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Phytoremediation potential of *Elodea canadensis* for reduction of chromium – optimization using response surface methodology

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ABSTRACT

Chromium is a toxic heavy metal which possesses carcinogenic threats to all living organisms. This heavy metal is found to be present in tannery wastewater. The reduction of chromium content in wastewater is evaluated using phytoremediation of *Elodea canadensis*. The experiments were carried out with variables namely number of days, number of plants/area, hydraulic retention time and concentration of chromium. Bioaccumulation and translocation factors were taken as measures for phytoremediation. The bioaccumulation factor of 3.89 and translocation factor of 2.8 were achieved. Both the factors indicate the ability of the macrophytes to act as phytoaccumulator. These results also pinpoint the mobilization of chromium from soil to roots and roots to shoot. The chromium content of the sample was reduced to 65% and total dissolved solids reduced up to 48%. The cost required for the treatment is very less as it doesn't require addition of chemicals and it works with zero energy consumption. Also, *E. canadensis* is easily grown and has aesthetic value. The treated water is free of contaminants and hence it is suitable for reuse in industries. Hence, the phytoremediation using *E. canadensis* is a sustainable strategy for detoxification and treatment of industrial wastewater.

Keywords: Phytoremediation; Chromium; *Elodea canadensis*; Bioaccumulation; Translocation; Total dissolved solids

1. Introduction

Leather and leather products are one of the most widely traded commodities that contribute to a global trade value of approximately $100 billion/y. The processing of leather involves pre-tanning, tanning and finishing of tanned leather to final products [1]. The process of tanning is produces large amount of solid and liquid chemical wastes, which are left untreated. It is estimated that Indian tannery industries contribute to the disposal of 2,000–3,000 tons of chromium to water bodies and landfills [2]. It is familiar that chromium is toxic to lives of all living organisms and intake of chromium by human beings can cause cancer [3]. Disposal of untreated solid and liquid wastes containing chromium and other heavy metals can disturb the ecosystem [4]. Hence, the treatment of tannery effluent is a paramount task for the industries and the society.

In the past decades, industries and researches have attempted to discover effective and economic strategies for the treatment of tannery effluent. Chemical coagulation using aluminum sulfate and ferric chloride has been found to be effective in removal of suspended solids [5]. Adsorption involves removal of contaminants in the effluent stream by using a solid substrate. Low-cost adsorbents developed out

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of wastes like neem bark, cotton shell, etc have been utilized for continuous treatment [6]. Groundnut shell activated with citric acid has been used for the adsorption of hexavalent chromium and it is found to be a cost-effective solution [7]. Adsorption of chrome solution using cement kiln dust has been found to be effective in removal of contaminants [8]. Even though, adsorption is simple and successful in removal of contaminants from effluent, it puts forth a new problem of disposal and recycling of adsorbents after treatment. Regeneration of saw dust-based adsorbents after chromium removal using acid and base solutions has been found to be successful. Further, chromium salts present in the acid–base solutions were precipitated [9]. However, the chromium precipitate disposal is once again troublesome. Contemporary approaches like capacitive deionization using bio-charcoal based electrodes have been attempted for removal of chromium from wastewater [10]. The effectiveness of electrochemical treatment is restricted to small scale and controlled environments.

Advanced oxidation processes using chemicals like Fenton reagent, ozone and many catalysts utilize hydroxyl radical for treatment of contaminants [9]. Similar advanced oxidation processes have been intensified by cavitation to improve the degradation [11]. Ultrasound has been utilized to induce cavitation in oxy-catalyst based advanced oxidation of tannery effluent and the de-contamination was found to be satisfactory [12]. Even though cavitation assisted advanced oxidation is found to be successful, the usage of this technique is subjective to considerable capital and operating expenses. Hence, application of these techniques in small and medium scale tannery industries is non-viable.

