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First report on bioaccumulation kinetics of Chromium (VI) and Malachite green by *Colchicum luteum* from aqueous medium

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Abstract

Colchicum luteum has the potential to remove Chromium (VI) and Malachite green (MG) from aqueous medium through Bioaccumulation (phytoremediation). Roots and arial parts of this plant, transport Cr(VI) and MG from standard solutions with and without MS (Murashige and Skoog) supplemented media. Amongst different treatment technologies for mitigation of Cr(VI) and MS from aqueous medium, *C. Luteum* has emerged as a potential mitigator of Cr(VI) and MG. Root intact live plants have been used for bioaccumulation in different concentration of Cr(VI) and MG separately for different time interval and residual concentration of standard solution was measured by spectrophotometric analysis. This mechanism is metabolic dependent and no any external source required for bioaccumulation. Significant result has been observed with high rate of removal percentage of Cr(VI) and MG. This experiment established a new way of using such type of perennial flowering plant as metallic decontaminant from their active sites. This plant may be used for other different metallic contaminants.

Keywords: *Colchicum luteum*, Chromium(VI), Malachite green, Bioaccumulation, Phytoremediation.

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1. Introduction

Under natural conditions plants essentially take up mineral nutrients together with contaminants from the soil solution. Plant nutrient uptake is an active process requiring energy to accumulate essential elements at higher level in plant tissues than in soil solution. Uptake of Cr(VI) by plants take place by bioaccumulation and there are probable mechanisms of removal of toxic elements[1 – 3]. The hexavalent chromium more than 500 times toxic than trivalent chromium is widely recognized to exert toxic effect for plants and living beings [3, 4], Chronic exposure of Cr(VI) in drinking water may cause adverse effect on health [5]. Although plants can uptake both Cr(III) and Cr(VI), the mechanism of uptake by plant remain unclear [6, 7]. Chromium is mostly absorbed by specific carrier for essential ions in plant metabolism [8 – 10]. Cr(VI) uptake by plants generally utilize either phosphate or sulphur transporter because of their structural resemblance to one another [11, 12]. Furthermore, Cr(VI) is reported to interface with the uptake of some essential plant nutrients (e.g., P, K, Mg, Ca, Fe and Mn) due to their ionic resemblance [13 – 15]. Cr(VI) uptake is regulated by various physiological processes including transport of Cr(VI) across the plasma membranes of root cells, detoxification, and sequestration of Cr(VI) in cell walls or vacuoles, xylem loading and translocated from root to shoot [16]. Once Cr(VI) absorbed by roots, it is initially retained largely within the roots and later transported throughout the plants by carrier ions [17]. Cr(VI) is principally transported through the xylem, and the reduction of Cr(VI) can occur in root with in the rhizospheres [18] or in the arial parts of plants [19, 20]. Therefore, extensive research is urgently needed on Cr(VI) accumulation and exploring novel chromium removal plant applying bioaccumulation. Several studies have explored the localization of Chromium in a range of plants and hyperaccumulators, including *Trifolium brachycalycium* [21] and *C. chnensis* etc.

Textile industries are the major industries worldwide which have become sources and reason for surface water contamination. Malachite green (MG), a cheap water-soluble dye used extensively for dyeing, belongs to triphenylmethane category [13, 22]. It is used for the dying of cotton, jute, paper, wool, silk, acrylic industries and leather products. Malachite green is environmentally persistent which causes damage to nervous system, liver, brain, eye burns and cancer.

