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Studies on reduction and removal of hexavalent chromium in industrial waste water by *Alternanthera sessilis* and *Tagetes erecta*

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ABSTRACT

Background: This study was taken up to remediate Cr^{6+} that is usually present in waste water of Industries that employs Chromium. This is a new venture in remediating Cr^{6+} using natural antioxidants which are usually overlooked. Also, it is an effort to both reduce and remove the Cr^{6+} from the waste water that gives an extra edge over other technologies. Antioxidant activity and sorption ability of the leaves of *Alternanthera sessilis* and *Tagetes erecta* were studied for reduction and removal of Cr 6+ from Industrial waste water. FRAP and DPC assays were conducted for determining, Antioxidant Activity and Cr^{6+} concentration respectively.
Results: Antioxidant activity of the aqueous (*A.sessilis*: 2657 AOU, *T. erecta:* 3071 AOU) and methanol (*A. sessilis*: 2932 AOU, *T. erecta:* 3127 AOU) extracts of both the plants was found to be almost similar. Untreated industrial waste water (106.07 ppm of hexavalent chromium) was collected from an electroplating unit and tests were conducted on the same. Extracts obtained by Ultra-Sonication for 5min showed the highest reduction efficiency (≥60% until 100ppm) when compared to other time intervals of sonication and conventional shaking, while Cake obtained by Ultra-sonication for 10min was found to have the highest adsorption efficiency (approximately 80%) for waste water. In the view of combining both reduction & removal, Synthetic and Industrial waste water were directly incubated with 2g of plant sample for various incubation periods and concentration levels. 1hr incubation for all the concentration levels proved to be the optimum incubation time for both plant samples with approximately 98% remediation efficiency. Conclusion: Current studies indicate that the plant samples adopted for our studies prove to be both cost effective and environment friendly alternative to the conventional techniques.

KEYWORDS

Antioxidant;
Chromium;
Reduction;
Removal;
Waste water.

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BACKGROUND

Chromium, a d-block transition metal is used in various forms in various industries including pigments manufacture, finishing of metals, in production of stainless steel, as corrosion inhibitors and for preservation of wood[1]. It is a known fact that Cr 6+ released by all these industries is 500 times more toxic than Cr 3+[2]. Cr 6+ has proven health effects not only on human beings but also all the organisms. Cr 6+ is a confirmed carcinogen and can cause Lung cancer, if sufficient protective measures against Cr 6+ exposure are not taken[3]. Different methods including Redox reactions, sorption, Precipitation, Biological Transformations and Physical Remediation processes are widely used in various industries for decreasing the level of Cr 6+ in Waste water[4]. Reducing Cr 6+ to its non-toxic form Cr 3+ is being achieved by various reducing agents including gaseous sulphur dioxide, sodium sulphite, sodium metabisulphite and ferrous sulphate[5]. Removing Cr 6+ by sorption is carried out by adsorbents ranging from Carbon Nanotubes[6], Algae (Biosorption)[7], coffee husk[8], eucalyptus bark[9] and others low cost adsorbents. Reduction and removal studies are carried out by using two plants *Alternanthera sessilis* and *Tagetes erecta* which have proven antioxidant activities[10].

Alternanthera sessilis (L.) known as sessile joy weed is commonly found in tropical and subtropical regions. Federal noxious weed list [USDA, APHIS] has listed *A.sessilis* as a terrestrial weed which belongs to the family *Amaranthaceae*. *A.sessilis* is a perennial plant which can grow under various soil conditions but thrives well in wet soil conditions[12]. Beneficial properties of *A.sessilis* are many, to list down a few; it is a widely used Leafy vegetable in many South Asian countries that can be grown commercially, to treat stomach ailments and as a dressing for wounds. The crushed plant material is used for headache, dizziness and as galactagogue. Leafy stems are ground and smeared for snakebites. *A. sessilis* is a popular folk medicine in few countries, often it is used to treat lung disorders[11].

Tagetes erecta which is a dicot like *A.sessilis* belongs to the family *Asteraceae*. The plant is habitually 1-3 feet tall and has a spread of 1-5 feet and has origin in North America[13]. It is grown for the purpose of showy flowers. After the harvesting of thereof, the rest of the plant usually is allowed to dry or removed. Flowers are also used for various commercial, traditional and medicinal purposes. As mentioned above, the leaves of both the plants have proven Antioxidant activities.

In the current report we present evidence that antioxidant activity of these plant materials is effectively used for reduction of Cr 6+ to its non-toxic Cr 3+. This is to determine the best extraction procedure with the optimised parameters for effective removal of Cr (both 6+ and 3+) from industrial waste water. In summary, reduction and removal put together provides an excellent alternative to the conventional methods that are being currently employed by the industries involving Hexavalent Chromium.