Biological remediation is a sustainable and eco-friendly approach to remove the contaminants in wastewater. Especially, heavy metals present in wastewater can be effectively removed using microbes or plant species, without huge expenses or power consumption or chemical usage [13]. Constructed wetlands have been put to use for carrying out such remediation processes using different plant species and they are found to be useful in removal of micro pollutants [14]. Phytoremediation of simulated textile effluent in a constructed wetland indicated considerable reduction of contaminants. Common plants like *Phragmites australis* and *Typha latifolia* have been utilized for the remediation process [15].

Constructed wetlands are utilized in two modes namely, vertical flow and horizontal flow and these wetlands are improvised by synergizing with other techniques like adsorption, microbial treatment, aeration, etc [16]. Plant species like *Typha domingensis* and *Canna indica* have been applied for the phytoremediation of chromium, zinc, and nickel under vertical flow conditions [17]. On the other hand heavy metals like cadmium, nickel and copper have been removed using *Nerium oleander* using horizontal sub-surface flow conditions [18].

Research evidence indicates the possibility of utilizing constructed wetland for removal of Direct Red 28 and kinetic models were evaluated for the treatment [19]. Hexavalent chromium which is a dominant pollutant in tannery wastewater is found to act as a micro-nutrient for plant growth when provided in limited quantities. Hence, the utilization of constructed wetlands for the removal of hexavalent chromium is a dexterous choice because it is reduced energy utilization and secondary pollutant generation [20]. However, detailed experimentation and analysis of the technique is required to capacitate it for real time utilization.

Submerged macrophyte plant species are resilient to stagnant water and marshy lands. It is suitable to utilize such submerged macrophytes for constructed wetlands [21]. Different species of such macrophytes like *Iris versicolor*, *Andropogon virginicus*, *Panicum virgatum*, etc have been put to use for treatment of pesticide wastewater [22]. Heavy metal rich industrial effluent has been treated with water hyacinth and it was observed that the treatment was effective for dye wastewater [23].

During the remediation process, the contaminants are removed via different mechanisms namely, phytostabilization, phytostimulation, phytofiltration, phytoextraction [24]. It is also understood that phosphate and sulfate transporters are crucial in the uptake of contaminants by plants [25]. A synergic effect of enzymes, transport through plant membranes, and plant metabolism is found to be responsible for the removal or reduction of toxicity of the effluent [26]. In order to understand the efficacy and extent of the phytoremediation few measures are suggested. The bioaccumulation factor is a measure of the capacity of the plant to absorb the contaminants in its tissues [27]. Translocation factor is another measure that indicates the distribution of the contaminants between the plant roots and shoots [28].

Based on the above findings, it is apparent that submerged macrophytes are capable of remediating contaminated wastewater. *Elodea canadensis*, commonly known as pond weed is chosen as the macrophyte for the de-contamination of hexavalent chromium solution. A horizontal sub-surface flow-constructed wetland is to be utilized for the treatment. Different operating variables namely treatment duration, the number of plants/area, retention time and, concentration of the chromium are considered. The effect of the above variables on chemical oxygen demand (COD), total dissolved solids (TDS), and conductivity of the wastewater are evaluated through response surface methodology analysis. The mechanism of the remediation is to be evaluated and the quantification of the treatment is done by the determination of the bioaccumulation factor (BAF) and translocation factor (TF).

2. Experiment

2.1. Construction of the wetland

In order to carry out the experiments in batch scale the wetland is designed with 4 small containers with the dimensions of 13 cm × 18 cm × 8 cm. Then for constructed wetland, it is filled with soil mixed with the biomass to a height of 7 cm. The horizontal flow of water is carried out in this process for getting better results than the vertical flow. Chromium solution is supplied through the wetland using drip irrigation tubes with perforations.

2.2. Preparation of synthetic effluent

It is intended to evaluate the phytoremediation of hexavalent chromium by *E. canadensis*. Therefore, a solution of potassium dichromate is prepared in 5 different concentrations between 2.5 and 7.5 ppm in distilled water. This synthetic effluent is prepared based on the concentration of hexavalent chromium in tannery wastewater [29].

