang *et al*., 2009.[24]

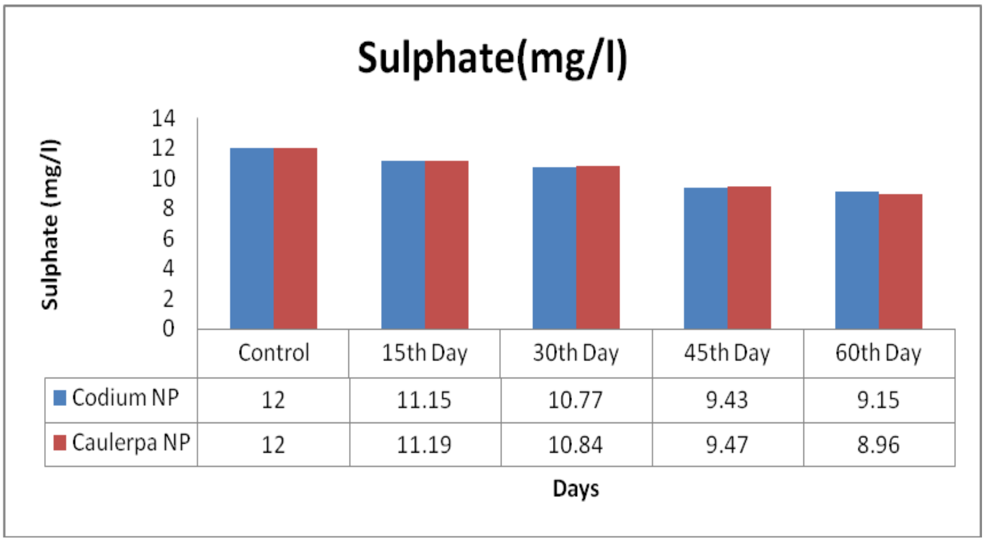


Fig. 13: Difference in the Sulphate of tannery effluent after treating with marine macro algae nanoparticles.

Sulfate removal is considered an important factor in almost all waste waters as they can lead to a bitter or a stringent taste and can also have laxative effects. From Fig 13, it was observed that the quantity of sulphate was declining significantly when treating with *Caulerpa* nanoparticles (8.96mg/l) when compared to that of *C.decorticatum* nanoparticles (9.15mg/l). The present study was found to be well coinciding with the work carried out by Rawat *et al*., 2010.

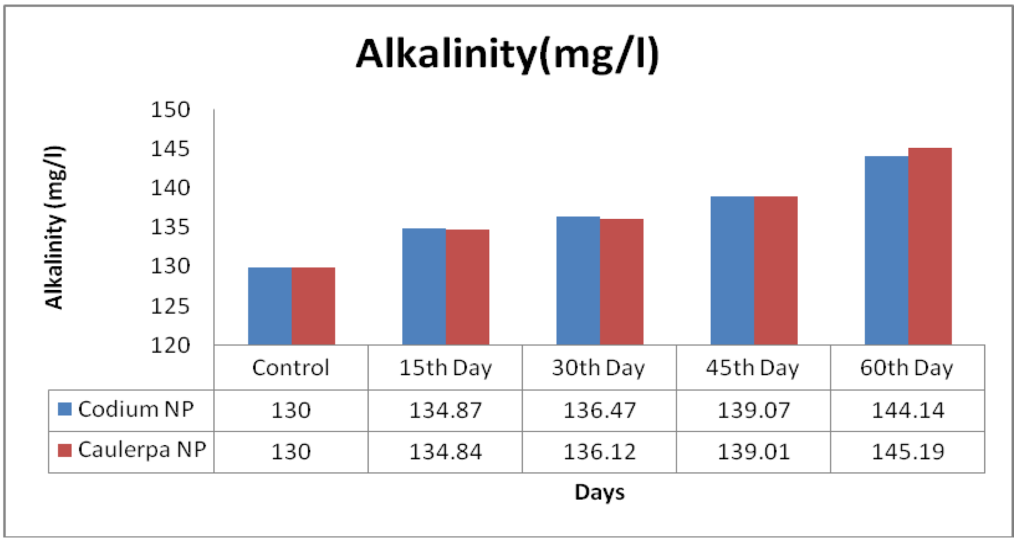


Fig. 14: Difference in the Alkalinity of tannery effluent after treating with marine macro algae nanoparticles.

