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Simultaneous Phytoremediation of Cr(VI) and Phenol Using Aquatic Macrophyte Water Hyacinth : Effect of PH and Concentrations

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ABSTRACT

In this study removal of Cr(VI) and phenol was carried out in single and binary synthetic solution using the phytoremediation process. For this purpose, plants were grown in photosynthesis chamber with 12 h light and 12 h dark period. The plants were grown in nutrient solution accompanied with Cr(VI) and phenol. The various initial concentrations of Cr(VI) (5, 10, 15, 20 mg/L) and phenol (10, 20, 30, 40, 50 mg/L) in single component synthetic solution of Cr(VI) and phenol and binary solution of Cr(VI) containing Cr(VI) and phenol in (1:2) ratio was used for the experimentation. The effect of initial concentrations o to the specific growth rate of the plant was investigated. The effect of pH on to the percentage removal of Cr(VI) in binary solution was also investigated.

Keywords : Water Hyacinth, Binary Solution, Chlorophyll, Phytoremediation, Specific Growth Rate.

I. INTRODUCTION

The discharge of wastewater from various industries to the main water bodies like river is the main focus of research now a days. The industrial effluents contain mainly toxic heavy metals and organic compounds. Industrial pollution is a potential threat affecting the water bodies, soil and air, which ultimately affects the whole ecosystem. Among these heavy metals and organic compounds, pollution caused by chromium (Cr) and phenol (C_6H_5OH) is of considerable concern because these are used in various industrial processes such as electroplating, leather tanning, textile, paints and pigments, paper, wood preservation and car manufacturing extra (Lin et al., 2009; Garg et al., 2013; Gunasundari et al., 2013). Chromium occurs in aqueous systems in two oxidation states trivalent [Cr^{3+} and $CrOH^{2+}$] and hexavalent [$HCrO_4^-$ and $Cr_2O_7^{2-}$] out of which hexavalent chromium is most toxic, carcinogenic and mutagenic to the living organism (Thinh et al., 2013; Talreja et al., 2014; Kumar et al. 2009). Trivalent, Cr(III) can be oxidized to hexavalent, Cr(VI) which is more mobile and toxic than Cr(III). Cr(VI) entered into the human body causes respiratory tract and lung carcinoma, epigastric pain, vomiting, nausea, corrosion of the skin and severe diarrhoea. Among the various harmful and toxic organic compounds like dyes, pesticides and fluorine, phenol is most toxic to the environment. Phenol is toxic aromatic organic compound and weak biodegradable pollutant. Exposure of phenol causes gastrointestinal disorder, lung damage, liver damage, kidney damage, heart attack and finally can lead to death (Gupta and Balomajumder 2015a; Gupta and Balomajumder 2015b; Gupta and Balomajumder 2015c). The US Environmental protection agency (US

EPA) recommends the maximum permissible limit for the discharge of Cr(VI) and phenol from industrial waste water is 0.05 mg/L and 1 mg/L, respectively (Vlyssides et al., 1997; Chaudhary and Balomajumder 2014). Hence, removal of Cr(VI) and phenol from industrial effluents become necessary before its discharge in to main water stream.

Generally, the industrial waste water containing high concentrations of Cr(VI) and phenol requires the use of costly chemical and physical processes for their disposal as ozonation, adsorption, ion exchange, membrane filtration, chemical oxidation, etc. (Dittert et al., 2014; Hamdan and El-Naas 2014; Cavaco et al., 2014). But these processes are having some disadvantages such as highly energy-intensive, not economic and release of effluents and waste water that will pollute the environment again. To overcome the disadvantages of these physical and chemical methods, biological methods have been recently developed as an alternative to these difficult and expensive treatments. Biological methods are simple and environment friendly processes and have the potential to completely degrade and reduce pollutants at relatively low capital and operating costs (Bhattacharya et al., 2014). Unlike this phytoremediation, i.e. removal of metals by plants is an effective methodology, respectful of the environment and cost effective. The production of renewable energy by this plant and some others sources is also a demand now a days (Jani et al., 2016(a); Jani et al., 2016(b). Therefore on this basis the objectives of this present study are (1) the removal of chromium & phenol by plant from single and binary synthetic simulated waste water. (2) Effect of initial concentrations of chromium, and phenol onto the percentage removal. (3) Effect of pH onto the percentage removal of chromium & phenol (4) to study the accumulation of chromium & phenol in leaf, stem & root of plants.

