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## Research Paper

# BIOREMEDIATION OF HEAVY METALS AND WASTE WATER TREATMENT USING LEAVES AND LATEX OF *CALOTROPIS PROCERA*

Bharati R Patil<sup>1\*</sup>, KeziaBuruga<sup>1</sup> and Gopal Mugeraya<sup>1</sup>\*Corresponding Author: **Bharati R Patil** ✉ [bharati.patil3@gmail.com](mailto:bharati.patil3@gmail.com)

Heavy metals are present in some industrial effluents, and are responsible for environmental pollution. Biosorption of heavy metals can be an effective method for the removal of heavy metal ions from wastewaters. In this study heavy metal biosorption by *Calotropisprocera* is being investigated. The effects of some important parameters such as pH, initial concentration of heavy metals by *Calotropisprocera* for various heavy metals and modification of biomass surface by autoclaving and acid treatment on biosorption capacity are studied. The biosorption of heavy metal ions on *Calotropisprocera* proves to be an efficient and low cost alternative to be considered in treatment of industrial wastewater containing heavy metal ions. The water treatment capacity of latex of *Calotropisprocera* on industrial and domestic waste water has shown that it has good clarifying properties. It can reduce the turbidity, color, odor, pH, hardness, alkalinity, microbial load, and total Coliforms of all highly turbid samples. The water treatment potential of latex of *Calotropisprocera* is not only of good economic importance but could be then most cost effective alternative method to prevent pollution. This biological treatment is therefore, preferred over and above other treatment methods because its techniques are cheap; do not need extensive training and controls. Thus, water treatment using *Calotropisprocera* may be a prescription for future problems.

**Keywords:** Bioremediation, *Calotropisprocera*, Biosorption, Heavy metals, Waste water treatment

## INTRODUCTION

Many industrial processes produce large amounts of wastewater, which are leading to detrimental effects on human life and environment. The major pollutants in wastewater are heavy metals such as lead, zinc, copper, cadmium, mercury,

chromium and arsenic. These metals accumulate in living tissues/organs and can cause accumulative poisoning and serious health problems such as, cancer and brain damage. There are numerous methods currently employed to remove these metals from aqueous

<sup>1</sup> Department of Chemical Engineering, National Institute of Technology, Surathkal-575025.

environment. Some of these methods are chemical precipitation and sludge separation, chemical oxidation or reduction, ion exchange, reverse osmosis, membrane separation, electrochemical treatment and evaporation. These techniques are very expensive and not environmentally acceptable, hence new biotechnological methods for remediation of these toxic metals are being considered recently (Chen *et al.*, 2005; Hawari and mulligan, 2006; Iyer *et al.*, 2005). Biosorption can overcome common problems associated with physicochemical processes (Costly and Wallis, 2001).

Biosorption as a wastewater bioremediation process has been found to be an economically feasible alternative for metal removal. This method offers the advantages of low operating cost, minimizing secondary pollution and high efficiency in wastes (Volesky and Holan, 1995; Tien, 2002). The use of nonliving biomass in biosorption is more practical and advantageous because living biomass cells often require the addition of fermentation media which increases the biological oxygen demand or chemical oxygen demand in the effluent. In addition, non-living biomass is not affected by the toxicity of the metal ions, and they can be subjected to different chemical and physical treatment techniques to enhance their performance.

Physical treatment methods such as heat, acid and base treatments have usually shown an increase in biosorption capacity of plant biomass due to reorganization of cell wall structure (Gong *et al.*, 2005). *Calotropisprocera*, also called rooster tree, giant milkweed or Sodom apple, is a member of flowering plant species in the dogbane family, *Asclepiadaceous*. This plant is a soft wooded, evergreen, perennial shrub. Giant milkweed is native to West Africa as far south as

Angola, North and East Africa. The leaves are widely used in Nigeria for coagulation of milk in preparing cheese (Ogugua and Muller, 1987).

In this study, the biosorption of heavy metals by *Calotropisprocera* are investigated. Also the effect of the system parameters such as pH, initial metal concentration and acid/heat treatment on the biosorption capacity of the biomass was investigated.

