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Potential of *Ipomoea aquatica* for elimination of phenol and cyanide from mono and binary component aqueous solution in a photosynthesis chamber

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ABSTRACT

Under the current study, water spinach *(Ipomoea aquatica*) has been tested for elimination of phenol and cyanide from mono and binary component aqueous solution in batch system. The plant was grown at six concentrations of phenol and cyanide in the ratio of (10:1), i.e., 100:10, 200:20, 300:30, 500:50, 700:70 and 1,000:100 in aqueous solution. The plant was found capable of eliminating up to 94.92% of phenol (300 mg/L) and 91.67% of cyanide (30 mg/L) during 13 d cultivation time. Removal of phenol was observed harmless at lower concentration upto 100–200 mg/L without any toxic effect; however, at 300–1,000 mg/L, plants have been indicated toxic effects. Moreover, *Ipomoea aquatica* indicated toxicity for all six concentration of cyanide. The effect of process parameters such as initial concentration of phenol and cyanide and pH was evaluated. In the *Ipomoea aquatica* plant, the biochemical parameters such as chlorophyll, protein and sugar content have been indicated a decreasing trend due to uptake of phenol and cyanide during cultivation. The calculated K_m of the root length elongation was 11.26 mM and the V_{max} was 7.24 μg phenol/g root/h. However, the calculated K_m of the root length elongation for cyanide was 6.65 mM and the V_{max} was 0.56 μg cyanide/g root/h. Toxicity to 100–1,000 mg/L of phenol and 10–100 mg/L of cyanide was measured by measuring the relative transpiration over 13 d. At 100 mg/L of phenol and 10 mg/L of cyanide, only a slight reduction in transpiration but no morphological changes were detected. In this study, phytoextraction/phytoaccumulation is found to be the mechanism of elimination since phenol and cyanide are accumulated into the root, stem and leaves of the plants. Pollutants are absorbed through the root of the plant by plasmalemma and become accumulated into the root cells and stem of a plant.

Keywords: Accumulation; Cyanide; Growth rate; *Ipomoea aquatica;* Phenol; Photosynthesis

1. Introduction

Increasing commercial growth and human population leads to the water supplemented with nutrients and pollution in the water environment [1]. The increasing industrialization, urbanization, and mining, and so on are the noteworthy sources of pollution in the water bodies [2,3]. The occurrence of toxicants in aquatic environments is the furthermost significant ecological anxieties for scientists. The removal of pollutants from wastewater is still a major environmental problem owing to occurrence of phenol and cyanide along with other pollutants. Phenol and cyanide are among the foremost toxicants found in effluents of coke ovens, coal conversion processes, petroleum refineries, and petrochemical industries. Wastewater released from these industries, usually comprises phenol and cyanide in concentrations ranging from 10 to 1,000 mg/L and 10–100 mg/L, respectively [3,4]. Phenol and cyanide both are carcinogenic, poisonous, and mutagenic and found related to the presence of the numerous health effects. Therefore, they are measured as significant contaminants in the list of US environmental protection agency (US EPA). Environmental protection agencies of many countries have proposed an acceptable limit for phenol and cyanide in effluent. US EPA and minimal

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national standard (MINAS) of central pollution control board (CPCB) limit cyanide in effluent as 0.2 mg/L and for phenol as 0.5 mg/L [2,4]. Various treatment technologies are used for the elimination of the phenol and cyanide viz, solvent extraction, membrane separation, photocatalytic degradation, electrochemical oxidation, and adsorption [5,6]. These approaches used are relatively expensive and energy demanding.

In present years, the use of plants for environmental clean-up has been commonly predictable all over the world. Phytoremediation is an eco-friendly approach for elimination of phenol and cyanide by plants from the contaminated environment and wastewater. Furthermore, it has various advantages in comparison with other treatment approaches [7]. Phytoremediation takes advantage of natural plant processes and involves less equipment and labor than other treatment methods since plants do most of the work. Also, the polluted site can be cleaned up without hauling and digging up soil or pumping groundwater, which saves energy. Among several plants utilized for phytoremediation, aquatic macrophytes achieve the greatest significant position. The marine macrophytes are floating aquatic plants, the roots of these plants are immersed in water. It has been established that some aquatic plants are effective at accumulating several toxic and organic elements in the tissues of plant. Numerous classes of aquatic macrophytes such as *Ipomoea aquatica*, *Typha angustifolia*, *Eichhornia crassipes*, *Potamogeton crispus*, *Ceratophyllum demersum*, and *Salvinia natans* have been used for the elimination of pollutants from wastewater [8–13]. Pollutants get accumulated into the roots and then transported to stem and leaves by ion exchange mechanism [14]. *Ipomoea aquatica*, locally known as water spinach, has hollow shoots that cultivate prostrate or floating and found in muddy waterbodies and lakes. *Ipomoea aquatica* is a free floating macrophyte having hairy root and can grow easily in polluted wastewater. It has arrowhead designed leaves of almost 2 cm wide and 15 cm long [15]. However, *Ipomoea aquatica* frequently cultivates in polluted water bodies of industrial effluents. This aquatic plant species cultivate as a harvest in areas where the temperature is found above 25°C. Some researchers experimentally verified that the aquatic plants are important for elimination of organic toxic contaminants from wastewater [7,16–17]. In aquatic plants, the contaminants are detached via underwater roots in residues and absorption from water bodies through the leaves [16].

Under the present study, phytoremediation capacity *of Ipomoea aquatica* for elimination phenol and cyanide from wastewater systems was evaluated. The effects of process parameters (initial concentration, pH, and biochemical parameters) on phenol and cyanide removal were also evaluated. The Normalized Relative Transpiration (NRT) and Michaelis–Menten kinetic parameters were calculated.

2. Materials and methods

Ipomoea aquatica were collected from local market roorkee, India, with almost the similar weight and size and 5–6 weeks old. All the plants were washed carefully with distilled water to eliminate soluble substances and dirt prior to the experimentation for the removal of phenol and cyanide. After washing, the plants were dried by using tissue paper and evaluate the initial weight of the plants. *Ipomoea aquatica* was grown in 4% Hoagland nutrient solution [18]. The plants were grown in the artificial photosynthesis chamber with controlled operating conditions 45 μ mol/m^2s^1 photon flux intensity and 60% relative humidity at 30 ± 1°C. *Ipomoea aquatica* plants were grown in 1 L experimental pots filled with 900 mL hoagland nutrient solution and kept in phytoremediation chamber.

2.1. Experimental method for the analysis of plant growth

Ipomoea aquatica was grown-up at different initial concentrations of 10–100 mg/L of cyanide and 100–1,000 mg/L of phenol both in mono and binary component solution of cyanide and phenol, respectively. In all studies, phenol at a concentration of 300 mg/L and cyanide concentration of 30 mg/L were used, excluding the effect of initial concentrations was tested. In phytoremediation chamber, artificial light was delivered for all plants with a light period of 12 h (light-on) and 12 h (light-off) maintained with fluorescent tube lights). The amount of water reduces due to evaporation and uptake was maintained everyday by adding desired concentration of nutrient solution. All experimental set was continued in triplicate. Samples were collected timely from 0 to 13 d from experimental pots for the evaluation of the pollutant concentrations with time period passed. The growth length of *Ipomoea aquatica* plant root and shoot were measured randomly with a ruler after every 4 d of culture to determine the effect on the physical health of the plant by phenol and cyanide concentration.