It is toxic to a wide range of aquatic and terrestrial animals. Many treatment technologies have been applied to decolorize MG from aqueous medium [23 – 26]. However, all physical, chemical, and mechanical techniques have certain limitations in term of design, dye separation efficiency, cost and effectiveness. Removal of MG dye has been reported based on experimental data [27, 28]. *Colchicum luteum* is a perennial flowering plant, whose 160 species are grown from bulb like corms. It is found in Europe, West Asia, India, and east coast of South Africa. *C. luteum* baker is an annual herb popularly called "*Suranjan*" because a Unani drug commonly in practice is derived from *Colchicum luteum* (Fig. 1). The word "*Suranjan*" probably come from Unani language and commonly known as "*Suranjan-e-Talkh*" in Urdu. The first mentioned report of *C. luteum* is in Ayurvedic literature by Acharya Shankar Dutt Gond in his text "*Shankar Nighantu*" 1935 [29]. *Colchicum* is an annual herb with underground dark brown sacly, oval shaped corm. Leaves are 6 to 12 inches in length and 1/3 to ½ inches in board [30]. Seeds and root both of *C. luteum* contain alkaloid colchicine in large amount. It also contains few amount of starch, oily resinous and alkaloid phenethlisoquinoline [31]. Its corms contain ~0.21 - ~0.25 % of alkaloid and ~0.41 - 0.43% in seeds. *C. luteum* also contains colchicine analogs decteyl thiocolchicine (DTC), decetylmetylcolcine (DMC) and trimetylcolcine acid (TMCA) that are effective in treatment of goat. Some phenolic compound also obtained from the *C. luteum* are 4–hydroxy 3–methoxybenzaldehyde (Vanilin), 3–(- 3–hydroxyphenyl) -2 propanoic acid (Coumaric acid), Caffeic acid and 3,4,5,7 Tetrahydroxy flavone (Luteolin).
Medicinal application of *C. luteum* has found its place in Unani system. This plant has high medicinal value. The dry corm of *C. luteum* is used in Joint pain, Inflammation, goat, swelling, sciatica, healing of wound, indignation, rheumatoid arthritis and osteoarthritis [32]. Besides this great medicinal importance of *C. luteum*, it is susceptible to growing ability in Gangetic plain. It will play an important role in bioaccumulation of metalic and dyes contamination from aqueous and soil medium.

The purpose of this experiment was to evaluate the potentiality of *C. luetum* as Cr(VI) and Malachite green (MG) bioaccumulator because *C. luetum* is very useful in medicinal purpose and it is unexplored as phytoremediator plant yet. In present research, the percentage removal and uptake capacity of Cr(VI) and Malachite green (MG) using living biomass of *C. luteum* has been studied under batch conditions.

2. Material and methods

2.1. Selection of plant for bioaccumulation studies on Cr(VI) and MG.

C. luteum Plant species has been obtained from Botanical Garden of Bihar Agricultural University, Sabour, Bhagalpur. The root intact plant was selected for bioaccumulation of Cr(VI) studies. Live plant was weighed in the range of 10-15 g. Whole plant was washed five times from running tap water and again washed thrice with distilled water to remove their soil and other particles. Meanwhile, healthy and green plant of *C. luteum* was selected for bioaccumulation studies of Cr(VI) and MG [1, 2].

2.2. Preparation of reagents

2.828 g of $K_2Cr_2O_7$ (Potassium dichromate) was dissolved in 100 mL distill water to get 1000 ppm Cr(VI) solution. The working solutions were obtained by diluting the stock solution to appropriate volume and prepared 1 ppm, 2 ppm and 3 ppm solution [33]. Murasige and Skooge basal salt solution (Himedia) was also dissolved separately in solution for nutrient support to plants. Standard aqueous solution for MG was prepared by MG crystal, 100 mg of MG crystal is taken in 1000 mL distilled water to prepare 100 ppm MG stock solution. 5 ppm and 10 ppm concentration of MG solution were prepared from 100 ppm stock solution by dilution. Both concentrations have been prepared separately in distilled water and Murasige and scoog (Murasige and scoog basal salt solution, Himedia) contain solution. Volume was made up by distilled water up to 1000 mL [1, 2].

2.3. Bioaccumulation of Cr (VI) and MG by of C. luteum

Batch experiments were carried using 10 - 15 g of root intact live biomass of *C. luteum*. Three different initial ion concentrations i.e., 1, 2 and 3 ppm of Cr(VI) in 100 mL solution were supplemented with MS nutrient media and without nutrient media in 250 mL conical flask. The experiment was conducted for 2 different time periods separately i.e. 24 h and 48 h. Experiments were carried in flasks of 250 mL containing 100 mL aqueous media separately for all above mentioned three different initial ion concentrations in which one flask containing MS nutrient media with initial Cr(VI) concentration and another one contain Cr(VI) solution in distilled water. Their respective blanks were run to know their actual initial ion concentration. After time bond treatment, plant was taken out from aqueous medium and residual concentration was measured by chromate test kit (Merck) using UV-Vis Double beam spectrophotometer (Pharo 300, Germany) with their respective blank solution to determine actual Cr(VI) ion concentration during treatment.