RESULTS AND DISCUSSION

Antioxidant activity

FRAP assay results indicate that conventional shaking for 24hrs has anti-oxidant activity higher than Ultrasonication carried out for 1 min, 3 min, 5 min and 10 min.

It is seen that, *A.sessilis* has a better anti-oxidant property than *T.erecta* in all the Ultra-Sonication meth-

TABLE 1 : Comparison of antioxidant activity of different extracts for various extraction methods. The values indicate the mean and standard deviation of triplicate samples.

	AS-Aq*	AS-Meth*	TE-Aq*	TE-Meth*
Shakeflask-24hrs	2657.704±57.36	2932.305±41.27	3071.05±63.74	3127.15±48.12
Ultra-Sonication-1 min	2617.145±0.007	408.56	66.53	766.92
Ultra-sonication-3min	2884.445±33.01	2550.595±8.25	1797.09±28.89	2280.36±28.89
Ultra-sonication-5min	2757.79±30.55	1276.99±29.36	1485.415±42.09	1090.6155±98.09
Ultra-sonication-10min	2889.12	2878.61±14.86	1103.115±3.3	1588.715±77.59

***AS-Aq : A. sessilis aqueous extract; AS-Meth : A. sessilismethanolic extract; TE-Aq : T. erecta aqueous extract; TE-Meth : T. erectamethanolic extract**

ods; while in conventional method *T.erecta* has a better Anti-oxidant activity (TABLE 1).

Amongst the two extraction solvents used, aqueous solvent was found to be effective for cell disruption. Also water being more economical, further extractions was made using aqueous solvent (i.e., water). Although there were variations found in the antioxidant activity of two plants in different extraction methods, they have proven activity which was again confirmed in our study. This characteristic of the plants was utilised in treating waste water containing toxic chromium to its non-toxic form.

Reduction of hexavalent chromium

The extracts were incubated with varying concentrations of Cr 6+ to study the reduction efficiency. It was found that the percentage reduction efficiency decreases with the increase in Cr 6+ concentration.

By comparing the extraction methods for effective reduction, Ultra-sonication for 5 min demonstrated better reduction efficiency than other methods for lower concentrations of Cr 6+ up to 100ppm (Typically, the concentration of Cr 6+ in untreated Industrial waste water). Ultra-Sonication for 7 min also gives a compa-

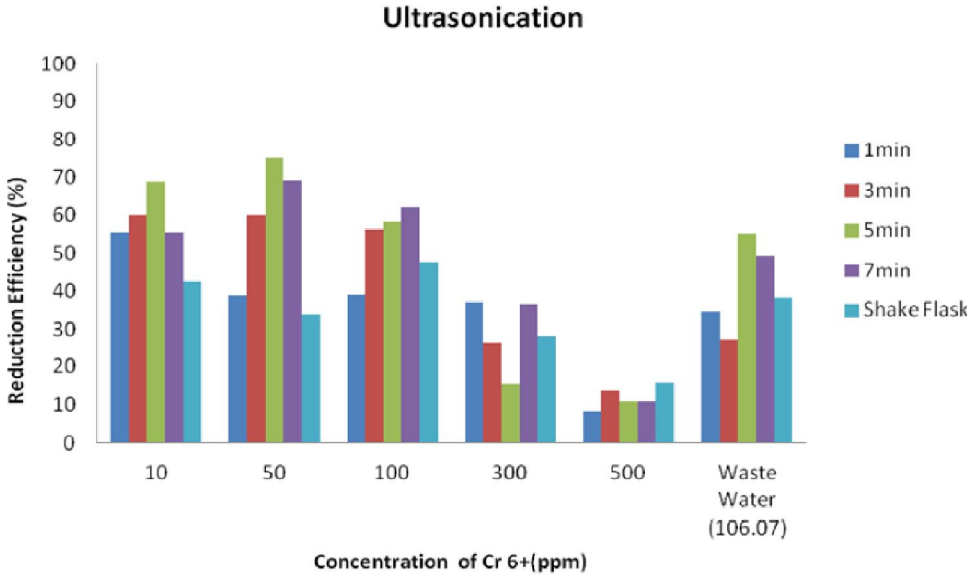

Figure 1 : Comparison of effect of extraction methods on reduction efficiency at various concentration levels of Cr 6+ in *A. sessilis*

