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PHYTOREMEDIATION OF CYANIDE AND PHENOL FROM WASTEWATER BY *E.CRASSIPES* (WATER HYACINTH)

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ABSTRACT: Cyanide and Phenol are most dangerous pollutants for the environment which discharges from various industries such as coke industry. In the present study cyanide and phenol were removed from the industrial wastewater using *E.Crassipes* by phytoremediation process. Study was conducted at controlled environmental conditions such as 25 ℃ temperature and 75 % relative humidity. Phytoremediation of cyanide and phenol showed significant effect on Normalized relative transpiration and removal rate of cyanide and phenol. Variation in percentage removal of cyanide and phenol at varying initial concentration and pH was observed. The metabolic properties such as chlorophyll, protein, carbohydrate and starch were changes at different concentration cyanide and phenol at different rate. The experimental data were examined for the biosorption studies by isotherms. The Langmuir and Temkin isotherms were used for the evaluation of the experimental data and their constants were calculated. The Langmuir isotherm was the best fitted model in the experimental data for cyanide and phenol both.

Keywords: *Chlorophyll, Cyanide, Isotherms, NRT, Phenol, Phytoremediation.*

I. INTRODUCTION

The increasing cost of wastewater treatment techniques is tending towards the search of alternatives of those wastewater treatment processes with low cost. But the lower cost treatment process is move towards the more strict nutrient discharge regulations. There is various numbers of biological, chemical and physical methods to remove cyanide from wastewater. These treatment methods have various advantages and disadvantages. The cost of treatment method increases with the decrease in required concentration of discharge [19]. The continuous increase in advances in science and technology has increases the interest towards the use of natural resources to a great extent. Recently various types of toxic chemicals are accumulated in the different environmental compartments i.e soils, ground water and atmosphere which increase the pressure on self-cleansing capacity of natural ecosystems. The exposure of these toxic pollutants is harmful for both human and ecosystem. In recent times various efforts are going underway in various countries [15,24] and they also try to accelerate the breakdown of pollutants by appropriate remediation process. The remediation process of contaminated ground water has been done through ex-situ method *i.e* extraction and treatment by activated carbon adsorption, microbes or air stripping. But the stimulation of anaerobic and aerobic microbial activities in the aquifer is called as in-situ methods. These methods of wastewater treatment are comparatively had high expenditure and manpower and also long term costs. Hence, alternatives of these methods are continuing in process to found more cost effective approaches to remove large amount of pollutants from natural ecosystem such as soil, ground water and wetlands. Phytoremediation is a remediation process of wastewater treatment which utilizes plants and then the corresponding rhizosphere microorganisms to transform and remove toxic chemicals accumulated in soils, ground water, surface water and the atmosphere. These days, phytoremediation is used to remove various types of pollutants such as petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radio nuclides and

landfill leachates. After studying the recent reports [4], it has been observed that approximately 80 % of the polluted ground waters are lies within the depth of 20m surface. This proves that there are various sites are suitable for the low cost phytoremediation process. Those plants which utilize various processes to remediate the wide range of chemicals at toxic waste sites are explained in [6,26]. These processes are modification of physical and chemical properties of contaminated soils, releasing root exudates, improvement of aeration by the release of oxygen directly to root zone and increasing the porosity of the upper soil surface, retardation of movement of chemicals, co-metabolic microbial and plant enzymatic transformations of chemicals and minimising the vertical and lateral migration of pollutants by extraction of available water and alteration of the hydraulic gradients. Phytoremediation process is most economical and effective for low concentration of pollutants and also suitable as a long term solution for sites with less toxic contaminates [10] but phytoremediation is used as a final polishing process after the initial treatment process for high concentration of contaminates.

Treatment of wastewater from various industries is of prime concern in these days because of the contaminants present in wastewater. Various types of toxic pollutants are discharges from some of the industries such as petroleum refinery, oil refinery, coke plant industry, chemical industry, plating industries, plating bath, gold ore extraction, electroplating industry, colour film bleaching, paint and ink formulation, explosive manufacture industry, steel plant industry. In a very high concentration, Cyanide and phenol are discharges from coke industry with several other contaminants. But cyanide and phenol has been considered as extremely toxic for human and natural environment. Hence treatment of cyanide and phenol contain wastewater is of major concern in recent days. The maximum contaminant limit of cyanide and phenol in effluent discharge has been set as 0.2 mg/l and 0.5 mg/l respectively by USEPA, WHO, and CPCB [7]. The toxic effects of cyanide with low concentrations can cause coma, heart pains, breathing disorders, thyroid gland enlargement, headaches and lastly death [2,3] and the toxic effects of phenol can cause headache, skin and eyes injuries, central nervous system depression, vomiting, heart, lung, lever, kidney damage and lastly death. Various physical, chemical, biological and combined have been employed for the wastewater treatment for the removal of cyanide and phenol from industrial wastewater. [5,7,21,25]

In the present study the removal of cyanide and phenol by plants has been studied and the toxicity of cyanide and phenol for those plants has been observed. Batch study has been assumed on the remediation process. The phytoremediation process is considered as single component adsorption process and hence equilibrium isotherm of adsorption such as Langmuir and Temkin models are applied on the experimental data.