II. MATERIALS AND METHODS

2.1 Selection of plant for Phytoremediation

The water hyacinth (Eichhornia crassipes) is a tropical plant belongs to the pickerelweed family (Pontederiaceae). It is a free floating aquatic plant well known for its reproduction abilities and removal of contaminants from water. It can very quickly grow to high densities (over 60 kg/m^2); thereby completely block water flow, which in turn may have negative effects on human health, environment, and economic development (Mishra and Tripathi 2009; Maine et al., 2001). These plants can tolerate variations in availability of nutrient, temperature and pH condition. The pH required for the growth of water hyacinth plants is 6-8. It can survive in a wide range of temperature from 1 to 40°C (optimum growth found at 25–27.5 °C) (Wilson et al., 2005).

2.2 Experimental setup and growth condition

Experiments were arranged to investigate the growth and phytoremediation properties of water hyacinth (E. crassipes) in different concentrations of Cr(VI) and phenol-enriched water in a thermostatically controlled, Plant Growth Chamber, make in Ambala, India. The image of water hyacinth plant and plant growth chamber is given in is given in Fig. 1(a) and Fig 1(b), respectively. Approximately the same size and weight, water hyacinth (E. crassipes) plants of 2-3 weeks old was used for experimentation. These plants were collected from Roorkee canal, Solani River. After collection and screening of water hyacinth plants of approximately same size and weight were washed thoroughly with tap water followed by millipore water prior to the experimentation. Before waste water treatment process, these plants were supplied with a nutrient solution for 3 days. The nutrient solution consisted of 1 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.5 mM $MgSO_4$, and 0.5 mM $NH_4H_2PO_4$ and consists of the following micronutrients: 25 μM Fe-EDTA, 23.1 μM H_3BO_3, 0.4 μM $ZnCl_2$, 0.18 μM $CuCl_2$, 4.57 μM $MnCl_2$, and 0.06 μM Na_2MoO_4 (Hoagland et al. 1950). The pH of the nutrient solution was adjusted to 6.5 -7.0 by using H_2SO_4 and NaOH of 0.1 N. Nutrient solution was changed in three days of operation and aeration of nutrient solution was carried out used during the growth of the water hyacinth plants. The experiments were carried out in an artificial photosynthesis chamber, with 12 h of photoperiod and 12 h of dark cycle, temperature of 27-30 °C, and relative humidity of 60-70%. For each experiment plants were remained in photosynthesis chamber for two weeks.

Fig. 1(a): Plant growth chamber used for phytoremediation

Fig. 1(b): water hyacinth plant used for phytoremediation

2.3 Chromium accumulation study:

To find out the concentration of Chromium accumulated in the various parts of water hyacinth plant, the plants were sorted into three parts, i.e., leaves, roots and stems. All samples were oven dried at 80°C for 48 h. Each sample of 1 g was digested in 10 mL of 1N HNO_3 and heated at 150°C for 1.5 h. After heating, 5 mL of 1N $HClO_4$ was added to the beaker. Further, the samples were heated at 150°C for 1 h. Then they were cooled and diluted to 100 mL by addition of distilled water.

2.4 Chlorophyll content measurement:

The chlorophyll content in leaves of water hyacinth was measured in spectrophotometer (Hach DR 5000) before and after uptake of Cr(VI) and phenol. The leaves of water hyacinth were cut into small pieces and 0.5 g of plant sample was added in 25 mL volumetric flask. After that, 80% acetone solution was filled to the 25 mL volumetric flask. All the flasks were kept in dark for 24 h in incubator cum shaker at room temperature. The OD (optical density) of each sample was taken at 663 and 645 nm in a spectrophotometer with an optical path of 10 mm against 80% acetone as a blank. All the readings were taken in triplicates and their average values were used. The amount of chlorophyll was calculated following the formulae below (Xiao-Zhang et al., 2009)

$$C_a = \frac{(12.3\ D_{663} - 0.86\ D_{645}) \times V}{d \times 1000 \times W} \tag{1}$$

C_a = Concentration of chlorophyll a (mg/g FW)
D = Optical density (OD) at the specific wave length
V = Final volume mL
W = Fresh weight of leaf material g
d = Length of light path cm

2.5 Specific growth rate (h^{-1})

The specific growth rate (h^{-1}) of the water hyacinth plant was calculated by measuring the chlorophyll content of the plant at various concentrations of Cr(VI) and phenol for both single and binary solution as per the following equation (Huertas et al., 2010).

$$\mu = \ln \frac{\frac{OD_2}{OD_1}}{t_2 - t_1} \qquad (2)$$

Where OD_2 is the OD at time t_2; OD_1 is the OD at time t_1; t_2 is the exponential phase time; and t_1 is the lag phase time.