2.3. Analysis of raw and treated effluents

Lab grade reagents were used for the analysis of the chrome solution before and after treatment. Key properties of a raw and treated solution, like pH, TDS, conductivity, and COD are measured for both streams. The pH meter and conductivity meter were used to measure the pH and conductivity of the streams, respectively. A titration procedure is followed for the determination of chemical oxygen demand [30]. The concentration of chromium in the samples is determined by atomic absorption spectroscopy. The key properties of raw synthetic effluent are listed.

2.4. Phytoremediation studies

First synthetic solution was prepared at different concentrations namely 2.5, 3.75, 5, 6.25, and 7.5 ppm levels. In that four containers which are filled with soil bed are planted *E. canadensis* a plant species. In each container, a different number of plant species, namely 2, 4 6, 8, and 10 were planted. The retention time of the effluent was varied between 0.5–1.5 h in five levels. The length of the stem, root, and number of leaves in the plants was noted before feeding water to the plant species. Fresh water is fed to the plant species in one container and grown for a week. The remaining three containers are fed with the synthetic chrome solutions and grown for different time intervals (30, 60, and 90 d). After a week comparative studies with grown freshwater plant species and grown synthetic chrome solution plant species are taken for analysis. Initial and final concentrations of chromium in each container are noted by using atomic absorption spectrometer (AAS).

2.5. Experimental design

Response surface methodology was used to optimize the reduction of chromium and TDS content of the water. Central composite design was used for the experimentation and result analysis and the experimental design was done using Design–Expert 7.0. The factors which were considered for the study are concentration of chromium, number of days of treatment, number of plants, and hydraulic retention time. The variables were altered in 5 levels and the experiments were replicated 5 times. Analysis of variance (ANOVA) study was used to find out the influence of variables on the reduction. R^2 and p-values as well as the lack of fit test were utilized to understand the significance of variables.

2.6. Determination of chromium in soil

In order to calculate the chromium content present in the soil where the plant species of *E. canadensis* were grown, toxicology analyses were done. The wet sand was taken from the wetland area and was dried by air dryer at 70°C and sent to Tamil Nadu Agricultural University, Coimbatore where the toxicology studies were performed in the Environmental Science Department to find out the total chromium content present in the soil.

2.7. Bioaccumulation factor and translocation factor

The bioaccumulation factor indicates that, the performance of the plant species which accumulates a metal into its tissues by the surrounding environment.

$$\text{BAF} = \frac{C_{\text{harvested tissue}}}{C_{\text{wastewater}}} \tag{1}$$

where $C_{\text{harvested tissue}}$ – concentration of the target metal in the plant-harvested tissue; $C_{\text{wastewater}}$ – concentration of the same metal in the wastewater.

Roots to shoots translocation of accumulated metal of the plant performance were indicated by translocation factor.

$$\text{TF} = \frac{C_{\text{shoot}}}{C_{\text{root}}} \tag{2}$$

where C_{shoot} – concentration of the metal in the plant shoots; C_{root} – concentration of the metal in the plant roots.

2.8. Percentage of chromium removal efficiency (%R)

The chromium removal of each plant performance was calculated by using the Eq. (3):

$$\%R = \left(\frac{C_i - C_f}{C_i}\right) \times 100 \tag{3}$$

where C_i – initial concentration and C_f – final concentration.

3. Findings

3.1. Bioaccumulation and translocation factors

The phytoremediation potential of a plant species is measured by bioaccumulation and translocation factors. For a plant to be used as a phytoaccumulator, both factors have to be greater than 1 [31]. For evaluation of these factors, the chromium content in soil, shoot, and root is measured and the factors are calculated. The chromium content in root and shoot is found to improve with retention time and concentration of the solution. With the increment in the number of plants per unit area, the chromium content in soil is found to reduce and chromium content in plant parts is found to distribute evenly. The number of days of the treatment is found to be influencing between 5–60 d. Beyond 60 d, the chromium content in soil was found to be increasing; this is due to retardation of phytoremediation. This resulted in the adsorption of chromium in soil which decreases the bioaccumulation factor. The translocation factor remained nearly invariant after 60 d.