Wastewater refers to liquid discharged from residences, business premises, small-scale industries and institutions. In general, wastewater can be characterized based on its bulk or organic contents, physical characteristics, and specific contaminants (Damelle, 1995). Each wastewater contains its unique quality and characteristics, which then suggests the type of treatment required. The two divisions of waste water—Domestic and Industrial effluent have different make-ups and often require various treatment processes such as physical, chemical, thermal and biological processes but the basic biological processes are mostly preferred over and above the three others (Aririatu *et al.*, 1999). However, the methods of water treatment from biological materials by exploring the active ingredients of natural coagulants will indeed be cost effective in providing water at a very cheap and affordable price and at all time in every household. More recently the problem of effluent from processing operation and their disposal has gained public recognition. In many areas of the world, especially the developing countries, the environmental issues are the same. The techniques involved are cheap and do not need extensive training and controls, Coagulants from agricultural materials can minimize the limitations of using expensive and prohibitive cost for developing economics by providing a less expensive means of flocculation

and coagulation to obtain the appropriate degree of pre-purification.

The rationale for this present study is to see how this bioremediation technique can be employed to treat and prevent water pollution in this country of ours. Since the environmental issues are the same, in many areas of the world, especially the developing countries, this project seeks in attempt to provide solution to how waste treatment alternatives can be selected that will provide environmental protection at an affordable price through the utilization of natural coagulants in water and wastewater treatment. Though, the initial waste treatments by physical, chemical and thermal means were fast and controllable but they require high energy and are cost prohibitive. These natural coagulants which can be readily propagated and easily accessible to common persons would offer solution to our most plagued environmental issues and water pollution.

This project seek to use the latex of *C. procera* as a natural coagulant and clarifying agent in the treatment of water and waste water following the determination of the physicochemical characteristics of public water, domestic waste water, and industrial waste water samples, and to evaluate and compare the effectiveness of *C. procera* latex (Sodom apple) on turbidity reduction, odor removal, pH reduction, microbial load reduction and total coliform reduction of the water and waste water. The establishment of this will generally serve as a contribution to the knowledge of water and waste water treatment and proffer alternative to the other methods of treatment which need extensive training and control.

## MATERIALS AND METHODS

### Biomass

*Calotropis procera* leaves were collected locally from NITK campus. Samples were washed twice with distilled water. The washed biomass was oven-dried at 50°C for 24 h, crushed with an analytical mill, sieved (size fraction of 0.5-1 mm) and stored in polyethylene bottles until use.

### Heavy Metals

Heavy metals Lead, Copper, Zinc, Nickel used for the experiment were obtained from NICE (NICE CHEMICALS Pvt. Ltd), CDH (central drug house Pvt. Ltd) and spectrum (spectrum reagents and chemicals Pvt. Ltd).

### Sample Collection

Samples of tap, well, stream, domestic waste water, and industrial effluent (Pulp and paper effluent from west coast paper mill, Dandeli, Karnataka), were collected. Water samples representing different turbidities were collected in sterile two water plastic containers. The samples were taken to the laboratory and were analyzed within 6 hours (maximum transit time – 4 h, maximum process time- 2 h), before treatment with the coagulant.

### Latex Collection

The latex of *C. procera* was collected by detaching the leaves from the stem at the petioles as described by Aworh *et al.* (1993). The latex was also obtained locally as exudates from plucked of *C. procera* plant and used immediately or stored at 4°C (Kareem *et al.*, 2002).

The latex was obtained in pre-weight bottles. A concentration of 2% was made and stirred to dissolve the active constituent and allowed for 5 min. Then, the effect of latex on odor, color, pH,

turbidity, microbial load and total coliform count was studied after treatment to determine the effect of treatment on the samples.

### Biosorption Studies for Heavy Metal Removal

The biosorption experiments were conducted in Erlenmeyer flasks containing 50 ml of heavy metal solutions and 0.1 g of *Calotropisprocera* biomass. The flasks were agitated on a shaker at 150 rpm constant shaking for 6 h to ensure equilibrium was reached. Studies were performed at a constant temperature of 25°C to represent the environmental conditions. At the end of experimental period, metal solutions were separated from the biomass by centrifugation at 4000 rpm and analyzed using Atomic Absorption Spectrometer. The amount of metal adsorbed on biomass,  $Q_m$  (mg/g), was calculated according to the following equation:

$$Q_m = (C_i - C_{eq}) V / m \quad \dots(1)$$

where  $V$  is the volume of metal solution (ml),  
 $m$  is the mass of dry *Calotropisprocera* (g),

$C_i$  and  $C_{eq}$  are the initial and equilibrium concentration of metal in solution (mg/l), respectively.