2.6 Analysis of concentrations of Cr(VI) and phenol

The residual concentrations of Cr(VI) and phenol after uptake was analyzed in UV spectrophotometer. For the analysis of Cr(VI) 1,5 diphenyl carbazide method and for phenol 4 amino-antipyrene method was used. The OD (Optical density) of Cr(VI) and phenol was measured at 540 nm and 510 nm, respectively (Ontanon et al., 2014).

III. Characterization of Water Hyacinth Plant

Fe SEM and EDX analysis of root and leaves of plant before and after uptake:

The Fe SEM and EDX analysis of root and stem of water hyacinth was carried out before and after uptake of Cr(VI) and phenol in binary solution. The image of Fe SEM of root and leaf of water hyacinth plant before uptake is shown in Fig. 2(a) and Fig. 2(b), respectively. The surface morphology of root and leaf of water hyacinth plant after uptake of Cr(VI) and phenol was different than before uptake. The surface of root and leaf of water hyacinth was became more flabby and rough in comparison to before uptake as shown in Fig. 2(c) and Fig. 2(d), respectively. The EDX image of root and leaf of water hyacinth before uptake is shown in Fig. 3(a) and Fig. 3(b), respectively. A peak of Cr(VI) was observed after uptake in root and leaf EDX spectrum as shown in Fig. 3(c) and Fig. 3(d), respectively. The observance of peak of Cr(VI) confirms the uptake of Cr(VI). The uptake of phenol was confirmed by the change in CK and OK wt% after uptake.