2.2. Chemicals and reagents

For the current study, chemicals was attained from Himedia Laboratories Pvt. Ltd. Mumbai, India. All chemicals used were at least of analytical reagent (AR) and used without additional purification. A stock solution of mono and binary component with a ratio of phenol and cyanide (10:1) were prepared by adding phenol and cyanide in double distilled water. To prepare stock solution containing 100 mg/L cyanide was prepared by dissolving 0.0189 g of NaCN in 1 L of millipore water (Q-H2O, Millipore Corp. with resistivity of 18.2 MX-cm). Stock solution comprising 1,000 mg/L of phenol was prepared by dissolving 1 g of pure phenol crystal in 1 L of millipore water. All solution was freshly prepared for each experiment. Solution of preferred concentration was prepared by diluting the stock solution.

2.3. Phenol and cyanide analysis in plant

To determine the cyanide and phenol content in the plants, the plants were discarded from experimental pots after 13 d of operation. The plants were washed and dried for final weighing. All plants were divided into roots, stem, and leaves and oven dried at 60°C. The dried samples were individually grounded and kept in deccicator. The analysis of cyanide and phenol was carried out using a UV spectrophotometer (HACH DR 5000) by calorimetric picric acid and 4-aminoantipyrene methods, respectively [19]. For the estimation of accumulation of phenol and cyanide in different parts of the plant, the samples were crushed by mortar and pestle. These samples were digested with HNO_3–$HClO_4$ in 2:1 ratio (v/v) and diluted to 100 mL with distilled

water [20,21]. Accumulation of protein, sugar and chlorophyll in roots, leaves and stem, was determined by using digestion method [20,21,23].

The phytoextraction/phytoaccumulation process was considered by calculation of cyanide and phenol removal capacity of the plants (Eq. (1)):

$$q_m = (c_f - c_i) \times v / w \tag{1}$$

where q_m is the plant removal capacity (mg/g), c_f is the final concentration of pollutants in plant and solution (mg/L), c_i is the initial concentration of pollutants in plant and solution (mg/L), v is the volume of solution (L), w is the fresh weight of plant (g).

The percent pollutant accumulation in *Ipomoea aquatica* was calculated using the formula:

$$\%A = \left\lfloor I_p - F_p / I_p \right\rfloor \times 100 \tag{2}$$

where F_p and I_p is the final and initial pollutant concentration in the plant (mg/L), %A is the percentage accumulation in plant (mg/L).

2.4. Analysis of sugar and protein content

Protein content was estimated before and after uptake of cyanide and phenol from mono and binary component solution by using bovine serum-albumin as standard [22] and sugar content were estimated by using phenol-sulphuric acid method [21].

2.5. Analysis of chlorophyll content

The analysis of chlorophyll contents in the leaves of *Ipomea aquatica* plants before and after uptake were examined spectrophotometrically by using the following equation according to Maclachalam and Zalik [23]:

$$C = \frac{\left(12.3D_{663} - 0.86D_{645}\right) \times V}{d \times 1000 \times w_f} \tag{3}$$

where C is the chlorophyll concentration (mg/g w_f), D is the optical density (OD) at the specific wave length specified, V is the final volume (mL), w_f is the fresh weight of leaves (g), and d is the length of the light path (cm).

For chlorophyll content measurement, frozen and dried leaves of each plant were cut into small portions, exactly weighed and 0.5–1.0 g fresh weight (w_f) of samples sited in 25 mL flasks. Then, 10 mL of 80% acetone was used to extract the pigments at room temperature. Samples were placed in the dark room for 24 h and flask were shaken thrice during this period. The absorbance of the samples was measured at 663 and 645 nm.

2.6. Measurement of relative growth rate (RGR), bioconcentration factor (BCF) and translocation factor (TF)

The RGR of the plant at different concentrations of phenol and cyanide was calculated by weighing the initial weight of fresh *Ipomoea aquatica* before uptake of phenol and cyanide and final weight of the plant after uptake of pollutant after 13 d. The RGR of *Ipomea aquatica* plant materials was calculated at different initial concentration of cyanide and phenol by using Eq. (4) [24].

$$\mathrm{RGR} = \left(m_2 - m_1\right) / \left(t_2 - t_1\right) \tag{4}$$

where m_1 and m_2 are the weight of the plant (*Ipomoea aquatica*) before uptake (t_1) and after uptake (t_2), respectively.

The BCF was calculated by dividing the concentration of the toxic pollutant phenol and cyanide in the plant to the concentration in synthetic/simulated nutrient solution given in Eq. (5). The concentration of toxic pollutant phenol and cyanide in the parts of the plant was estimated by acid digestion with HNO_3–$HClO_4$ in 2:1 ratio (v/v) [25]. BCF was considered for different parts of plant as root, stem, and leaves by the following equation:

$$\mathrm{BCF} = C_{p,plant} / C_{p,solution} \tag{5}$$

where $C_{p,plant}$ is the pollutant concentration in the part of the plant (mg/kg) and $C_{p,solution}$ is the pollutant concentration in the solution (mg/L).

TF [26] for pollutants inside a plant was calculated by the given equation as:

$$\mathrm{TF} = C_{p,stem} / C_{p,root} \tag{6}$$

where $C_{p,stem}$ is the pollutants concentration in stem of plant and $C_{p,root}$ is the pollutant concentration in root of plant.

2.7. Estimation of phenol and cyanide uptake kinetics parameters

The uptake of phenol and cynaide were carried out for two growth stages of *Ipomoea aquatica* plants. Outlet solution containing phenol and cyanide was collected and the rate of phenol and cyanide uptake was estimated from the difference in concentration at the inlet and outlet. At each stage, the weight of the absorbing roots of the plant was also determined [27]. The rate of phenol and cyanide uptake by *Ipomoea aquatica* plants were calculated by linear regression analysis of four consecutive sampling times of phenol and cyanide solutions measured from the pots during depletion. It was assumed the uptake experiment that uptake of water by the plant roots, which further transports towards the plant body and due to transpiration some water may get lost, did not affect the phenol and cyanide concentrations in the liquid medium. The relationship between pollutant concentration (C) and uptake rates (V) was plotted and fitted to the equation proposed by Michaelis–Menten model [28].

$$V = (V_{\max} \times C) / (K_m + C) \tag{7}$$

where V is the rate of phenol and cyanide absorption when its concentration in solution is C, V_{max} is the maximum rate of phenol and cyanide uptake, C is phenol and cyanide concentration in the uptake solution, and K_m is the Michaelis–Menten constant.