To study the bioaccumulation of MG by live biomass of *C. luteum* in two different initial concentrations, i.e. 5 and 10 ppm for 24 h and 48 h contact time period, MG concentrations were dissolved in MS basal salt containing solutions and another in distilled water only with their respective blanks. 100 mL of standard solutions was taken in conical flask and root portion of *C. luteum* put on solution for 24 h and 48 h contact time. After completion of time-based treatment, residual concentration was measured λ max at 618 nm [34] by using UV single beam spectrophotometer (GENWAY Genova Plus, Agilent technologies, Malaysia).

3. Result and discussion

3.1. Bioaccumulation of Cr(VI) by live biomass of C. luteum

In this experiment Cr(VI) with MS containing (nutrient) media show effective removal of Cr(VI) by *C. luteum*. In 24 h experiment, root of *C. luteum* uptake is 83.16% Cr(VI) from 1 ppm initial ion concentration (Table.1). 48.27% from 2 ppm (Fig. 2 a) and 47. 65% removed from 3 ppm initial ion concentration (Table 3, Fig. 2 a). These results confirmed that chromium complexation with organic compound was involved in facilitating chromium accumulation by plants [35] while in 48 h treatment with nutrient containing media chromium reduced up to 89.24 % in 1 ppm (Table 1) 77.08 % from 2 ppm (Table 2) and 55.53% from 3 ppm initial ion concentration (Table 3). In aqueous medium containing Cr(VI) ion only showed less removal efficiency than with nutrient media. Experiments in aqueous medium of Cr(VI) in 24 h shows just 5.26% removal percentage in 1 ppm initial concentration (table 1), in 2 ppm (Table 2) is up to 33.33% and 19.72 % in 3 ppm initial ion concentration (Table 3). The overall Cr(VI) removal percentage trends were in agreement with wheat (*Triticum vulgare*) plants that grow in hydroponic culture with chromium concentration along with other organic compound, that accumulated more chromium in their roots than plant parts grown in only chromium concentration [36].

3.2. Effect of initial ion concentration on bioaccumulation of Cr(VI)

The accumulation and translocation of Cr inside the plants also depend upon the concentration of Cr in the medium [37]. In this study initial ion concentration present in aqueous medium played an important role in bioaccumulation of Cr(VI) by *C. luteum*. In 24 h treatment initial ion concentration without nutrient media shows interesting results, at 1 ppm without MS only remove 5.26% whereas at 2 ppm it increased up to 33.33% and at 3 ppm results again lightly decreased up to 19.72% (Fig. 2 b) whereas vice versa trends were found with MS media of 24 h treatment. At 1 ppm initial concentration reduced to 83.16% whereas, at 2 ppm it decreased up to 48.27% and it remained in 3 ppm concentration up to 47.65%. On other hand, effect of initial ion concentration with 48 h bioaccumulation treatment showed that with MS media experiment set, from 1 ppm concentration Cr(VI) removal was 89.24%, 77.08% from 2 ppm and 53.53% from 3 ppm initial concentration (Tables 1-3). Initial ion concentration of Cr(VI) ion without MS media, 48 h treatment showed 83.83% removal in 1 ppm, 77.65% in 2 ppm and 37.81% removal in 3 ppm initial ion concentration (Tables 1-3). Above results from both nutrients and without nutrients media show that lower initial ion concentration was easily removed in both 24 h and 48 h treatment. Results presented that *C. luteum* plant have ability to accumulate Cr(VI) at lower concentration. When chromium ions concentration increased in media, their accumulation efficiency changed.