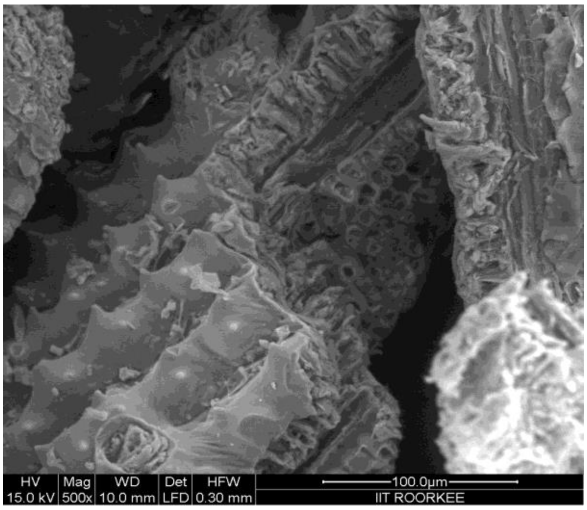

Fig. 2(a) Fe SEM image of root of water hyacinth before uptake

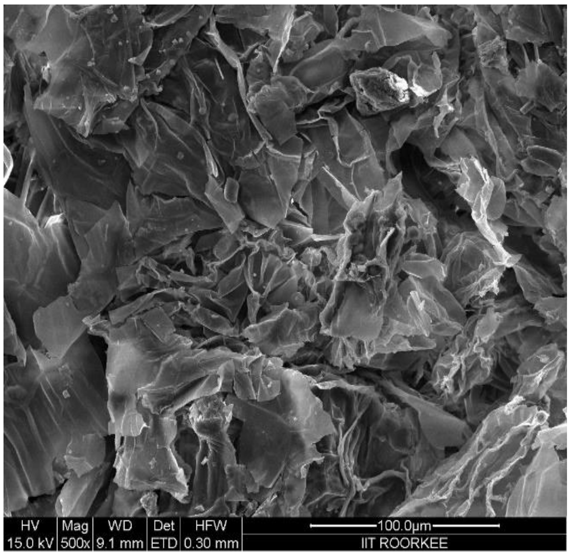

Fig. 2(b) Fe SEM image of leaf of water hyacinth before uptake

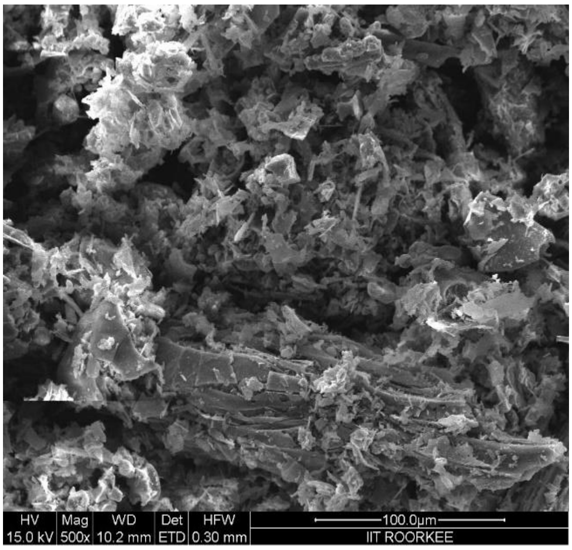

Fig. 2(c) Fe SEM image of root of water hyacinth after uptake of Cr(VI) and phenol

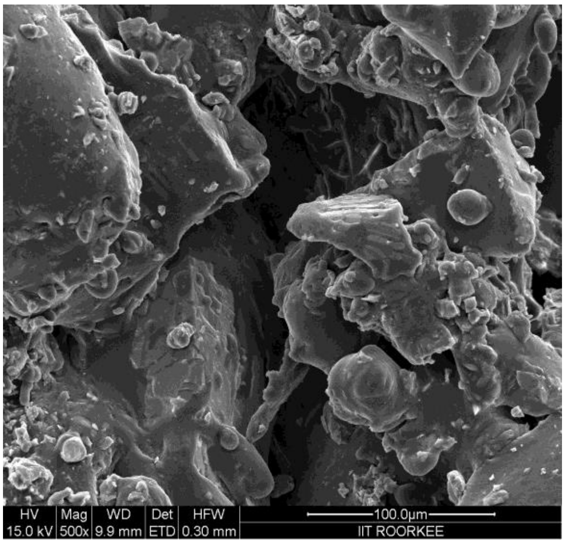

Fig. 2(d) Fe SEM image of leaf of water hyacinth after uptake of Cr(VI) and phenol

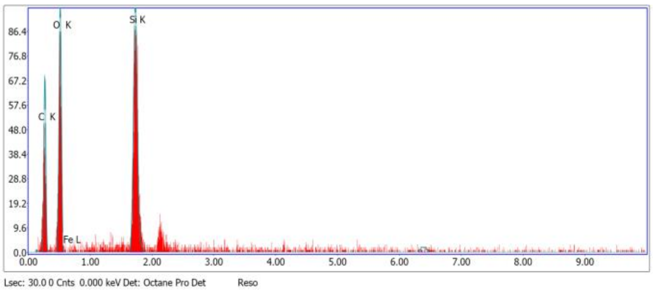

Fig. 3(a) EDX spectrum of root of water hyacinth before uptake

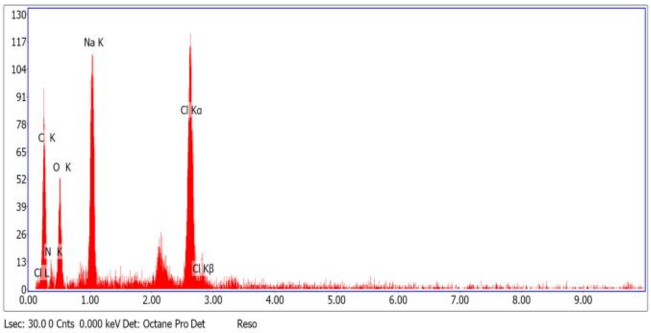

Fig. 3(b) EDX spectrum of leaf of water hyacinth before uptake

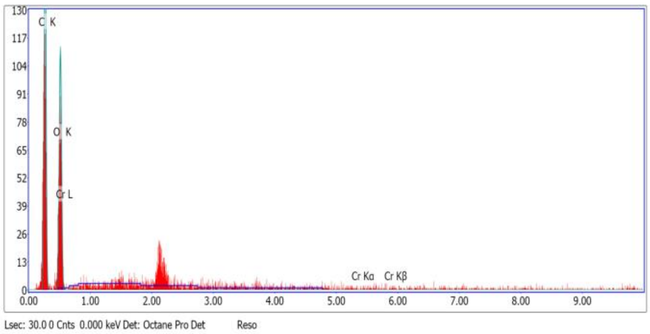

Fig. 3(c) EDX spectrum of leaf of water hyacinth after uptake of Cr(VI) and phenol