2.8. Estimation of NRT

Transpiration is expressed as the measure of the toxicity of pollutants in the plants. Transpiration can be calculated directly by measuring the weight of the plant with pot [29]. The transpiration is normalized with respect to initial transpiration. To consider the fact that healthy trees grow rapidly and thus increase transpiration, the NRT is calculated. The mean NRT is calculated by Eq. (8).

$$NRT(C,t)=\frac{1/n\sum_{i=1}^{n}\frac{T_i(C,t)}{T_i(C,0)}}{1/m\sum_{i=m}^{m}\frac{T_j(0,t)}{T_j(0,0)}} \quad (8)$$

where C is the concentration of pollutants in mg/L, it is the time period in h till the end of the experiment, T is the absolute transpiration in g/h and i is replicate 1, 2, … , n and j is control 1, 2, … , m for plants.

3. Results and discussion

3.1. Characterization of root, stem and leaves of Ipomoea aquatica

For the characterization of roots, stem and leaves of fresh *Ipomoea aquatica* before and after uptake of cyanide and phenol were individually dried in an oven at 60°C and grounded to the fine particle sizes. If the temperature is high, then biochemical compounds/proteins/sugars may be affected. Very high temperature may destroy some of the constituents, whereas lower temperatures have the affinity to encourage fungal growth. After drying and grinding, they were individually stored in vacuum desiccator.

3.1.1. FTIR analysis of root, stem and leaves of Ipomoea aquatica

The FTIR spectrum is used to find the functional groups existing in parts of *Ipomoea aquatica*, i.e., root, stem, and leaves that might be accountable for the uptake of phenol and cyanide. Fig. 1(a)–(c) demonstrates the FTIR spectrum of leaves, stem, and roots of *Ipomoea aquatica*, respectively. The strong band around 3,500.00 cm^{-1} in FTIR spectrum of leaves, root, and shoot of the *Ipomoea aquatica*, before uptake of phenol and cyanide ascribed the vibrations of N-H and O-H functional groups [30]. The change is found in peak area around N-H and O-H functional group after uptake of phenol and cyanide. In addition, the peak around 1,200.00 cm^{-1} to 2,100.00 cm^{-1} in the FTIR spectrum of *Ipomoea aquatica* parts, i.e., leave, stem, and roots signifies the C-C bond stretch. The peak around 2,900.00 cm^{-1} due to C-H group was found change after the uptake of phenol and cyanide. Peak at 1,600 cm^{-1} relates to –CH stretching owing to the occurrence of conjugated hydrocarbon groups, carboxylic groups, aromatic hydrocarbons, and carboxyl and carbonate structures representing uptake of phenol however, peak at 1,353 cm^{-1} corresponds to inorganic nitrates marking a possible uptake site for cyanides. An increase in absorbance around peaks at 1,353 cm^{-1} reveals the uptake of cyanide in the form of nitrates and amines. Further peak around 700.00 cm^{-1} relates to uptake of pollutants owing to the occurrence of methylene groups. These shifts in the peaks indicated that there was pollutants binding process taking place at the surface of the plants. It could be observed from FTIR spectrum of parts of *Ipomoea aquatica* that more uptake of pollutant was found in stem and leaves than roots. Table 1 indicates the elemental composition of *Ipomoea aquatica* before and after 13 d uptake of phenol and cyanide.

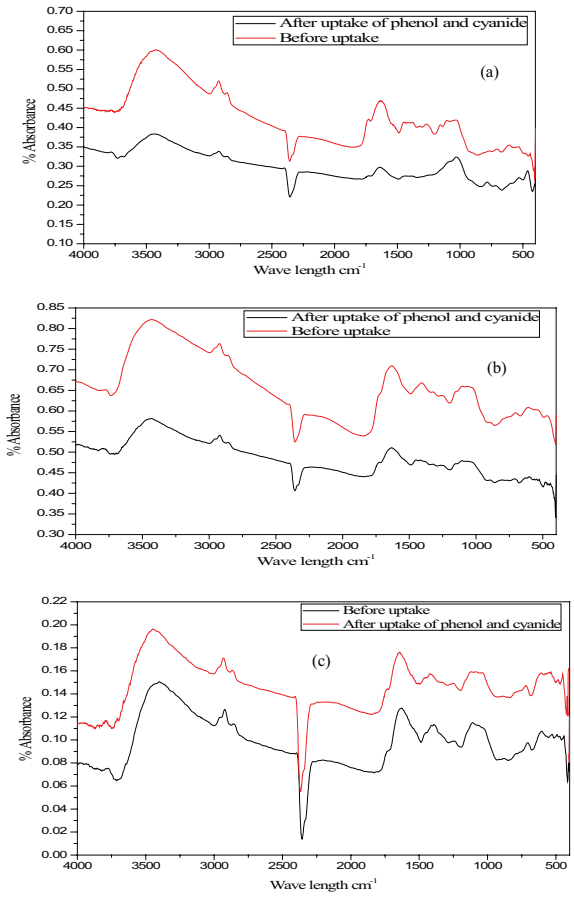

Fig. 1. FTIR Spectrum of *Ipomoea aquatica* (a) leaves, (b) stem, (c) roots.

Table 1
Elemental composition of *Ipomoea aquatica* before and after 13 d uptake of phenol and cyanide

Element C K	Uptake	Wt% root	Wt% leaves	Wt% stem
C K	Before uptake	37.12	44.40	52.44
	After uptake	45.38	46.91	53.96
O K	Before uptake	36.91	34.81	28.46
	After uptake	34.65	37.33	31.40
N K	Before uptake	2.53	5.85	5.13
	After uptake	2.40	5.27	5.10
Na K	Before uptake	0.59	0.00	0.68
	After uptake	0.82	0.76	0.94

3.2. Factor affecting phenol and cyanide accumulation in *Ipomoea aquatica*

The potential use of water spinach (*Ipomoea aquatica*) for elimination of phenol and cyanide from mono and binary component synthetic/simulated wastewater was tested experimentally in this current study. There are several factors such as age of plant, pH, temperature, initial concentration, light intensity, competition with other pollutants, and contact time, which affect the pollutant accumulation in *Ipomoea aquatica*. Six different concentrations ranging from 100 to 1,000 mg/L (100, 200, 300, 500, 700, and 1,000 mg/L) of phenol and 10 to 100 mg/L (10, 20, 30 50, 70 and 100 mg/L) of cyanide was used for the experimentation purpose. The chlorophyll, sugar, and protein content in leaves, shoots and roots of *Ipomoea aquatica* prior and after 13 d of exposure to phenol and cyanide from mono and binary component solution are given in Tables 2, 3, and 4, respectively.