Also the effect of solution pH on biosorption of metal ions was studied. For this purpose aqueous solutions adjusted to the predetermined pH values in the range of 2.0 to 6.0 were used.

To determine the effect of initial metal concentration on biosorption capacity of *Calotropisprocera*, five different heavy metal concentrations (50-200 ppm) of metal solutions were prepared.

### Analysis of Physico-chemical Properties and Bacteriological Properties

Raw water samples were analyzed for physiochemical and bacteriological quality. Physical tests of water were carried to find out color, odor, temperature, pH, turbidity, hardness and conductivity using various methods. Chemical properties such as alkalinity, acidity, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), were carried out using various chemical methods. Biological properties such as microbial load and total coliform count were determined.

### Treatment Methods

The latex was obtained in pre-weight bottles. A concentration of 2% was made and stirred to dissolve the active constituent and allowed for 5 min. Then, the effect of latex on odor, color, pH, turbidity, microbial load and total coliform count was studied after treatment to determine the effect of treatment on the samples. After treatment with this coagulant, the treated water and waste water samples were analyzed for physiochemical and bacteriological quality as described before. The turbidity absorbance readings at 540 nm wavelengths and the pH (at 25°C) readings were taken at 1 h and 24 h interval after treatment with the coagulants. The microbial load of the water samples were determined by the pour plate method using both Plate Count Agar (PCA) and nutrient agar (Arinatu *et al.*, 1999).

The coliform count and faecal coliform count was determined by the multiple tube fermentation technique/ method (Prescott *et al.*, 1999, Lamikanra, 1999, Fawole and Oso, 2001), for Most Probable Number (MPN) counts of the water

samples the results obtained were compared with the MPN index table of Seeley and Demark (1981) to estimate the number of coliform to estimate the number per ml (Edema *et al.*, 2001). The confirmatory test for the presumptive positive tubes of the MPN in total coliform enumeration was to verify the presence of coliform and to detect any false positive results (Edema *et al.*, 2001). The sterility of each batch of test medium was confirmed by incubating one or two uninoculated tubes or plates along with the inoculated tests. The uninoculated tubes or plates were always examined to show no evidence of bacterial growth. Any uninoculated tube or plate that showed evidence of bacterial growth was discarded. Also, untreated water and waste water

samples were left to stand for 24 h as control test for turbidity.

## RESULTS

### Heavy Metal Removal

#### pH

pH has a very significant effect on biosorption of metal ions from solutions. It is known that the solution of pH affects the surface charge of the biomass, the specification of the functional groups such as carboxylate, phosphate, hydroxyl and amino groups of the cell wall (Vilar *et al.*, 2005). The maximum biosorption of Lead, Zinc, Copper and Nickel were at pH 4.0, 6.0, 5.0, 3.0, respectively.

**Table 1: Removal Efficiency of *Calatropisprocera***

Heavy metal	Initial-metal concentration (ppm)	Metal concentration after treatment (ppm)	Removal efficiency (%)
Lead (Pb)	50	7.00	85.87
	100	13.90	
	150	21.25	
	200	28.85	
Zinc (Zn)	50	7.43	85.06
	100	14.95	
	150	22.45	
	200	29.95	
Copper (Cu)	50	7.9	84.61
	100	15.02	
	150	23.56	
	200	30.05	
Nickel (Ni)	50	7.45	85.02
	100	14.94	
	150	22.45	
	200	29.95	

### Initial Metal Concentration

The metal distribution at equilibrium is important to determine the maximum biosorption capacity of the biomass for Pb, Zn, Ni and Cu metals. The effect of initial metal concentration on the biosorption capacity was investigated at maximum pH values.

A higher initial concentration provides an important driving force to overcome all mass transfer resistances between the metal solution and *Calotropisprocera* cell wall, and therefore the biosorption capacity increases. In addition, the number of collisions between metal ions and the biosorbent increases with increasing initial metal concentration so the biosorption process is enhanced (Aksu, 2005). Biosorption rate was decreased with increasing initial concentration from 100 to 200 mg/l and this can be explained by the saturation of the biosorption sites on the biomass surface.