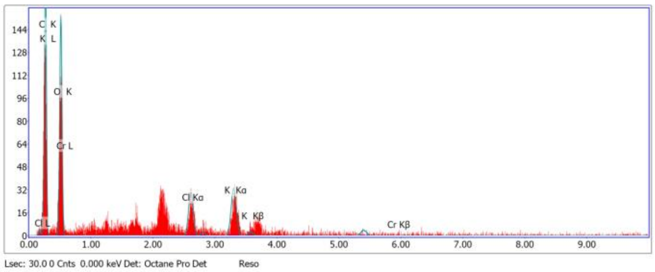

Fig. 3(d) EDX spectrum of root of water hyacinth after uptake of Cr(VI) and phenol

IV. RESULTS AND DISCUSSION

In the present study, the Cr(VI) and phenol removal capability of water hyacinth at different concentrations has been studied. The removal study has been carried out for a certain period of time (15 days), the effect of pH on the pollutants (Cr(VI) and phenol) uptake by plants has been measured and the accumulation of chromium in different parts of the plant body has also been investigated. The results of the study are described below.

4.1. Effect of initial concentrations on to the percentage removal

The amount of Cr(VI) and Phenol present in water for different initial concentrations at different time interval after uptake is shown in Fig. 4(a) and Fig. 4(b), respectively. The percentage removal of Cr(VI) at different concentrations of 5, 10, 15, 20 mg/L are 79, 75, 69 and 58 %, respectively. The percentage removal of phenol at various concentrations of 10, 20, 30, 40 and 50 mg/l are 83.2, 69.2, 58.67, 45.6 and 41.8%, respectively. From Fig. 4(a) it can be seen that after 10 days, water hyacinth can remove chromium at 5, 10, 15 and 20 mg/L to 1.053, 2.48, 4.56 and 8.33 mg/L, respectively. Toxic effect was observed for 30 and 50 mg/L of Cr(VI) concentrations onto the water hyacinth. Some morphological symptoms of metal toxicity such as yellowing, chlorosis of leaves and root shedding were seen in the plants exposed to the 10.0, 15.0 and 20.0 mg/L of Cr^{+6} concentrations. It was observed that with the increase in initial concentrations of Cr(VI), percentage removal was decreased and maximum percentage removal of Cr(VI) was obtained at 5 mg/L. From Fig. 4(b) it is observed that water hyacinth can remove phenol at initial concentrations of 10, 20, 30, 40 and 50 mg/L to 1.67, 6.16, 12.4, 21.76 and 29.1 mg/L, respectively during seven days' time period. Same effect was observed for phenol as the percentage removal was decreased with the increase in initial concentrations of phenol and maximum removal observed at 10 mg/L. The probable reason behind this fact was due to the toxic effect of Cr^{+6} and phenol on the plant as shown in Fig. 4(c).

Phenol uptake initially occurred at slower rate than Cr^{+6} uptake and plant uptake rate approximately same during first 4 days, then it gradually decreases and formation of new roots and leaves are observed. However, the uptake during this period compared to Cr^{+6} was higher. This phenomenon indicates that at a neutral pH (7±0.3) range chromium uptake mechanism and phenol uptake mechanism is different from each other. The reason for greater uptake of phenol than chromium is probably due to the fact that phenol can act as a carbon source for the plant which provides the protection against the toxic pollutant (Mishra and Tripathi 2009). Another study on young poplar foliage reveals that phenol metabolism is directly linked with the rates of carbohydrate translocation. A consistent positive correlation between rates of carbohydrate import and phenolic metabolism was evident (Arnold et al., 2014).

The residual concentrations of Cr(VI) and phenol obtained at various concentrations of Cr(VI) and phenol in binary solution (1:2) is shown in Fig. 4(d) and Fig. 4(e), respectively. In binary solution, concentrations of Cr (VI) and phenol was varied from 2.5 to 25 mg/L and 10 to 50 mg/L, respectively. It was observed that in case of binary solution, plant can survive up to 25 mg/L of Cr(VI) concentration and for single component solution plant can survive up to 20 mg/L of Cr(VI) concentration. This indicates that the presence of phenol reduces the toxicity effects of Cr(VI). Presence of phenol in binary solution probably act as essential ligands for Cr(VI) uptake. Another study carried out in literature established the fact that organic compound such as phenol forms the complexes with heavy metals such as Cr(VI), making them available for plant uptake [Bartlett and James 1988]. The toxicity of chromium in Lycopersiconesculentum showed that in the presence of organic acids like carboxylic acid and amino acids is reduced and the Cr uptake in roots is enhanced (Paul and Srivastava 2006).