Table 2
Chlorophyll, sugar and protein variations in *Ipomoea aquatica* 13 d after exposure to phenol in mono component solution

Plant part	Phenol concentration (mg/L)	Chlorophyll content (mg/g dry weight)	Sugar content (mg/g dry weight)	Protein content (mg/g dry weight)
Roots	100	–	40.82 ± 0.34	47.25 ± 0.32
	200	–	35.77 ± 0.34	41.64 ± 0.88
	300	–	27.86 ± 0.54	31.05 ± 0.45
	500	–	20.99 ± 0.13	25.65 ± 0.64
	700	–	15.64 ± 0.72	20.01 ± 0.99
	1,000	–	9.54 ± 0.26	14.43 ± 0.02
Stem	100	–	34.77 ± 0.93	91.38 ± 0.66
	200	–	26.55 ± 0.27	80.92 ± 0.45
	300	–	20.59 ± 0.17	56.65 ± 0.94
	500	–	17.54 ± 0.38	41.87 ± 0.56
	700	–	13.59 ± 0.88	34.45 ± 0.88
	1,000	–	7.54 ± 0.82	21.08 ± 0.91
Leaves	100	10.53 ± 0.23	43.22 ± 0.32	110.97 ± 0.94
	200	9.24 ± 0.67	38.91 ± 0.87	99.58 ± 0.87
	300	7.87 ± 0.54	30.43 ± 0.31	90.93 ± 0.66
	500	5.66 ± 0.29	23.65 ± 0.11	79.12 ± 0.98
	700	3.45 ± 0.87	19.83 ± 0.42	60.18 ± 0.76
	1,000	2.45 ± 0.80	10.98 ± 0.65	47.45 ± 0.77

Table 3
Chlorophyll, sugar and protein variations in *Ipomoea aquatica* after 13 d exposure to cyanide in mono component solution

Plant part	Cyanide concentration (mg/L)	Chlorophyll content (mg/g dry weight)	Sugar content (mg/g dry weight)	Protein content (mg/g dry weight)
Roots	10	–	37.88 ± 0.63	41.05 ± 0.12
	20	–	29.87 ± 0.08	32.45 ± 0.98
	30	–	20.54 ± 0.11	27.85 ± 0.32
	50	–	15.78 ± 0.99	20.12 ± 0.92
	70	–	10.97 ± 0.43	14.87 ± 0.12
	100	–	8.76 ± 0.72	9.87 ± 0.87
Stem	10	–	31.99 ± 0.39	85.18 ± 0.76
	20	–	25.78 ± 0.83	74.65 ± 0.67
	30	–	19.84 ± 0.55	59.23 ± 0.11
	50	–	14.83 ± 0.52	49.87 ± 0.54
	70	–	10.99 ± 0.11	29.86 ± 0.15
	100	–	5.88 ± 0.09	15.38 ± 0.53
Leaves	10	12.83 ± 0.83	49.8 ± 0.25	102.77 ± 0.81
	20	11.63 ± 0.55	42.98 ± 0.73	95.98 ± 0.32
	30	10.88 ± 0.87	37.84 ± 0.72	83.65 ± 0.21
	50	8.87 ± 0.13	30.82 ± 0.62	68.77 ± 0.43
	70	7.76 ± 0.48	23.82 ± 0.99	55.98 ± 0.34
	100	5.88 ± 0.76	17.76 ± 0.58	41.43 ± 0.13

Table 4
Chlorophyll, sugar and protein variations in *Ipomoea aquatica* after 13 d exposure to phenol and cyanide in binary component solution

Plant part	Phenol concentration (mg/L)	Cyanide concentration (mg/L)	Chlorophyll content (mg/g dry weight)	Sugar content (mg/g dry weight)	Protein content (mg/g dry weight)
Roots	100	10	–	39.02 ± 0.73	45.18 ± 0.77
	200	20	–	32.44 ± 0.25	40.21 ± 0.23
	300	30	–	27.55 ± 0.62	37.87 ± 0.33
	500	50	–	20.94 ± 0.22	29.76 ± 0.57
	700	70	–	15.82 ± 0.66	20.12 ± 0.65
	1,000	100	–	10.99 ± 0.65	12.32 ± 0.83
Stem	100	10	–	34.09 ± 0.42	86.08 ± 0.81
	200	20	–	29.87 ± 0.42	74.76 ± 0.23
	300	30	–	20.93 ± 0.49	61.25 ± 0.64
	500	50	–	15.76 ± 0.33	49.65 ± 0.37
	700	70	–	12.66 ± 0.85	35.76 ± 0.16
	1,000	100	–	6.88 ± 0.38	27.54 ± 0.22
Leaves	100	10	11.73 ± 0.87	52.76 ± 0.98	110.97 ± 0.94
	200	20	10.87 ± 0.98	45.87 ± 0.91	99.58 ± 0.87
	300	30	9.12 ± 0.54	39.85 ± 0.76	90.93 ± 0.66
	500	50	8.54 ± 0.87	29.87 ± 0.71	79.12 ± 0.98
	700	70	7.76 ± 0.21	24.02 ± 0.92	60.18 ± 0.76
	1,000	100	6.87 ± 0.94	18.88 ± 0.55	47.45 ± 0.77

Table 5
Measurement of shoot and root length of the *Ipomoea aquatica* at different growth period under the treatment of various phenol and cyanide concentrations

Age (days)	Length	Control	Phenol:100 Cyanide:10	Phenol:200 Cyanide:20	Phenol:300 Cyanide:30	Phenol:500 Cyanide:50	Phenol:700 Cyanide:70	Phenol:1,000 Cyanide:100
4	Roots (cm)	7.1	7.0	6.8	6.7	6.2	5.8	5.0
	Shoots (cm)	9.4	9.4	9.3	9.0	8.5	7.5	6.7
8	Roots (cm)	11.4	11.2	11.0	10.5	7.6	6.5	5.9
	Shoots (cm)	10.5	10.2	10.0	9.6	8.7	7.7	6.9
13	Roots (cm)	12.6	12.5	12.1	11.3	8.1	6.9	6.2
	Shoots (cm)	13.5	13.2	13.0	12.7	8.9	7.8	7.2

3.2.1. Effect of initial concentration of phenol and cyanide

Table 5 indicates the growth analysis of *Ipomoea aquatica* plant at different age of plant and treatment with various phenol and cyanide concentrations ranging between 100–1,000 mg/L of phenol and 10–100 mg/L of cyanide. It was seen that the length of shoot and root of *Ipomoea aquatica* plants increased gradually at the initial stage of low concentration of phenol and cyanide, this might be due to the uptake of phenol and cyanide species as a nutrient material for the growth of plant. However, increase in phenol and cyanide concentration causes significant decrease in shoot and root length of plants. From 13-d-old *Ipomoea aquatica* plants presented in Table 5, drastic reductions in shoot and root length of the plant was observed at 1,000 mg/L of phenol and 100 mg/L of cyanide concentration as compared with the control plants viz. 13.5 cm to 7.2 cm of shoot length and 12.6 to 6.2 cm of root length. The results revealed that the growth rate of *Ipomoea aquatica* plants are highly affected by phenol and cyanide. Percentage removal of the plant for mono and binary component at varying concentrations (100–1,000 mg/L for phenol and 10–100 mg/L for cyanide) used for experimentation are revealed in Table 6. The kinetics of phenol and cyanide uptake from mono component aqueous solution by *Ipomoea aquatica* shown in Fig. 2(a) and 2(b). Uptake of phenol and cyanide for binary component solution are shown in Fig. 3(a) and 3(b). Throughout the experiment, the concentration of both pollutants was observed to decrease with increasing time of exposure and then becomes constant. It could be observed from figures that the reaction may be slower due to the reduction of the pollutant concentration. This is due to the fact that at initial stage, there was a high concentration gradient available between bulk solution and *Ipomoea aquatica* and as time proceed equilibrium was maintained between bulk solution and *Ipomoea aquatica*. The phytoremediation capability of the plant was stated by the observation of pollutant concentrations before and after uptake. The study