The maximum bioaccumulation factor was found to be 3.89 which indicates the bioaccumulation capacity of the plant. In comparison with *Ficus infectoria* and

Rumex dentatus, the bioaccumulation factor is less [28]. But *F. infectoria* is an edible fruit-bearing plant and it is not suitable for phytoremediation. *R. dentatus* is a non-native species to Asian countries. Therefore, *E. canadensis* is suitable for the study.

The maximum translocation factor of 2.8 was obtained. This indicates the ability of the plant to mobilize the chromium from its root to shoot. In comparison with many pond based macrophytes like *Parthenium hysterophorus*, *Brassica napus* this translocation factor is better. Alternatively, research works report the usage of microbes to aid phytoremediation. *Zea mays* along with *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas pseudoalcaligenes* and *Bacillus halotolerans* have been used for the detoxification of chromium rich soil. The translocation factor was observed to be 1.29 for the treatment duration of 2 months [31]. Remediation with *E. canadensis* indicates improved translocation rates, proving that it is a better phytoaccumulator. *Sorghum bicolor* aided with *B. subtilis*, *B. pumilus*, *P. pseudoalcaligenes* and *B. halotolerans* exhibit a translocation factor of 100 [32]. This indicates that microbial assistance can boost the activity of the accumulator in the uptake of contaminants. However, *S. bicolor* is an edible millet and nutrient supplement. This poses serious concerns about toxicity as chromium is carcinogenic. Studies conducted using different variety of plant species indicate that trees are better hyper-accumulators [33]. But trees require a long duration of time to grow. On the other hand, *E. canadensis* reach maximum size within 2 months. Hence, the usage of *E. canadensis* as a phytoaccumulator is a discreet strategy for the remediation of chromium-rich wastewater.

3.2. Effect of phytoremediation on the chromium content

The chrome solution prepared at 3 different concentrations of 2.5, 5, and 7.5 ppm was treated using *E. canadensis*. The phytoremediation was carried out using 4 independent variables namely the concentration of a solution, number of days, number of plants/area, and hydraulic retention time. The maximum reduction of chromium was obtained for 7.5 ppm. 65% of chromium content was consumed during 90 d of phytoremediation at the lowest retention time. In comparison with chemical coagulation and adsorption the reduction is comparatively low [5,8]. But in phytoremediation, the problem of secondary pollutant generation in the form of sludge or used adsorbent is eliminated. The reduction of chromium content was optimized using response surface methodology and the reduction is modeled using the Eq. (4).

$$\begin{aligned}\%\text{Reduction of Cr} = {} & 70 - 3.46A + 8.17B + 2.92C \\ & + 7.13D + 0.75AB - 1.63AC - 1.5AD \\ & + 0.5BC - 0.75BD + 0.875CD + 2.98A^2 \\ & - 1.08B^2 - 0.083C^2 + 0.8542D^2 \end{aligned} \quad (4)$$

The residual plot for the above study is given in Fig. 1. The regression analysis was found to be satisfactory with an R^2 value of 0.94.

Analysis of the variance study indicated that all the 4 independent variables namely concentration of solution, number of days, number of plants/area and hydraulic retention time are significant. However, the interactions of the above parameters don't influence the chromium reduction. The ANOVA results are summarized in Table 2. The response surface plot is given in Fig. 2.

3.3. Effect of phytoremediation on TDS content

During phytoremediation, the change in TDS is an intriguing phenomenon. The dissolved chromium is