The generation of reactive oxygen species or ROS by the plant is one of the common response to reduce the toxicity of Cr(VI). ROS are formed in the cells of water hyacinth plant as an intermediate product during the reduction of O_2 to H_2O (Dietz et al., 1999). Chromium is a toxic heavy metal which can cause the oxidative damage to plants overcome by the formation of ROS. A study on tomato hairy root culture established a direct relationship between the basic and ionically bound to cell wall peroxidase and phenol removal efficiency (Oller et al., 2005).

4.2. Effect of initial concentrations on to the specific growth rate (h^{-1})

Graphs are plotted between specific growth rate (h–1) vs. initial concentration of Cr(VI) solution (mg/L), specific growth rate vs. initial concentration of phenol, as shown in Fig. 5(a) and Fig. 5(b), respectively. From figures, it is observed that the specific growth rate (h–1) decreases with the increase in initials concentrations for both Cr(VI) and phenol. This shows that both Cr(VI) and phenol are the inhibitory type of substrates. The concentration of Cr(VI) and phenol was varied from 5 to 20 mg/L and 10 to 50 mg/L, respectively. The specific growth rate of water hyacinth plant decreases very rapidly after 15 mg/L of Cr(VI). In case of phenol, the specific growth rate decreases very rapidly after 30 mg/L of phenol. Therefore both Cr(VI) and phenol was found to be inhibitory type of substance for the growth of the water hyacinth (Gupta and Balomajumder 2016).

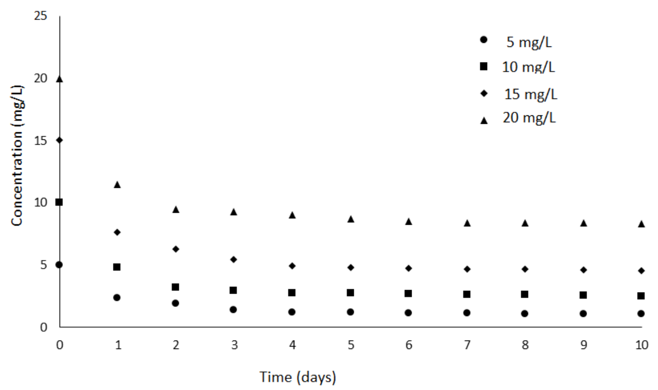

Fig. 4(a): Residual Cr +6 concentration with respect to time at pH 7± 0.03 for single component solution of Cr(VI)

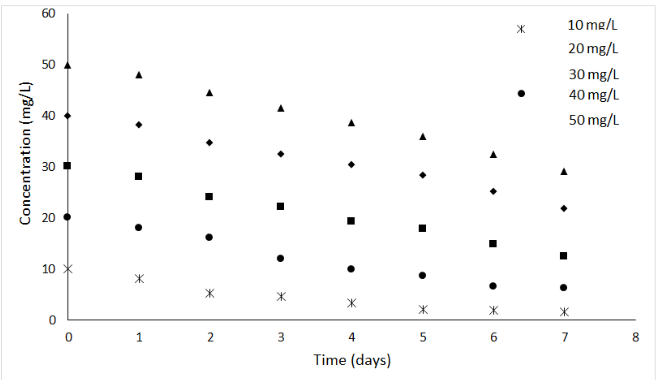

Fig. 4(b): Residual Phenol concentration with respect to time at pH 7± 0.03 for single component solution of phenol

Fig. 4(c): Chlorosis of leaves observed in the plants exposed to 10.0, 15.0 and 20.0 mg/L of Cr+6.