The alkalinity of water is primarily a function of the carbonate, bicarbonate, and (or) hydroxide content in the water. The determination of alkalinity is a measure of the excess basic constituents over the amount necessary to balance the strong acid constituents. From the above figure it could be observed that, *C.scalpelliformis* nanoparticles were found to increase the alkalinity (145.19mg/l) in the effluent water higher than the *C.decorticatum* nanoparticles. Similar results were obtained with *P.zopfii* which was studied by Rawat *et al*., 2010.[21]

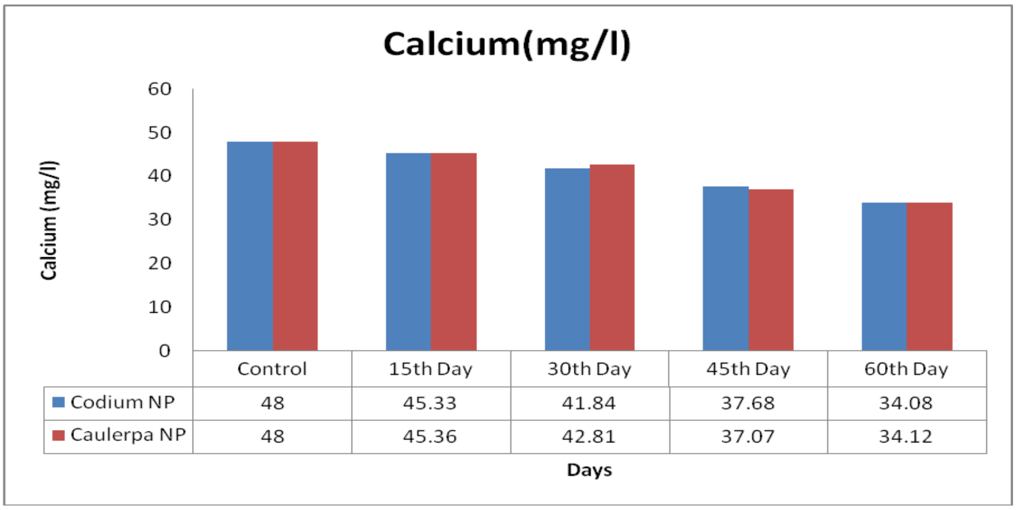


Fig. 15: Difference in the Calcium of tannery effluent after treating with marine macro algae nanoparticles.

Calcium imparts the property of hardness to water. The levels of calcium were significantly reduced by *C.decorticatum* nanoparticles (34.08mg/l) which is evident from the above graphical representation. *C.pyrenoidosa*is also efficient in removing the calcium salts from the waste water which was studied by Liang *et al*., 2009 which corroborates with the current work.[24]