Table 6
Percentage Removal of phenol and cyanide from mono and binary solution by *Ipomoea aquatica* plant after 13 d of treatment

pollutants	Mono component solution		Binary component solution	
	Concentration (mg/L)	% Removal	Concentration (mg/L)	% Removal
Phenol	100	97.50	100	96.07
	200	96.78	200	95.89
	300	95.59	300	94.92
	500	94.28	500	92.35
	700	93.36	700	90.81
	1,000	89.96	1,000	87.50
Cyanide	10	99.70	10	95.7
	20	98.50	20	92.35
	30	97.00	30	91.67
	50	96.20	50	90.03
	70	95.86	70	89.73
	100	93.10	100	87.01

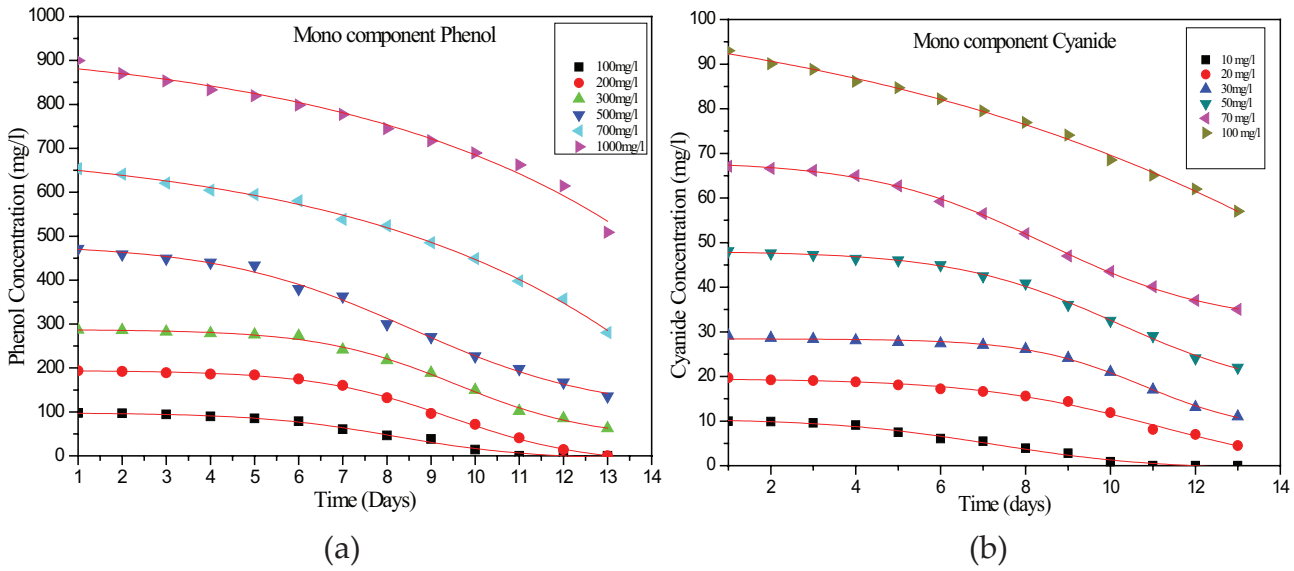

Fig. 2. (a) Uptake of phenol from mono component aqueous solution by *Ipomoea aquatica*; (b) Uptake of cyanide from mono component aqueous solution by *Ipomoea aquatica*.

of pollutant concentration with growing time has recommended that phenol and cyanide concentration decreased from day 1 to day 13. The results indicated that the removal percentage of phenol was higher than cyanide in binary component solution. The reduction in percentage removal of phenol and cyanide with the increase in the initial concentration was depends on the uptake capacity of the particular plant site, transport mechanism and tolerance limit of the plant [31,32]. Pollutants utilized by the plants are elevated from the root to stem and then to the leaves and become accumulated. The percentage removal of phenol and cyanide for aquatic plants was compared from previous works is specified in Table 7. The decrease in initial growth, could be due to the decrease of a fraction of the cells, produced by the pollutant toxic effect [32]. Accumulation of phenol and cyanide onto surface of *Ipomoea aquatica* is due to biosorption monitored by vigorous uptake into cells of the plant [33]. The accumulation capacity of pollutants depends on the basis of variation of affinity and competition between ions during uptake [33].

3.2.2. Effect of phenol and cyanide concentration on plant growth

The effect of phenol and cyanide concentration on plant growth was evaluated by the measurement of plant weight. The increase of the pollutant concentration had a substantial influence on the weight of *Ipomoea aquatica*. At increasing pollutant concentrations, the plants spread chlorosis and the leaves of *Ipomoea aquatica* started to turn dull after 8 d. Increasing pollutant concentration, reduced the plant growth after 9-d treatment (Table 5). At a low initial concentration of phenol and cyanide, the plant growth was