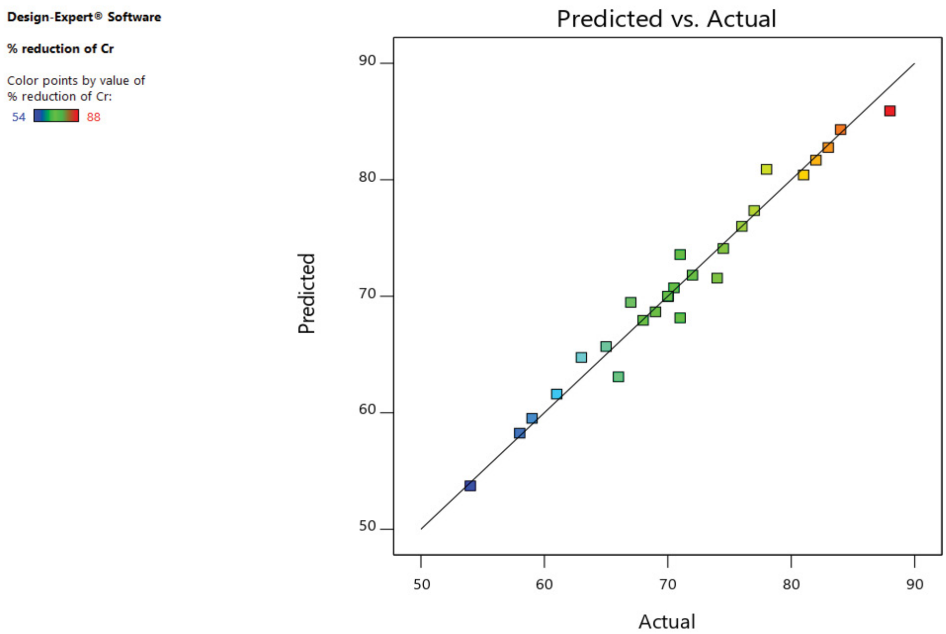

Fig. 1. Residual plot for reduction of chromium.

Table 1
Properties of raw synthetic effluent

Property	2.5 ppm	3.75 ppm	5 ppm	6.25 ppm	7.5 ppm
pH	8.79	9.30	9.77	10.26	10.73
Total dissolved solids (ppm)	1.38	1.61	1.81	1.95	2.2
Conductivity (mS)	1.86	2.18	2.42	2.71	3.3

Table 2
Analysis of variance for reduction of chromium and total dissolved solids

Source	Chromium reduction					Total dissolved solids reduction				
	Sum of squares	df	Mean square	F-value	p-value	Sum of squares	df	Mean square	F-value	p-value
Model	1,762.67	14	125.9	32.93	<0.0001	1,704.14	14	121.72	234.55	<0.0001
A-Concentration of Cr	143.52	1	143.52	37.54	<0.0001	190.4	1	190.4	366.88	<0.0001
B-No. of days	800.33	1	800.33	209.35	<0.0001	640.94	1	640.94	1,235.02	<0.0001
C-No. of plants/area	102.08	1	102.08	26.7	0.0001	240.76	1	240.76	463.91	<0.0001
D-Hydraulic retention time	609.19	1	609.19	159.35	<0.0001	577.55	1	577.55	1,112.86	<0.0001
AB	2.25	1	2.25	0.5886	0.4557	0.01	1	0.01	0.0193	0.8916
AC	10.56	1	10.56	2.76	0.1187	0	1	0	0	1
AD	9	1	9	2.35	0.1472	0	1	0	0	1
BC	1	1	1	0.2616	0.617	2.25	1	2.25	4.34	0.0562
BD	2.25	1	2.25	0.5886	0.4557	3.06	1	3.06	5.9	0.0292
CD	3.06	1	3.06	0.8011	0.3859	1.27	1	1.27	2.44	0.1407
A^2	57.57	1	57.57	15.06	0.0017	0.0205	1	0.0205	0.0395	0.8452
B^2	7.61	1	7.61	1.99	0.18	33.02	1	33.02	63.63	<0.0001
C^2	0.045	1	0.045	0.0118	0.9151	2.96	1	2.96	5.69	0.0317
D^2	4.73	1	4.73	1.24	0.2846	8.39	1	8.39	16.17	0.0013
Residual	53.52	14	3.82			7.27	14	0.519		
Lack of fit	53.52	10	5.35			7.27	10	0.7266		
Pure error	0	4	0			0	4	0		
Cor. total	1,816.19	28				1,711.4	28			