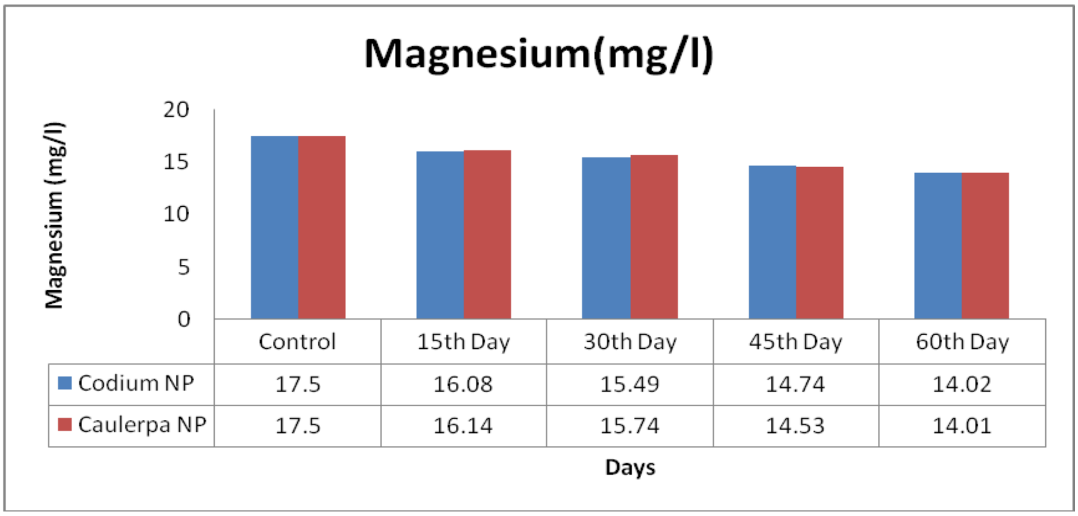


Fig. 16: Difference in the Magnesium of tannery effluent after treating with marine macro algae nanoparticles.

High concentrations of magnesium may cause scouring diseases among livestock. It also imparts the property of hardness to water. With the known effects of hard water, removal of magnesium is considered to be an important factor for industries. From fig 16, it was found that the magnesium content was declined to a great extent on treating the effluent water with both the algal nanoparticles. A similar study was conducted by sing *et al*., 2010 in which he showed the effective use of *C.vulgaris*in reducing the magnesium levels in the waste water.[23]

Anti Microbial Test

The result of the anti microbial test is given below in table 1 and figure 17

Table 1: Inhibition zone by the algal nanoparticles.

NAME OF ORGANISM	ZONE OF INHIBTION (cm)
CAULERPA SCALPELLIFORMIS	Clear Zone
CODIUM DECORTICATUM	1.1

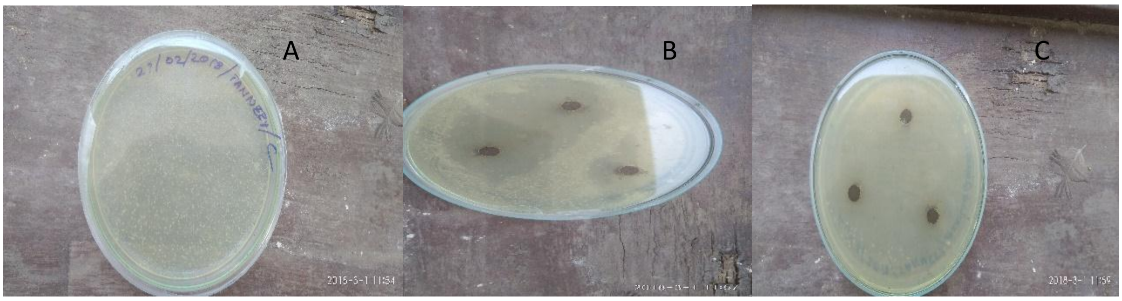


Fig. 17: Control with the growth of microorganisms(A). Inhibition zone formed by *Codium decorticatum*(B).Clear zone observed with *Caulerpa scalpelliformis*

From the above, it can be observed that *Caulerpa scalpelliformis*has better anti microbial activity giving clear zone of inhibition while *Codium decorticatum*gave 1.1cm zone of inhibition.

CONCLUSION

From the present prototypic study, the optimized (5mM) silvernanoparticles prepared from *Codiumdecorticatum*, showed higher efficacy intreating the tannery effluent at the end of 60 days compared to that of the *Caulerpascalpelliformis* nanoparticle. However, *Caulerpa scalpelliformis* had better anti microbial activity with that of *Codium decorticatum*.

The extension of the current study in proving the efficacy of nanoparticles prepared from *Codium decorticatum*, *Caulerpa scalpelliformis* could be carried out for along period for more than 6 months to 1 year and it may show muchimprovement in the treatment of tannery effluent.

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