A. Phytotoxicity

Phytotoxicity measurement can be done by using three parameters such as growth of biomass, transpiration and water use efficiency. As we know that the growth and life of plants depends upon the carbon dioxide, Nutrients, light and water. Several plants take up water and nutrients via their roots. The process of loss of water from plants via stomata is known as the transpiration which starts from soil and then roots of plants and at last ends by the evaporation into atmosphere. The driving force for the water transpiration is the potential difference between soil and air. Through the osmosis process, water enters into the roots of plants. Through the cells of the plant or between the cells within the cell walls, water is distributed throughout the plant and then fills the xylem. Minerals are transported in the plant by the tissues of plant by diffusion process. Water is also used to avoid wilting of leaves due to loss of water from the cell walls. The stomata is used to regulate the resistance in the diffusion and to maintain a favourable ratio between the water transpired and uptake of CO_2. Hence transpiration efficiency is defined as the ratio between the transpired water and the produces dry matter which is relatively constant [11]. The inverse of the transpiration efficiency is known as the water use efficiency of production

(WUEP). Transpiration can be measured by weighing the plant with the pot. The effect on biomass of plants can be measured by weighing the plant directly. In the case of seeds, the parameters for the toxicity are related to germination and produced biomass *i.e* root, stem, leaves, fruit and photosynthesis [22].

B. Transpiration Measurement

Different types of trees have different value of transpiration rates. Hence the relative transpiration is defined in the terms of the weight loss due to the toxic effects on plants in comparison with the initial transpiration when pollutants were not mixed. The transpiration is then normalized with respect to initial transpiration and the transpiration of control plants. The normalized relative transpiration (NRT) is calculated by the following expression

$$NRT(C,t) = \frac{\frac{1}{n}\sum_{i=1}^{n}\frac{T_i(C,t)}{T_i(C,0)}}{\frac{1}{m}\sum_{j=1}^{m}\frac{T_j(0,t)}{T_j(0,0)}} \quad (1.1)$$

Where C is the concentration in mg/l, t is the time period in h, T is the absolute transpiration in g/h and n and m are the number of replicates for exposed and control plants [22].

C. Study of Metabolic Parameters

The parameters of metabolic studies are Chlorophyll a(), Chlorophyll b(), Protein, Carbohydrates and starch. The concentration of these parameters is examined before the exposure of plants to the pollutants and after the exposure of plants to the pollutants at different concentration [9].

D. Chlorophyll measurement

The chlorophyll content in leaves was determined spectrophotometrically at the end of the experiments. The amount of chlorophyll a and chlorophyll b in plant leaves was calculated by the equation (1.2) and (1.3). [14,27,28]

$$C_a = \frac{(12.3D_{663} - 0.86D_{645})V}{100*d*W} \quad (1.2)$$

$$C_b = \frac{(19.3D_{645} - 3.60D_{663})V}{100*d*W} \quad (1.2)$$

here C_a is the concentration of chlorophyll a (mg/g FW), C_b is the concentration of chlorophyll b (mg/g FW), D is the optical density (OD) at the specific wave length indicated, V is the final volume (mL), W is the fresh weight of leaf materials (g), and d is the length of the light path (cm).

E. Biosorption Studies

The phytoremediation experiment of pollutants were done on the healthy Water Hycainth(*E.Crassipes*) plants of different biomass. The biosorption studies has been done by the studying the effect of pH, Initial concentrations from aqueous solution. The initial concentration of cyanide and phenol were taken as 20,40,60,80 and 100 mg/l.

F. Adsorption Isotherms

Capacity of any biosorbent can be determined by the equilibrium study. Biosorption isotherm is examined by surface properties and affinity of biosorbent which is explained by by certain specific values and the comparison of the biosorptive capacities of different biosorbent for different pollutants can also be explained through the biosorption isotherm. Equilibrium data is analyzed by biosorption systems. The amount of the biosorbed pollutants per unit plant weight q_e can be calculated by the following relation at the equilibrium condition at constant temperature.

$$q_{e=}\frac{(C_i - C_e)V}{g} \quad (1.4)$$

Where C_i and C_e are the initial and final concentration, V is the volume of solution and g is the weight of plants. In the present study the plants are considered as the biosorber.

Hence some of the mathematical models can be used to trace experimental data of biosorption isotherms. In the present study Langmuir and Temkin isotherms are studied for the equilibrium analysis [16].

Various equilibrium models studied in the present study are listed in table 1.[16,18]

Table 1:-Isotherm models

Isotherm model	Expression	Parameter
Langmuir Model	$$\frac{C_e}{q_e} = \frac{1}{Bq_m} + \frac{C_e}{q_m}$$	q_m (mg/g) and B(L/mg) are constants.
Temkin Model	$$q_e = B \ln A + B \ln C_e$$	A and B are constants.

Where C_e is the equilibrium concentration (mg/l) and q_e is the amount of biosorbed pollutants (mg/g).