### The Effect of the latex of *Calotropisprocera* on the Odor and Color of the Samples

The latex of *Calotropisprocera* reduced the objectionable odor associated with domestic effluent as well as the color of the waste samples while the colorless samples remain very clear.

### The Effect of the Latex of *Calotropisprocera* on the pH of the Water Samples

A reduction in the pH (at 25°C) was obtained in the range of 0.93-20.64% and an increase in pH of some waste water sample was also obtained in the range of 27.10-32.90%. The latex of *Calotropisprocera* reduced the pH of most water samples in the range of 1.84-20.65% and increased the waste samples in the range of 27.10-31.61%. As the time interval after treatment increased, so did the degree of pH reduction and increment increases.

**Table 2: Properties of Waste Water on Treatment With *Calotropisprocera* Latex**

Samples	Tap Water		Well Water		Stagnant Water		Domestic Water		Effluent Water	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
pH	6.55				8.0	7.11	5.6		8.56	7.10
Turbidity	0.08	0.06	0.14	0.07	0.13	0.07	0.5	0.03	0.99	0.25
Conductivity (µs/cm)	184	45	885	705	800	698	956	850	1300	950
Total Hardness (mg/l)	50	20	108	91	560	350	240	180	280	120
DO	5.4	4.40	6.3	5.7	5.05	4.75	5.22	4.85	7.43	6.25
BOD	30	13	50	15	200	150	240	100	380	100
COD	50	15	134	85	380	150	450	150	1861.8	950
TDS	120	50	356	300	200	120	800	500	2950	987
TSS	100	38	64	48	150	88	115	95	1120	480

### **The Effect of the Latex of *Calotropisprocera* on the Turbidity of the Water Samples**

The effect of the *Calotropisprocera* latex on the turbidity of the water and waste/effluent water samples is determined. A considerable reduction in turbidity was obtained. As the time interval after treatment with the coagulant increased, so did the degree of the clarification until the maximum time above which there was no further decrease in turbidity. The maximum time varied for the different coagulant to the samples treated but for these two coagulants it was 24 h.

### **The Antimicrobial Effect of the Latex of *Calotropisprocera* on the Microbial load of the Water and Effluent/Waste Water Samples**

The antimicrobial effect of the coagulant is also determined. The results indicate a reduction in the microbial load of both the water samples and the effluent/waste water samples by as much as 98.42% for 1 h interval after treatment and by 100.00% for 24 h interval after treatment. *Calotropisprocera* latex reduced the microbial load in the range of 98.82% for 1 h interval after treatment and 100.00% for 24 h interval after treatment respectively. Also, the coagulant exert antimicrobial effect on the total viable count of all samples incubated at both 24 h and 48 h, and showed a reduction as much as averagely 99.39% and 96.96%, respectively.

### **The Antimicrobial Effect of the Latex of *Calotropisprocera* on the Total Coliform Counts of the Water and Effluent/Waste Water Samples**

The antimicrobial effect of the coagulants is determined. The results indicate a reduction in the most probable number (MPN/100 ml) of the presumptive total coliform count of both the treated samples of water samples and the

effluent/waste water samples. It showed a reduction in the range of 84.21% - 100%. Viability studies indicated that the reduction in microbial load and total coliforms was broad based hence it affected all types of water samples.

## **CONCLUSION**

Biosorption technology, utilizing natural materials for effectively removing metals from aqueous media, offers an efficient alternative compared to traditional chemical and physical treatments. The goal of this work was to explore the potential use of *Calotropisprocera* biomass as a low-cost sorbent for the removal of various heavy metal ions from aqueous solutions. Experiments showed that *Calotropisprocera* have a remarkable ability to take up heavy metal ions.

The results obtained showed that the pH and initial metal concentration affected the uptake capacity of the biosorbent. And also acid/heat modification affected the biosorption yield and metal uptake positively. *Calotropisprocera* shows great promise for the removal of heavy metal ions.

Conclusively, waste water treatment using *Calotropisprocera* an effectively reduce color, odor, turbidity, hardness, toxicity. This biological treatment (bioremediation) is therefore, preferred over and above other treatment methods because its techniques are cheap; do not need extensive training and controls. Thus, any water treatment solution that does not start with a good water analysis is a prescription for future problems.

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**Hyderabad, INDIA. Ph: +91-09441351700, 09059645577**

**E-mail: editorijerst@gmail.com or editor@ijerst.com**

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