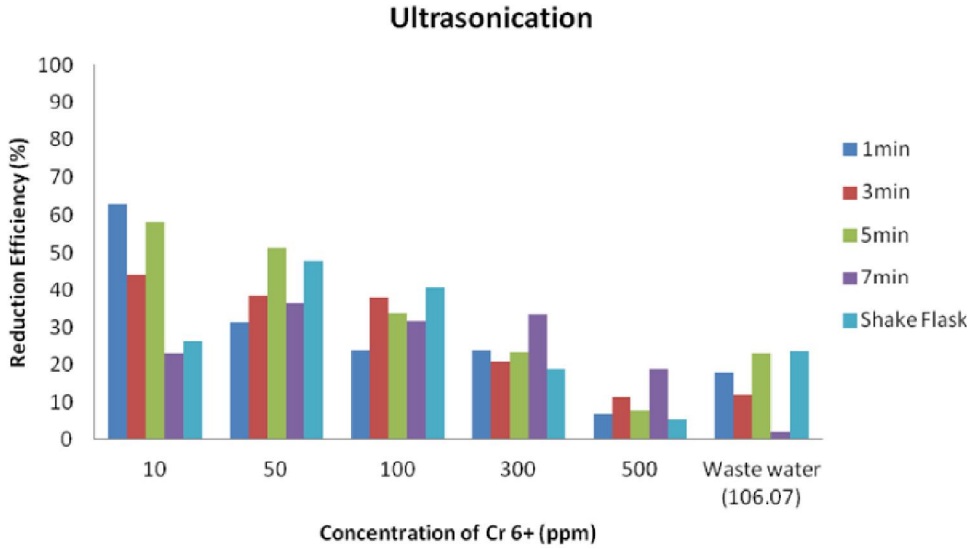

Figure 2 : Comparison of effect of extraction methods on reduction efficiency at various concentration levels of Cr 6+ in *T. erecta*

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rable efficiency. It was also evident that, *A.sessilis* had a better reducing efficiency than *T.erecta* irrespective of extraction methods (Figure 1 and 2).

Effect of time on remediation efficiency in direct incubation of plant samples

The waste water samples were incubated directly with the processed plant material at various incubation periods by shake flask method. The water was filtered out conventionally and % remediation efficiency was calculated. It was found that % Remediation decreased as the concentration of Cr 6+ increased.

Remediation efficiency for all the concentration levels was found to be more than 50% for all the incubation times considered. However 1 hr incubation showed an overall effective lessening of Chromium (>80% efficiency for all Cr 6+ concentrations) when compared to all the other incubation periods (Figure 3 & 4).

Waste water and 100ppm were found to have similar % remediation efficiency in all the experiments. This indicates that the other constituents of waste water like Zn^{2+}, Cl^{-}, SO^{4-}, Na^{+}, F^{-}etc. has no effect on Cr 6+ remediation.

EXPERIMENTAL

Preparation of plant extracts

Fresh mature leaves of *A.sessilis* and *T.erecta* were

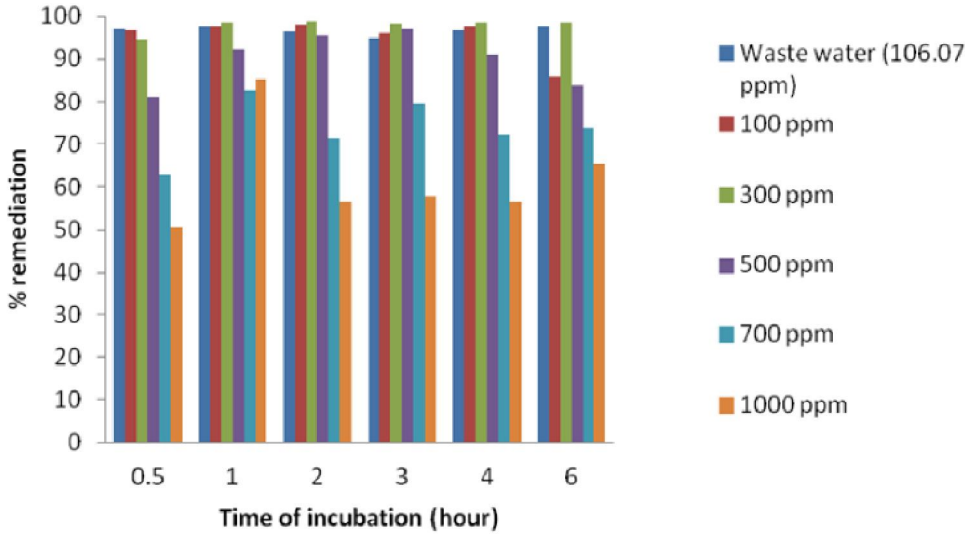

Figure 3 : Comparison of effect of incubation time on % remediation at various Cr 6+ levels using *Alternanthera sessilis* sample