consumed during the phytoremediation, but the minerals present in the soil tend to dissolve in exfiltrated water. It was observed that the dissolution was rapid during the beginning of the process and it was stabilized after 1 week. Later, the TDS content was found to be decreasing. The maximum reduction was achieved in 90 d of treatment and the TDS was reduced up to 48.5%. In comparison with the reduction in TDS achieved by ultrasound assisted advanced oxidation, the reduction in TDS is low [13]. But, phytoremediation is advantageous as it doesn't require energy or chemical addition. Therefore, this method can be effective and economical for the removal of TDS from wastewater streams. The reduction of TDS was optimized using response surface methodology and the reduction is modeled using the Eq. (5).

$$\text{TDS} = 22 - 3.98A + 7.31B + 4.48C + 6.94D + 0.05AB + 0.75BC = 0.875BD + 0.5625CD - 0.0562A^2 + 2.26B^2 - 0.675C^2 + 1.14D^2 \quad (5)$$

The residual plot for the above study is given in Fig. 3. The regression analysis was found to be satisfactory with an R^2 value of 0.97.

Analysis of the variance study indicated that all the 4 independent variables namely concentration of solution, number of days, number of plants/area and hydraulic retention time are significant. However, the interactions of the above parameters don't influence the reduction in TDS. The duration of treatment is found to strongly change the chromium reduction. The ANOVA results are summarized in Table 2. The response surface plot is given in Fig. 4.

For the removal of chromium and reduction of TDS, the concentration of a solution is found to strongly change the chromium reduction. Therefore, for practical applications of the treatment technique, equalization becomes essential. Further, the hydraulic retention rate is identified to be significant. Since the plant can be grown in swamp wet land, stagnation of wastewater can be carried out to achieve maximum removal. It is also evident that increasing the number of plants used for treatment can improve the remediation.