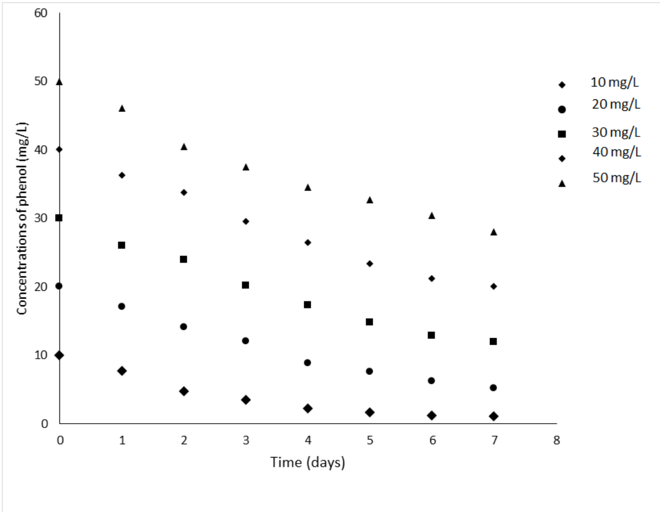

Fig. 4(d): Residual concentration of phenol with respect to time in binary solution of Cr(VI) and phenol

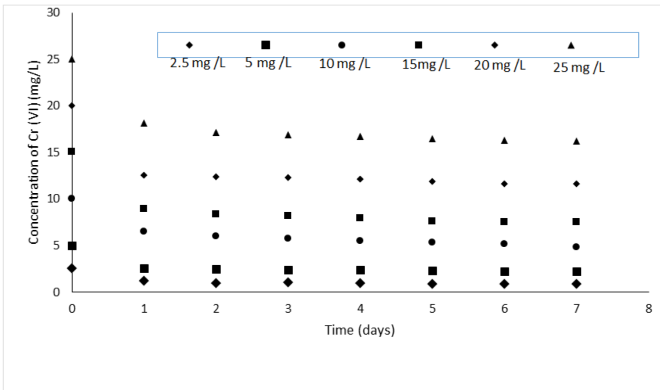

Fig. 4(e): Residual concentration of Cr (VI) with respect to time in binary solution of Cr (VI) and phenol

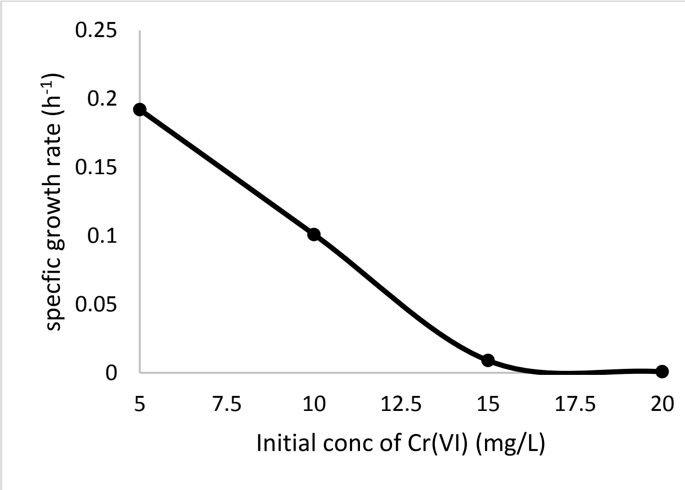

Fig. 5(a): Initial conc. of Cr(VI) vs. Specific growth rate (h^{-1})

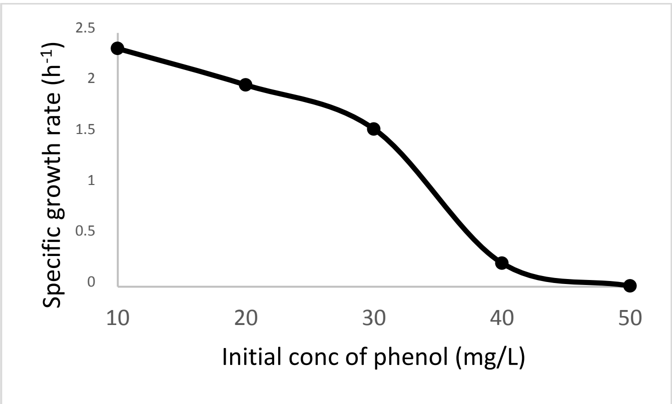

Fig. 5(b): Initial conc. of Phenol vs. Specific growth rate (h^{-1})