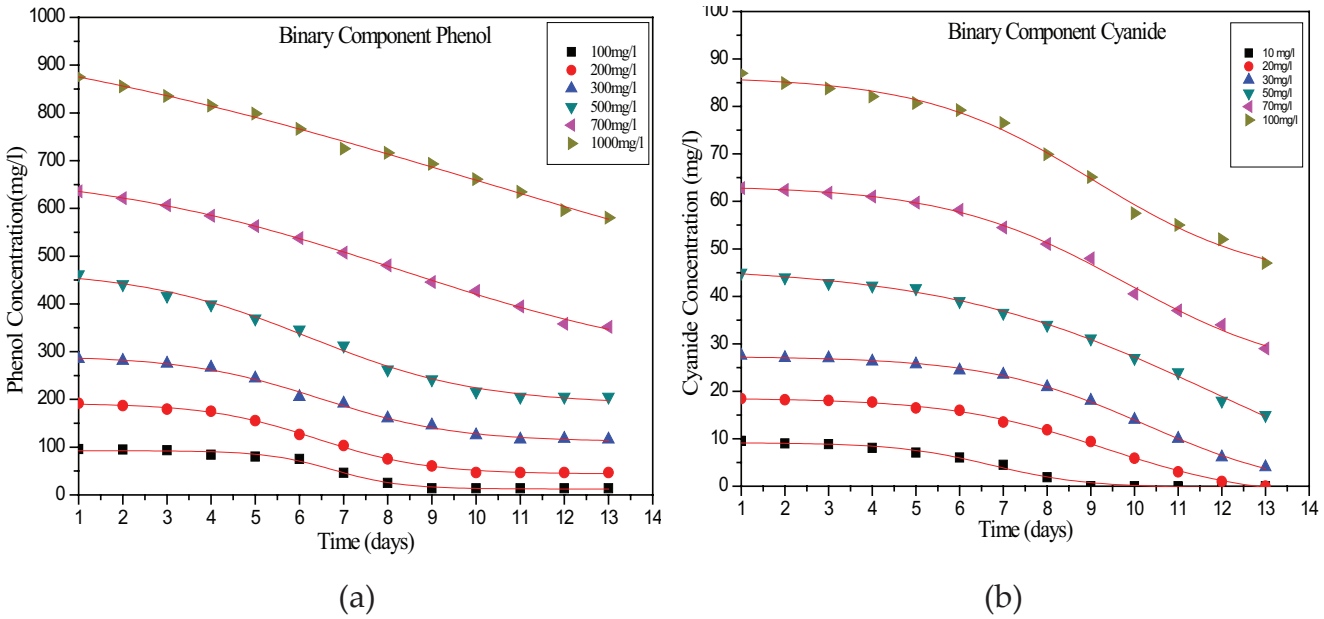

Fig. 3. (a) Uptake of phenol from binary component aqueous solution by *Ipomoea aquatica*; (b) Uptake of cyanide from binary component aqueous solution by *Ipomoea aquatica*.

Table 7
Comparison of percentage removal of aquatic plants for phenol and cyanide removal from literature

Plant name	Pollutant uptake	pH	Initial concentration (mg/L)	% Removal	Uptake capacity (mg/g)	Time (days)	References
Eichhornia crassipes	Phenol	7	10–40	99.80	2.46	16	Gupta et al. [34]
Vetiveria zizanoides L. Nash	Phenol	7	10–1,000	70	–	1–12	Singh et al. [25]
Potamogeton crispus	Phenol	7	200	–	65	6	Hafez et al. [12]
Ipomoea aquatica	Cyanide	3.97	3.38	76.92		37	Indrayatie et al. [10]
Cyperus iria	Cyanide	3.97	3.38	74.85		37	Indrayatie et al. [10]
Commelina nudiflora	Cyanide	3.97	3.38	76.92		37	Indrayatie et al. [10]
Oryza sativa	Cyanide	3.97	3.38	71.30		37	Indrayatie et al. [10]
Vetivera zizanioides	Cyanide	3.97	3.38	81.07		37	Indrayatie et al. [10]
Eichhornia crassipes	Cyanide	8.5	5–50	72%	0.035	4	Ebel et al. [9]
Ipomoea aquatica	Phenol	8	300	94.92	28.47	13	Present study
Ipomoea aquatica	Cyanide	8	30	91.67	6.88	13	Present study

unaffected. Though, addition of pollutant concentration increased signs of phenol and cyanide toxicity therefore, reduction in biomass [32]. Translocation of pollutants has been reported in the process of phytoextraction it may not be the main mechanism of pollutant transport in aquatic vascular plants. The containment, immobilization and accumulation of pollutants in the root structures may be due to the process of rhizofiltration, which is commonly observed in aquatic plants. Roots exudates in the rhizosphere may also cause the pollutants to precipitate onto the surface of roots [34]. Pollutants can be actively absorbed into the root cells via plasmalemma, and adsorbed on the cell walls via passive diffusion or moved acropetally in the roots of aquatic macrophytes.

3.2.3. Effect of pH on removal of phenol and cyanide

To evaluate the effect of pH on phenol and cyanide removal process, the plants were grown at pH range (2–12), under the identical conditions. It is identified that the pH controls the plant growth through affecting availability and movement of ions [35]. Effect of pH on removal of phenol and cyanide are shown in Fig. 4(a) and 4(b), respectively. Among six designated pH values, the highest phenol and cyanide removal by *Ipomoea aquatica* was detected at pH 8 [9,10]. The adsorption of either hydrogen ions or hydroxyl ions takes place quite strongly. At higher pH, hydroxyls are favored, whereas hydrogen ions are adsorbed favorably at lower pH. Therefore, hydration of plants surface gives the properties of an ion-exchanger to the plants surface, freely

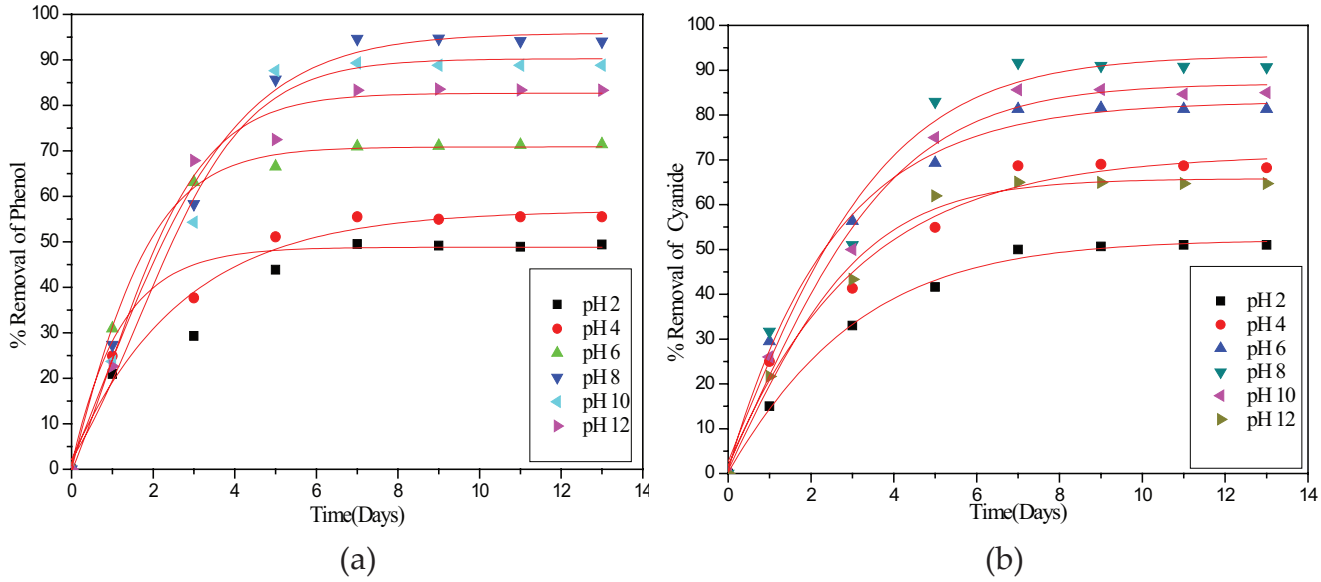

Fig. 4. (a) Effect of pH on removal of phenol from binary component aqueous solution by *Ipomoea aquatica*; (b) Effect of pH on removal of cyanide from binary component aqueous solution by *Ipomoea aquatica*.