3.3. Effect of Contact time on bioaccumulation of Cr(VI)

Contact time is an important parameter that influences the rate of bioaccumulation by plants. In this study 24 h and 48 h of bioaccumulation treatment were provided. Cr(VI) ions alone in distilled water showed effective removal in 48 h time while during first 24 h it was unable to remove markable percentage of Cr(VI) (Fig. 2 b). Initial concentration of 1 ppm with distilled water in flask, removal was 5.26% during 24 h of contact time while after 48 h rapid accumulation takes place by *C. luteum* and it reached up to 83.83%. Similar results were with 2 ppm where 33.33% removal happened in 24 h and it rapidly increased in next 24 h that is 77.65%. Same trends were also observed in 3 ppm initial concentration with only distilled water as a solution where 19.72% removal took place in 24 h and it increased to 37.81% during 48 h (Fig. 2 b). These data state that proper accumulation of Cr(VI) by *C. luteum* takes place in 48 h. Effect of contact time determines the rate of bioaccumulation where Cr(VI) is only present in aqueous medium. In another case Cr(VI) ion dissolved in nutrient media does not show remarkable difference during both 24 h and 48 h of contact time, It shows minor difference in rate of bioaccumulation except in 2 ppm initial ion concentration. Cr(VI) with nutrient media uptake by plant is 48.27% in 24 h and 77.0.8% in 48 h. These data clearly established that effect of contact time along with initial ion concentration both played important role in rate of bioaccumulation by *C. luteum*.

3.4. Bioaccumulation of MG by live biomass of C. luteum

The bioaccumulation study of MG by *C. luteum* was conducted in 100 mL aqueous medium in conical flask with weighing amount of plants ranged from 10 to 15 g. The decolorization results are plotted in Fig. 3 and 4 from the results. It was observed that the decolorization potential was found to be function of both 24 h and 48 h time period, Table 4 state that the equilibrium is attained at 24 h treatment. In 5 ppm initial MG concentration with MS nutrient media shows 78.68% removal in 24 h and 77.98% removal in 48 h, these data clearly indicated that the absorption equilibrium is achieved with in 24 h whereas at 10 ppm initial MG concentration with MS nutrient media,

Decolorization potential of *C. luteum* was decreased with respect to 5 ppm MS contain MG solution. It has been observed that decolorization percentage is 58.84% in 24 h and 67.18% in 48 h (Fig. 3). Further study on removal efficiency with MG concentration with distilled water only, at 5 ppm initial MG concentration shows 72.43% removal of MG within 24 h and 86.96% in 48 h. These data state that effect of MS nutrient media does not influence the rate of bioaccumulation. MG can enter independently in root cell of plants whereas at 10 ppm initial concentration, rate of bioaccumulation increased with respect to 10 ppm MS contain solution. It was observed that decolorization percentage is 74.28% in 24 h treatment and 77.24% in 48 h treatment. These data indicate that initial MG concentration increased independently (Without supporting any nutrient media) and the rate of bioaccumulation was unaffected and achieved their equilibrium (Fig. 4). In all set of 5 ppm MG initial concentration experiment, removal percentage was

Table 1: Removal data of Cr(VI) at initial concentration of 1 ppm by biomass of *C. luteum* upto 24 h and 48 h time treatment.

100 ml Cr(VI) containing aqueous medium	1 ppm Cr(VI) conentration					
	24 h treatment			48 h treatment		
	Actual initial Cr(VI) concentration (ppm)	Residual Cr(VI) concentration (ppm)	Removal %	Actual initial Cr(VI) concentration (ppm)	Residual Cr(VI) concentration (ppm)	Removal %
Cr(VI) with MS media	1.01	0.17	83.16	0.93	0.10	89.24
Cr(VI) with D.W.	0.95	0.90	5.26	0.99	0.16	83.83

Table 2: Removal data of Cr(VI) at initial concentration of 2 ppm by biomass of *C. luteum* up to 24 h and 48 h time treatment.

100 ml Cr(VI) containing aqueous medium	2 ppm Cr(VI) concentration					
	24 h treatment			48 h treatment		
	Actual initial Cr(VI) concentration (ppm)	Residual Cr(VI) concentration (ppm)	Removal %	Actual initial Cr(VI) concentration (ppm)	Residual Cr(VI) concentration (ppm)	Removal %
Cr(VI) with MS media	1.74	0.90	48.27	1.92	0.44	77.08
Cr(VI) with D.W.	1.83	1.22	33.33	1.88	0.42	77.65