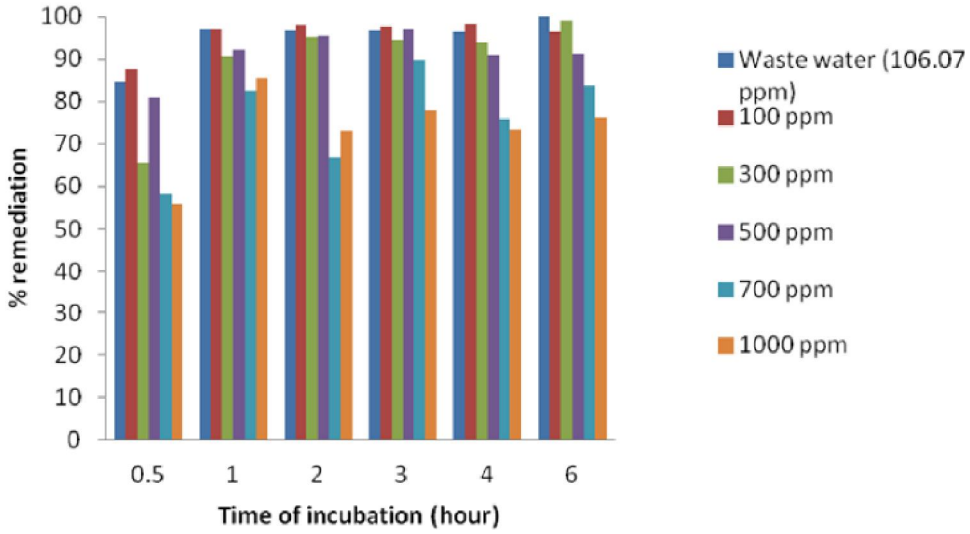

Figure 4 : Comparison of effect of incubation time on % remediation at various Cr 6+ levels using *Tageteserecta* sample

collected from the farms of Anekal taluk, Bengaluru (R), India. The leaves were extensively washed and dried (at 50^0C for 48hrs) and were finely powdered. The powder was suspended in water/ methanol (1:10 w/v) and subjected to different extraction procedures (shake flask and ultra-sonication for varying time intervals). The extracts were then filtered using Whatman paper No.1. The residue obtained as cake was dried and stored at room temperature for removal studies.

Preparation of synthetic waste water

A stock solution of Cr 6+ of concentration 1000 mg/l was prepared using an accurately weighed quantity of the $K_2Cr_2O_7$ in distilled water. Different concentrations of Chromium required for the experiments were prepared by diluting the initial stock solution.

Antioxidant activity by FRAP assay

Antioxidant activity of the samples was determined by the FRAP assay as described by Benzie and Strain[14]. Vitamin C (ascorbic acid) standard ranging from 0-1200 μM, with an interval of 200 μM was prepared. The activity of the samples was found with the help of standard graph after reading the absorbance at 592 nm.

Estimation of Cr 6+ by DPC (Di-phenylcarbazide) method

The total Cr 6+ content in the waste water was determined by reaction with Di-phenylcarbazide in acidic pH using a colorimeter[15].

To obtain Cr 6+ concentration of unknown sample, a known volume of sample was taken in 100 ml standard flask, pH was adjusted to 0.7 to 1.3 using 0.2 N sulphuric acid and distilled water was added to make up the volume. Then Diphenyl Carbazide was added so that final volume became 102 ml. The instrument was set by keeping blank in absorption cell. The absorbance was read at 540nm for both blank and test samples. The absorption was interpreted with the calibration curve and Cr 6+ concentration of unknown sample was obtained by the following formula

[mg Cr(VI)/l] (ppm) = μg Cr (in 102 ml final volume) / A

Where A= ml of original sample taken for analysis.

Direct Incubation of the plant sample with chromium

In the view of combining both reduction and removal, the plant powders were directly incubated with Cr 6+. Plant powders (2 grams each) were incubated with varying known levels of Cr 6+ concentrations (100-1000ppm), for various incubation periods (1hr, 2hr, 3hr, 4hr and 6hr) in shake flasks at room temperature. DPC method was carried out to obtain the total percentage remediation of Cr 6+.

LIST OF ABBREVIATIONS

AOU : Antioxidant units (μM of Ascorbic Acid/gram dry weight of the sample)
Cr^{6+} : Hexavalent Chromium
Cr^{3+} : Trivalent Chromium

CONCLUSION

This technology that is devised to apply the antioxidant potentials of *A. sessilis* and *T. erecta* can be used to both reduce and remove the toxic hexavalent chromium from the industrial waste water. The two step process including antioxidant extraction and extract incubation with waste water is efficient for Chromium concentration upto 100 ppm and gradually deteriorates thereafter. The efficiency can be enhanced by directly incubating the processed plant material with waste water containing chromium. This not only renders the process simpler (by eliminating the extraction step) but also improves the remediation efficiency at low incubation time (here, 1 hour) and higher Cr 6+ concentrations (approximately 85% for 1000ppm Cr 6+). By appropriate assessment of the antioxidant potential of the biological material, this technology can be scaled up and efficiently used in eliminating the hazards caused by Cr 6+ in industrial waste water.

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