adsorbing cations or anions from the solution. It is also observed from FTIR study that the surface of plant contains a number of functional groups like carboxyl, phenolic, sulphate, phosphate, carbonyls etc., which are responsible for increasing negative charges on its surface. Phenol and cyanide are easily dissociable in aqueous solutions at measured pH. Phenol remains constant in acidic pH range where the phenol remains in undissociated forms. However, at pH more than its pKa (9.96), a reduction in removal percentage is detected representing phenol removal mostly in its undissociated form. Since cyanide has a pKa of 9.39 maximum removal of cyanide is found at pH 8–10. In pH range (4–8), there is a reduction in percentage removal of cyanide which could be recognised to hydrolysis of weak acid dissociable cyanides to hydrogen cyanide. Subsequently, hydrogen cyanide is very hydrophilic; its affinity to be removed at low pH is noticeably reduced. However, at higher pH, the cyanide occurs in undissociated form. It may be due to the maximum removal of phenol found in pH range 7–8 and cyanide removal occurs in pH range 8–11 [2,4]. The uptake increases with the pH increases due to the deprotonation of the active sites [36,37]. Pollutant removal through bioaccumulation is strongly depends on pH and found maximum at pH above 8 for this current investigation. Lower removal of phenol and cyanide at higher pH is owing to the occurrence of excess OH^- ions competing with pollutants for the adsorption sites. At pH 8, significant bioaccumulation of the phenol and cyanide still occurred (Fig. 4(a) and (b)).

It can be described by formation of covalent bonding between surface –OH group of plant and negatively charged pollutants molecules. This can be recommended that the another mechanism, i.e., chemisorption might be effective [37]. Therefore, strong pH-dependence of phenol and cyanide bioaccumulation onto plant composed with FTIR results show to possible participation of physical forces such as Vander Waals, hydrogen bonding and covalent chemical bonds in this process.

3.2.4. Effect of phenol and cyanide on biochemical parameters of Ipomoea aquatica

The toxic effect on the plant growth was detected due to reduction in chlorophyll, sugar, and protein content of the test plants for mono and binary solution. Chlorophyll contents are significant indicators of loss to the photosynthetic system of the plants encouraged by the environment [38]. In the current study, due to toxic effect of phenol and cyanide on plant, the changes in biochemical parameters of *Ipomoea aquatica*, i.e., chlorophyll content, sugar content and protein content after uptake of phenol and cyanide from mono and binary solution are shown in Table 2, 3, and 4, respectively. The strong reducing ability of phenol and cyanide may cause phytotoxicity to the plants. The reduction in parameters chlorophyll, sugar, and protein contents of the *Ipomoea aquatica* indicated a comparable tendency of deterioration corresponding to the increase in the concentration of phenol and cyanide. The reduction in parameters was detected with the increasing exposure time [39]. It could be due to deterioration in sugar content at the higher concentration; therefore, the energy of the plant was reduced. Similar clarifications on reduction in chlorophyll, protein and sugar contents in several aquatic plants for phenol and cyanide uptake were described by Xiao-Zhang and Ji-Dong [40]. After 5 d, the new young branch was grown for both mono and binary solution of phenol and cyanide, this might be owing to the fact that at initial stage plants were stressed situation, after 5 d of operation the plants were accommodated in to the toxic environments. The formation of reactive oxygen species (ROS) such as superoxide and H_2O_2 throughout the stressed condition was owing to the biochemical mechanism of the plant.

3.3. RGR, BCF and TF

The growth of *Ipomoea aquatica* was increased with decrease initial concentration of phenol and cyanide with time. The plant growth was inhibited at the higher concentration of

phenol and cyanide due to decrease in dissolve oxygen (DO) and the osmotic influence on the cell of the plant [41]. The measurement of RGR can be used to determine the plant health throughout the experimental period and BCF was calculated to determine the capability of *Ipomoea aquatica* for the accumulation of phenol and cyanide. RGR can be calculated according to Eq. (5). The RGR value of the plant was observed 0.067, 0.067, 0.05, 0.033, 0.0167 and 0.011 mg/d at 100, 200, 300, 500, 700 and 1,000 mg/L, respectively, for phenol in mono component solution. Hence, there is no toxic effect of phenol on the *Ipomoea aquatica* found for the concentration ranging from 100 to 200 mg/L, and then constant RGR value 0.067 mg/d. RGR value for cyanide in mono component solution was found to be decreased from 0.056 to 0.0067 mg/d at increase initial concentration 10 to 100 mg/L. Similarly, when the pollutant concentration in water increases, the amount of pollutants accumulation in plants increases; therefore, the RGR value decreases. The RGR value for binary component solution of phenol and cyanide ratio (10:1) was observed to be 0.043, 0.037, 0.026, 0.011, 0.003, and 0.0013 mg/d with increase initial concentration. Therefore, it was found that RGR value is more for the mono component solution of phenol and cyanide than the binary component solution. This is due to fact that both component show antagonistic effect on each other; as a result, the growth of plant reduced in binary solution. Therefore, RGR value is reduced for binary component solution. From the opinion of phytoremediation, a noble accumulator should have the capability to concentrate the pollutants in its tissue.

In monocomponent solution, the BCF value for phenol was found to be 870, 600 and 430 in stem, leaves and roots, respectively. While BCF value for cyanide was found to be 800, 650 and 410 in stem, leaves and roots, respectively. Therefore, the BCF value of phenol and cyanide in stem of *Ipomoea aquatica* was more than other parts of the plant. This demonstrates that phenol was more accumulated in the stems of *Ipomoea aquatica* than cyanide. BCF was calculated to determine the ability of *Ipomoea aquatica* for the accumulation of phenol. BCF for phenol in mono component solution was 430 but for binarycomponent solution BCF was found to be 532 which shows high ability of *Ipomoea aquatica* for the accumulation of phenol. This shows that phenol was more accumulated in the root of the plant in the presence of cyanide. However, since the stem biomass was higher than root biomass, phenol and cyanide in the stem part had the maximum proportions of the pollutant in the entire plant. In addition, the value of BCF for phenol and cyanide in binary component solution was found more than mono component solution.

The TF is used to define the translocation of pollutants from roots to stem and stem to leaf [42]. The value of TF greater than 1 shows that the plants has able to translocation of pollutants [41]. The value of TF of phenol and cyanide ranged from 1.21 to 0.83 and 1.00 to 0.84, respectively, in the roots to stem of *Ipomoea aquatica* in mono component solution. The data show that the phenol translocation is more than cyanide in roots to stem of the plant. The TF vary species to species and metal to metal [40]. The value of TF more than 1 defines that the plant has capability to transfer pollutant from root to stem [42,43]. The TF value more than 1 indicated that *Ipomoea aquatica* effectively transfer polltants from root to stem. The TF of phenol and cyanide in binary solution is 1.08 to 0.81 and 0.99 to 0.35, respectively, indicate TF is reduced in binary component solution for both phenol and cyanide. Low value of TF of pollutants to parts of plant could be due to adoption of pollutants inside root cell to reduce it non-toxic.