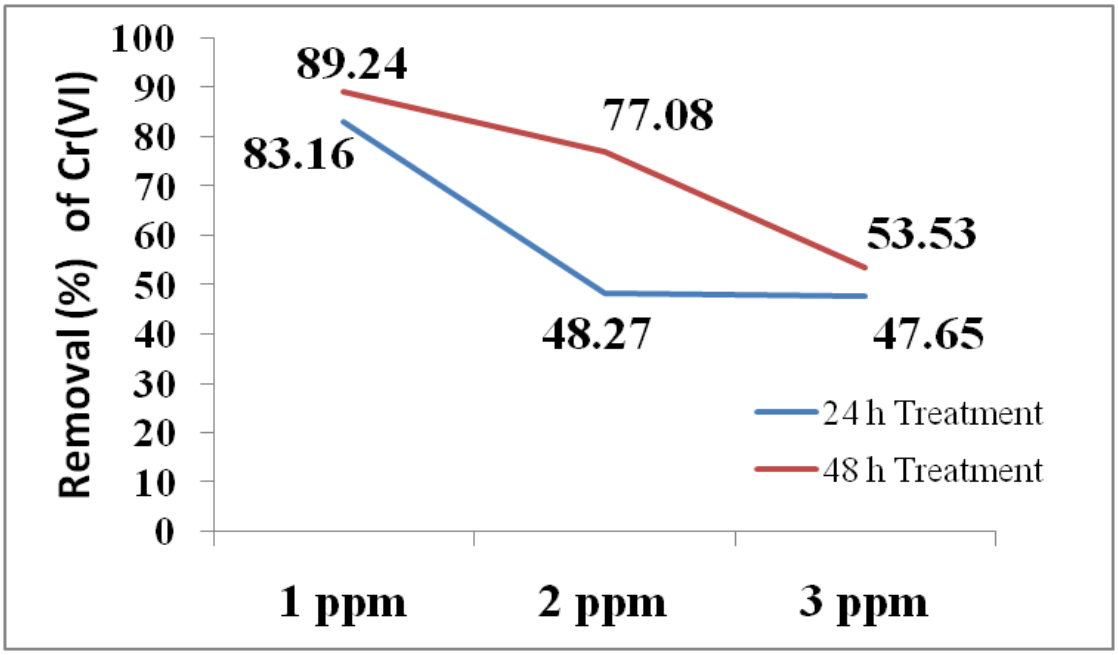
Table 3: Removal data of Cr(VI) at initial concentration of 3 ppm by biomass of *C. luteum* up to 24 h and 48 h time treatment.

100 ml Cr(VI) containing aqueous medium	3 ppm Cr (VI) concentration					
	24 h treatment			48 h treatment		
	Actual initial Cr(VI) concentration (ppm)	Residual Cr(VI) concentration (ppm)	Removal %	Actual initial Cr(VI) concentration (ppm)	Residual Cr(VI) concentration (ppm)	Removal %
Cr(VI) with MS media	2.77	1.45	47.65	2.97	1.38	53.53
Cr(VI) with D.W.	2.94	2.36	19.72	2.75	1.71	37.81

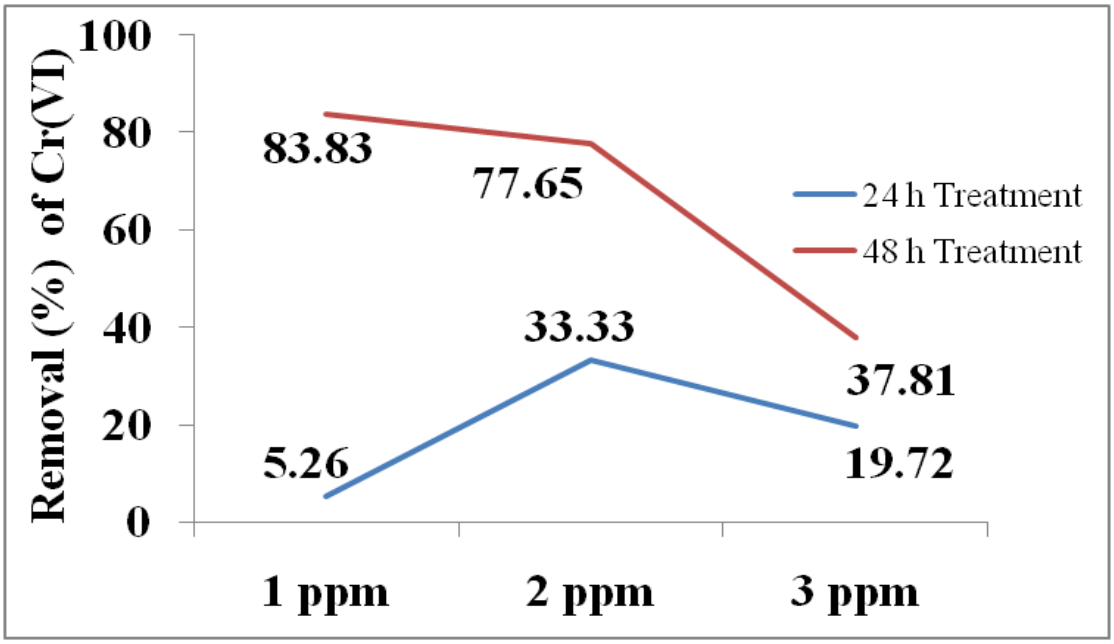
Table 4: Decolorization results of Malachite green by live biomass of *C. luteum*.