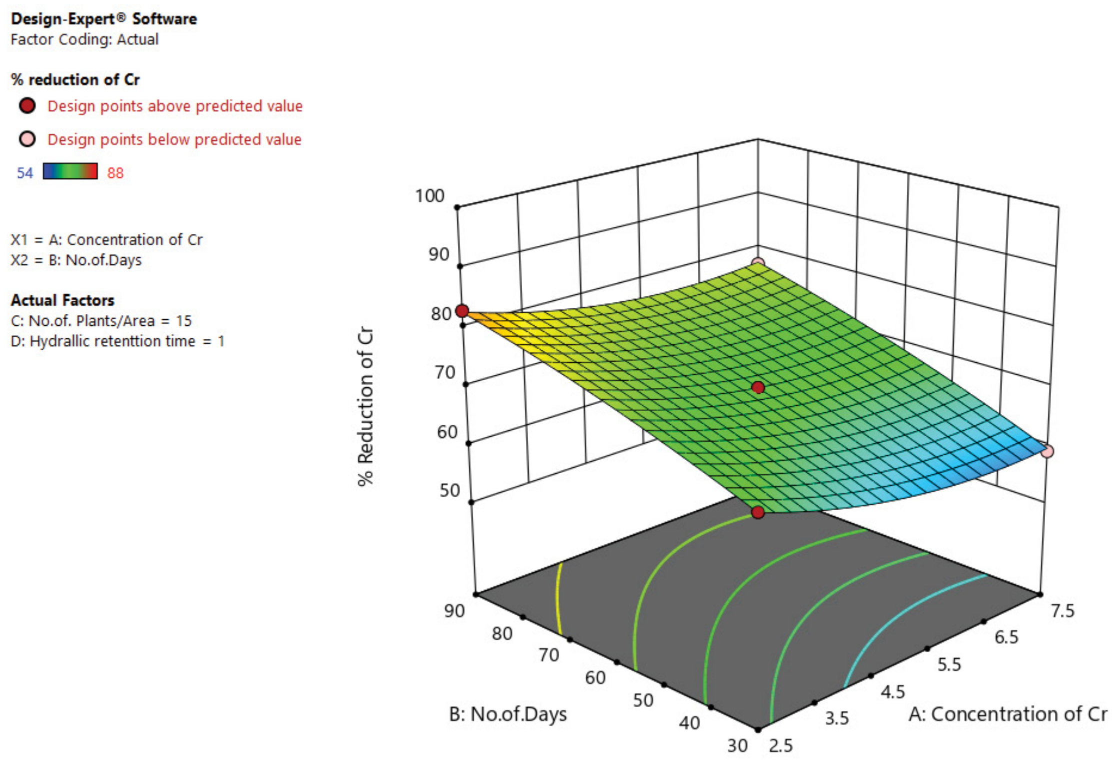

Fig. 2. Reduction of chromium against independent variables.

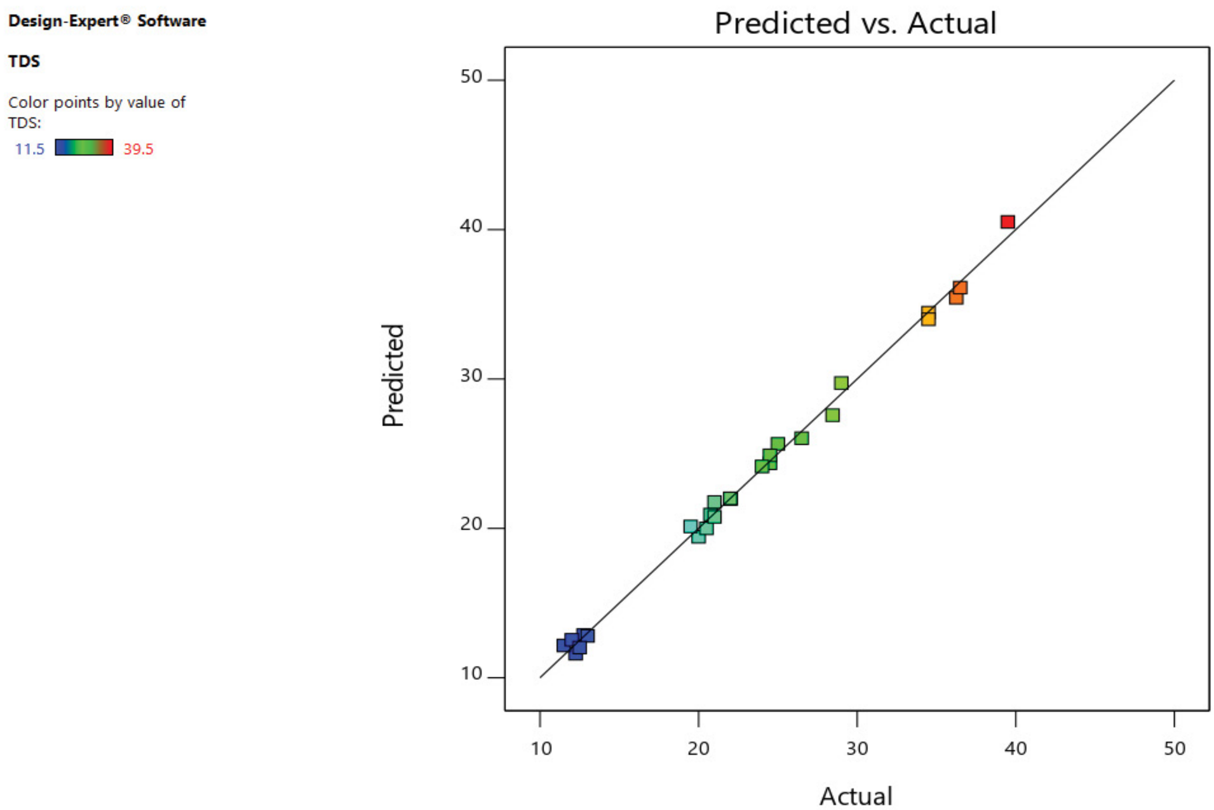

Fig. 3. Residual plot for reduction of total dissolved solids.