3.4. Phenol and cyanide uptake kinetics parameters

The phenol and cyanide ions uptake kinetic parameters (V_{max} and K_m) of *Ipomoea aquatica* plants are given in Table 8. Phenol and cyanide solution concentrations and uptake rates at different growth stages were fitted in Michaelis–Menten equation. Then the V_{max} and K_m were calculated with the help of fitted coefficients obtained by fitting the solution phenol and cyanide concentration and uptake rate data on the Michaelis–Menten equation [28]. At root length elongation stage, the values of V_{max} were obtained as 7.24 μg/g root/h for phenol and 6.65 μg/g root/h for cyanide, which increased to 11.15 μg/g root/h and 12.24 μg/g root/h for phenol and cyanide, respectively, at total height change stage. Also, K_m values at 11.26 mM and 0.56 mM for phenol and cyanide at root length elongation stage decreased to 5.75 mM and 0.39 mM for phenol and cyanide at total height change stage (Table 8). Also, during 4 to 13 d of *Ipomoea aquatica* during the root length elongation stage, the maximum phenol and cyanide

Table 8
Values of V_{max} and K_m for phenol and cyanide uptake equation at different growth stages of *Ipomoea aquatica*

							V_{max}	K_m
	Initial concentration of phenol (mM)							
Growth stage of *Ipomea aquatica*	1.062	2.123	3.186	5.309	7.433	10.619	(μg phenol/g root/h)	(mM)
	Uptake rate (μg/g root/h)							
Root length elongation	0.018	0.028	0.046	0.054	0.065	0.071	7.24	11.26
Total height change	0.006	0.012	0.019	0.023	0.029	0.035	11.15	5.75
	Initial concentration of cyanide (mM)							
Growth stage of *Ipomea aquatica*	0.051	0.103	0.154	0.257	0.360	0.514	(μg cyanide/g root/h)	(mM)
	Uptake rate (μg/g root/h)							
Root length elongation	0.015	0.024	0.043	0.051	0.062	0.068	6.65	0.56
Total height change	0.005	0.010	0.015	0.020	0.024	0.031	12.24	0.39

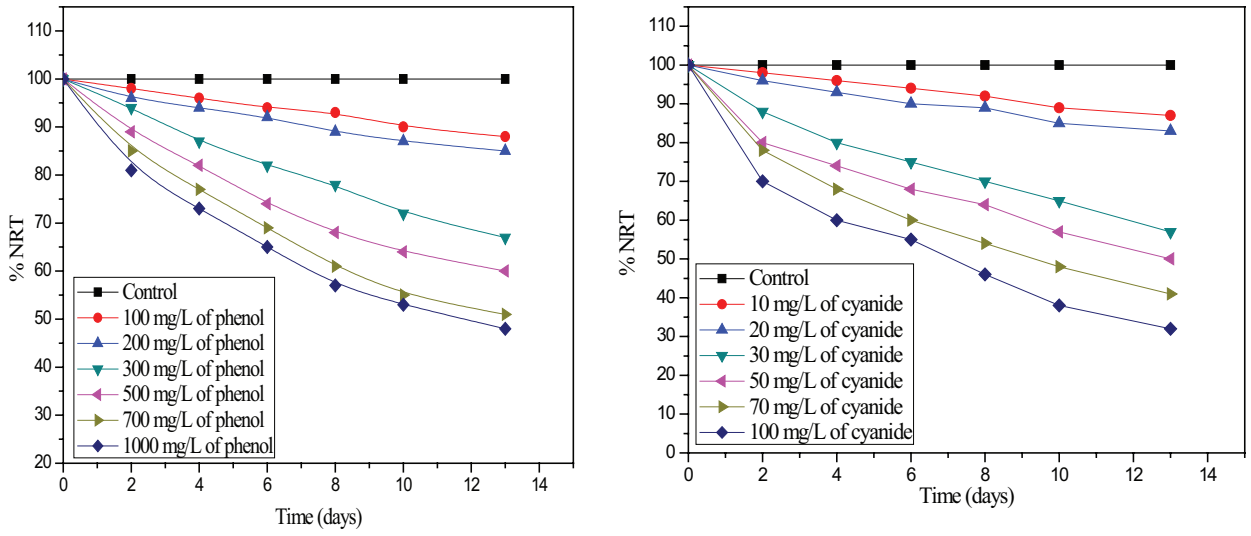

Fig. 5. (a) Relative transpiration of *Ipomoea aquatica* supplied with phenol at different concentrations; (b) Relative transpiration of *Ipomoea aquatica* supplied with cyanide at different concentrations.

uptake per unit weight of the roots were 0.071 and 0.068 μg/g root/h for phenol and cyanide with 10.619 m*M* of phenol and 0.514 mM of cyanide concentration.

3.5. NRT

Fig. 5(a) and 5(b) shows the variation in % NRT with time for the removal of phenol and cyanide, respectively. Plants with 1,000 mg/L of phenol show % NRT, i.e., 48.10% and plants with 100 mg/L of cyanide show % NRT, i.e., 50.09%. This variation is due to toxicity of phenol and cyanide for plant [9]. In the phenol and cyanide toxicity experiments, phenol and cyanide decreased the relative transpiration of *Ipomoea aquatica*, higher concentrations led to lower transpiration (Fig. 5(a) and (b)). At the lowest concentration (200 mg/L of phenol and 20 mg/L of cyanide), transpiration was only slightly reduced and the plants survived without any morphological changes. The transpiration rate is a very sensitive toxicity parameter because stress factors, e.g., toxic substances, have a high impact on the water balance of plants. Therefore, chemical stress makes a quick decrease in transpiration and drying of leaves.

4. Conclusions

The current investigation highlighted that *Ipomoea aquatica* as a good accumulator of phenol and cyanide. The plant was effectively removed up to 94.92% of phenol and 91.67% of cyanide. During 13 d contact with phenol and cyanide, biochemical parameters such as chlorophyll, sugar and protein was found decrease. The effect of physicochemical parameters with time such as pH and initial concentration of phenol and cyanide were also evaluated on the removal efficiency by the plant. Phenol accumulation was found toxic above concentration 200 mg/L indicate toxicity symptoms, i.e., delay in growth and yellowing of the leaves at pH 8. However, cyanide was indicated toxic symptoms above 20 mg/L concentrations. The NRT and Michaelis–Menten kinetic parameters were also evaluated. Overall, on the basis of these results *Ipomoea aquatica* is harmless for elimination of phenol and cyanide. The main disadvantage of this technology is taking more time. Therefore, the plant with higher adaptation capacity and long roots could be used. High pollutant concentrations can be harmful to the plants, while some plants have better adaptation to toxicity than others. Pollutant concentration are not too high then this technology will successfully use at industrial level.

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