Aqueous Solution (100 ml)	λ_{max} 618 nm				
	λ_{max} of MG concentration (Blanks)	24 h treatment		48 h treatment	
		λ_{max} of residual MG concentration	decolorization (%)	λ_{max} of residual MG concentration	decolorization (%)
5 ppm MG with MS nutrients	1.004	0.214	78.68	0.221	77.98
10 ppm MG with MS nutrients	1.883	0.775	58.84	0.618	67.18
5 ppm MG with distilled water only	0.468	0.129	72.43	0.061	86.96
10 ppm MG with distilled water only	0.914	0.235	74.28	0.208	77.24

Figure 1: Plant of *Colchicum luteum*.



a



b

Figure 2: Removal percentage of Cr(VI) accumulated by *C. luteum* from 100 ml Cr(VI) with MS media **(a)** and with D.W. **(b)** from different initial Cr(VI) concentration.

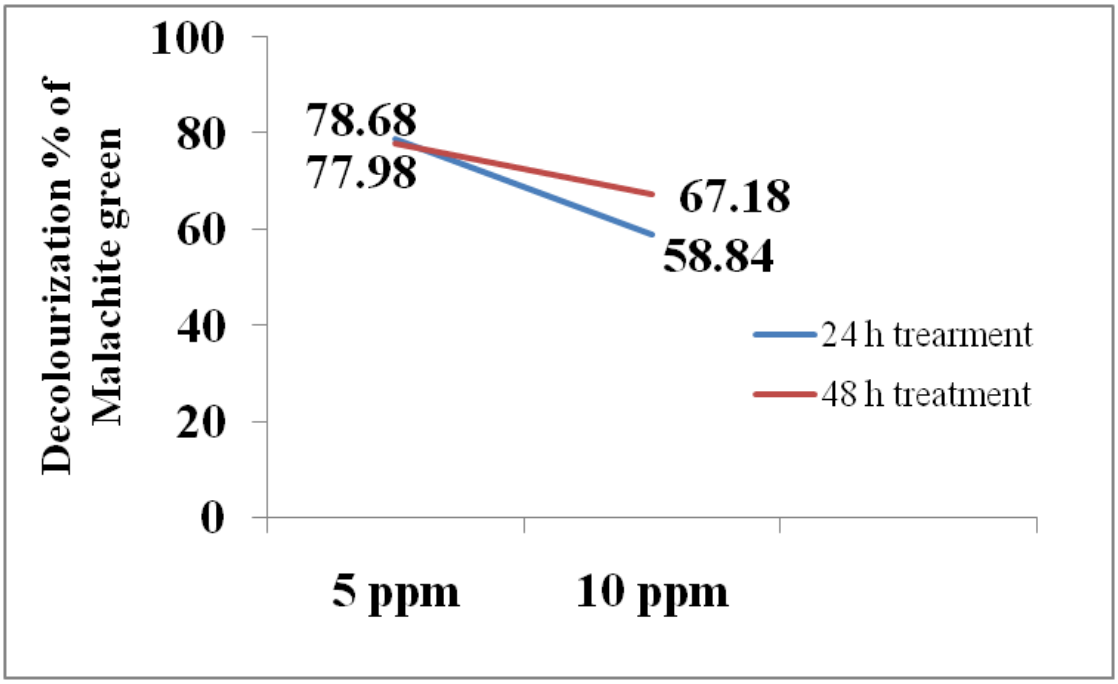


Figure 3: decolorization percentage of Malachite green with MS nutrients accumulated by *C. luteum*.