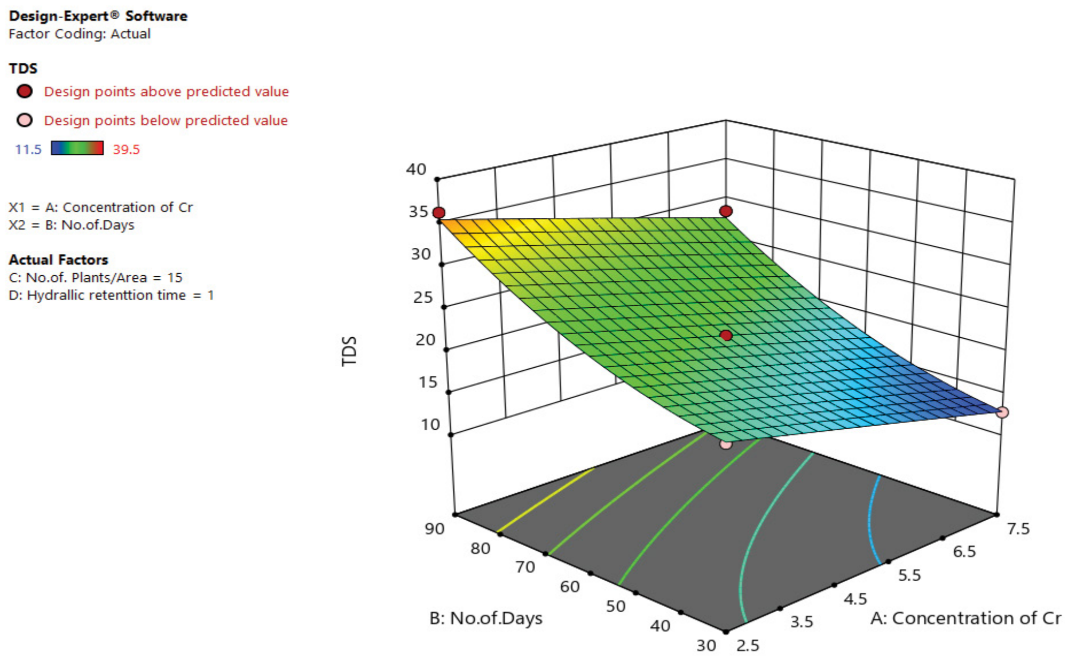

Fig. 4. Reduction of total dissolved solids against independent variables.

Phytoremediation using water hyacinth indicates that the chromium content was reduced up to 46% and TDS was reduced up to 50% [24]. In comparison with the afore-mentioned results, *E. canadensis* indicates superior remediation characteristics. Hence, the potential for the development of treatment units with macrophytes can be fruitful for the remediation of wastewater.

4. Conclusion

Phytoremediation is a potential technique for the treatment of wastewater with toxic metal content. In this work, the phyto-accumulating capability of *E. canadensis* in a constructed wetland is evaluated. A detailed study on the effect of variables like the number of plants, the concentration of chromium, retention time, and number of days on the phytoremediation is studied. Both the bioaccumulation factor and translocation factor were found to be greater than 1 for the species. This substantiates the ability of the plant to act as a phyto-extractor. The chromium content of the solution was reduced up to 65% and the TDS content was reduced up to 45%. Response surface analysis was carried out and it was determined that all the variables influenced the phytoremediation. Upon optimization, it was revealed that the treatment is effective for 90 d with 7.5 ppm chromium solution. The optimized retention time was 1.5 h and the best results were obtained with 2 plants in the container. Phytoremediation is an excellent alternative for the treatment of wastewater as it doesn't consume energy. There is no secondary waste generation and it also improves the ecosystem. Further studies can be carried out to identify the optimized conditions for the treatment in pilot scale with the large number of plants. Also, the treatment can be coupled with other techniques to achieve zero pollutant discharge to the environment.

Conflict of interests

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper.

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