4.2. Effect of pH

It was observed that water hyacinth plant can survive with at a pH ranging from 4 to 10 which includes most naturally occurred water bodies. The change in various initial pH used for the experimentation is given in Fig. 6(a). Free diffusion of carbon dioxide into the atmosphere and nutrient salts uptake by water hycinth plants will affect the pH levels. Measuring the final pH indicated that the water hyacinth plant had a tendency to change the pH of water i.e. acidic or alkaline or towards neutrality. The addition of 3 M NaOH to the nutrient solution at pH 12 produced a precipitate. This phenomenon can be explained using Le Chatelier's principle of chemical equilibrium. In the aqueous solution of potassium dichromate ($K_2Cr_2O_7$) the dichromate ion is in equilibrium with the chromate ion, and this can be described with the following equation:

$$Cr_2O_7^{2-} + H_2O \leftrightarrow 2CrO_4^{-} + 2H^{+}$$

The dynamic equilibrium established between the chromate and dichromate ions is disturbed by the acidity or basicity of the solution. Shifting the equilibrium with change of pH is a classic example of Le Chatelier's principle. The more acidic the solution, the more the equilibrium is shifted towards the left of dichromate ions. In the pH range 2–7 the dominant form of Cr(VI) was $Cr_2O_7^{2-}$ and $HCrO_4^{-}$, at pH higher than 7 Cr(VI) is present in the form of $CrO4^{-2}$ ion. The nutrient solution contained different metal ions (Ca, Mn, K, Fe, Zn, Cu, Na, Mo etc.) and at lower pH $CrO4^{2-}$ solubility is very less. Due to this reason chromium removal was very less at higher pH. The maximum percentage removal of Cr(VI) in binary solution of Cr(VI) and phenol was obtained at pH 7 as shown in Fig. 6(b), therefore all the experiments were carried out at this pH.

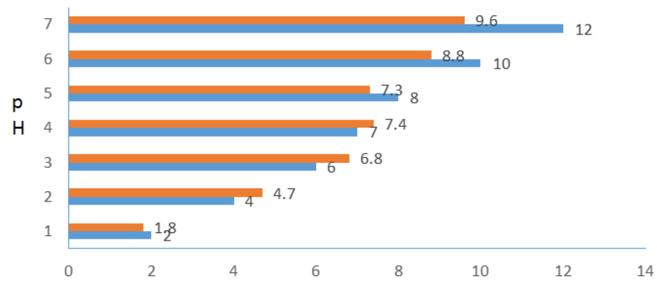

Fig. 6(a): bar chart of Initial and final pH level after 2week exposure of water hyacinth plant in growth chamber for 20 mg/L Cr (VI)

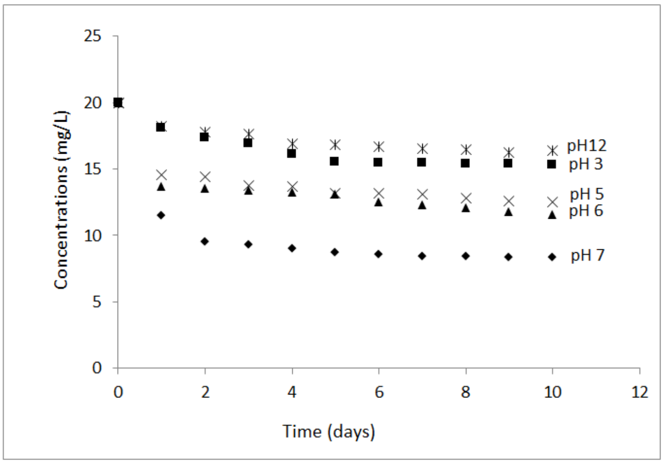

Fig. 6(b): Removal of Cr concentration 20 mg/l with respect to pH with 10 days exposure

V. CONCLUSION

In this study phytoremediation is proved to be a potential method for the accumulation and uptake of Cr(VI) and phenol in water hyacinth plant. The plants were easily grow in phytoremediation chamber at temp of 27-30 °C, and relative humidity of 60-70% during the 12 h light and 12 h dark cycle. Approximately 79.9 % removal of Cr(VI) and 83.3 % removal of phenol was obtained at an initial concentration of 5 mg/L of Cr(VI) and 10 mg/L of phenol, respectively. The percentage removal of Cr(VI) is observed to be higher in the presence of phenol. The less toxic effect of Cr(VI) onto the water hyacinth plant was observed in the presence of phenol. It can be due to the fact that phenol was used as carbon source by the plant and get stored in the leaves therefore provide the energy to the plant at more stressed condition. The maximum percentage removal of Cr(VI) and phenol was obtained at pH 7 in binary solution.

VI. ACKNOWLEDGEMENT

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