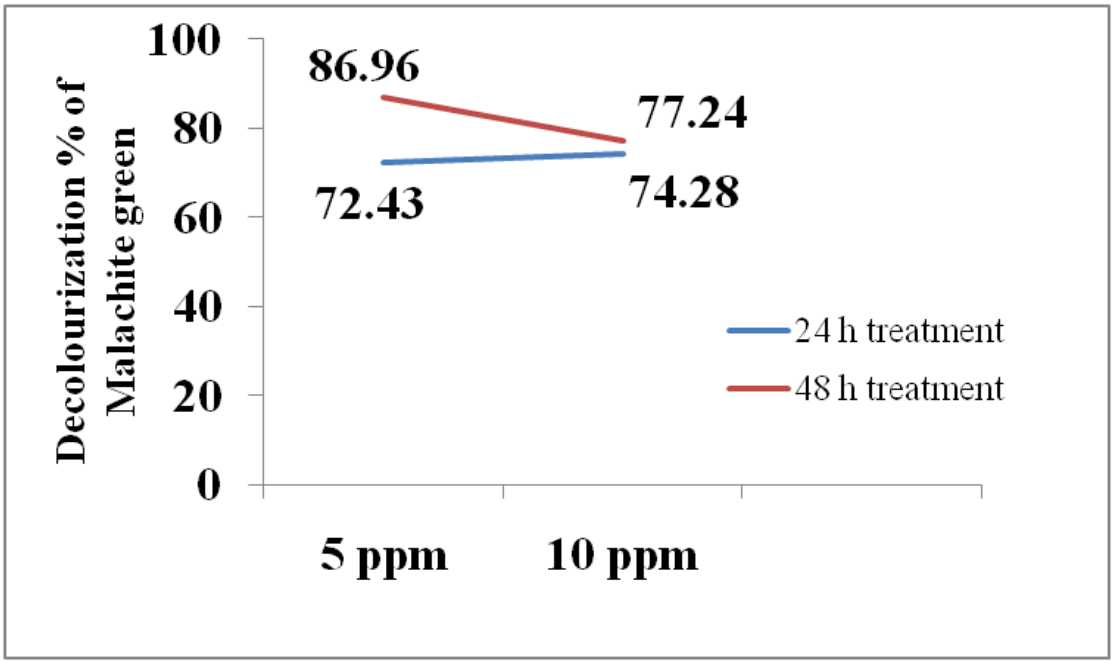


Figure 4: decolorization percentage of Malachite green with distilled water accumulated by *C. luteum*.

more than 70% whereas in 10 ppm set decolorization potential was better without nutrient media. After analysis of complete data of decolorization of MG by *C. luteum*, it was concluded that *C. luteum* have a great ability to uptake MG from hydroponic system. It may be applicable in *In-situ* remediation. One important point obtained from spectrophotometric data is that, no reverse release of MG concentration takes place after uptake that means *C. luteum* completely decompose and permanently absorb MG in their tissues. It has been reported that many plant species usually have high antioxidant enzyme activity upon exposure to toxic substances [38 – 41]. So, *C. luteum* has ability to degrade and

successfully tolerate MG dye 5 ppm and 10 ppm in this research. If so, the reuse of contaminated water with MG could be applied by using *C. luteum*. Other studies reported such type of results like Cabbage and *Brassica rapa* could take up and accumulate some toxic substances in their tissues, especially in their roots [42, 43].

4. Conclusion

It has been reported that *Cabbage* and *Brassica* rape could accumulate toxic substance in their tissue [38]. But *C. luteum* has greater potential to decontaminate wastewater with Cr(VI) and MG. *Ricinus communis* a type of medicinal herb has been reported as potential herbal drug properties and established as chromium accumulator. Despite this, from the experimental findings it has been crystal clear that *C. luteum* can be utilized as a potential remover of Malachite green. In addition, *C. luteum* has the ability to remove Cr(VI) from aqueous medium with nutrient media [44]. Thus, *C. luteum* has been explored as an eco-friendly and low-cost absorbent of organic dyes and hexavalent chromium as a first report.

Conflict of interest

The authors declare that they have no conflicts of interest.

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