II. Materials and methods

A. Chemicals and Plants

All of the chemicals utilized in this study were of analytical grade and taken from Himedia Laboratories Pvt. Ltd, Mumbai, India. Concentration of 100 mg/l of cyanide stock solution was prepared by dissolving 0.189 g of NaCN in 1 L of Millipore water(Q-H_2O, Millipore Corp. with resistivity of 18.2 MX-cm). Concentration of 100 mg/l of phenol stock solution was prepared by dissolving 9.72µl in 200 ml of Millipore water(Q-H_2O, Millipore Corp. with resistivity of 18.2 MX-cm).

B. Preparation of Hoagland solution

Hoagland solution is used in the experiment to provide the nutrients to the plants for their growth. The contents of Hoagland solution are taken as given in [15].

C. Plant preparation

E.Crassipes(Water Hyacinth) is a harmful plant which attracted attention because of its fast rate of growth and spread and this fast rate in growth creates serious problems for the environment. But it has several unique properties for the utilization in the phytoremediation process. *E.Crassipes* recognise as one of the most useful aquatic plants for the phytoremediation of wastewater. This plant has amazing ability to extract pollutants from wastewater [1].

E.Crassipes(Water hyacinth) plants were taken from nearest solani river of roorkee and washed with running tap water to remove all surface contaminants present on their roots, leaves and stem. Damaged leaves were detached from the plants. The weight of each plants were measured 20 g approximately. Six washed and healthy plants of water hyacinths were dried for some time and weigh. Solution of different cyanide and phenol concentration were prepared and used for the remediation process. Plants were grown in Hoagland solution in the phytoremediation chamber under 18 h light periods with 25℃ light and 75 % relative humidity.

III. Results and Discussion

Water Hyacinth plants had a length of approximately 18cm and weight of approximately 20 g are transplant into 100 ml aluminium oil-wrapped Erlenmeyer flasks with solution of cyanide having different cyanide concentration. These plants are planted in a phytoremediation chamber under artificial light at temperature 25℃ and 65 % of relative humidity. The plants were positioned as such that roots were submerged in the solution. The weight of whole apparatus was done by weighing machine. After 2, 4, 6, 8, 12, 14 days, the weight of apparatus were measured again to determine the transpiration. Biomass production was calculated by weighing the plants before and after the experiment. Water use efficiency was determined by the ratio of growth and transpiration.

A. Normalized Relative Transpiration

In the present study the normalized relative transpiration of Water Hyacinth (*E. Crassipes*) , has been calculated for different cyanide and phenol concentration of 0,20,40,60,80 and 100 mg/l. Figure 1 and 2 shows the variation in % NRT with time for the removal of Cyanide and Phenol respectively.

It has been observed that the transpiration was decreases with time and the plants cannot be survived after 12 days for lower concentration but at higher concentration, plants get affected by their health after 2-6 days and at last died. When experiment was being carried down the plants get green sick and leaves become pale and after sometime disappear from plants. At the concentration of 60,80 and 100 mg/l, the transpiration rate decrease in lower rate and plants died during the experiment. For the control plants the normalized transpiration rate did not get effective change from initial value and plant survived during the experiment. At higher concentration the transpiration was decreases in lower rate than the lower concentration. At lower concentration of cyanide the transpiration decreases to 70% approximately but for higher concentration of cyanide the transpiration approximately decreases to 40 % only. Similarly at lower concentration of phenol the transpiration decreases to 80% approximately but for higher concentration of cyanide the transpiration approximately decreases to 38 % only.

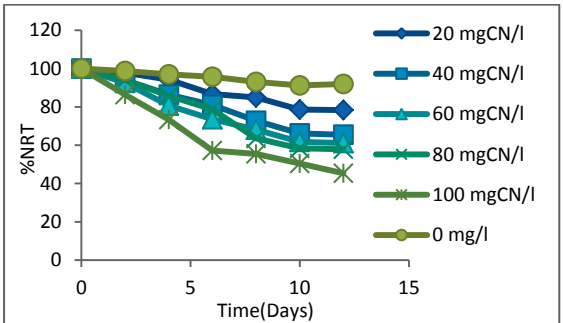

Fig.1Variation in %NRT with Time for the removal of Cyanide

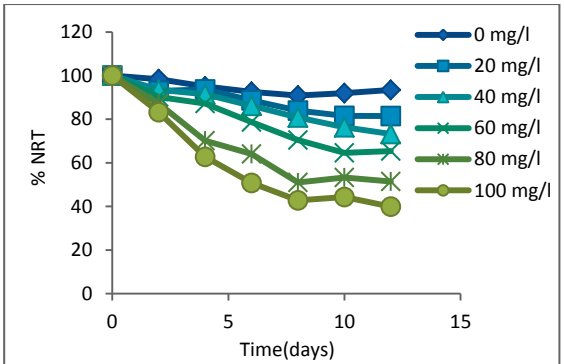

Fig.2 Variation in % NRT with time for the removal of Phenol

B. Removal with time

The initial concentration of cyanide and phenol has been taken as 0, 20, 40, 60 ,80 and 100 mg/l. Plant in concentration of 0 mg/l has been consider as control plant. Figure 3 and 4 shows the decrease in cyanide and phenol concentration with time. After 2 days, there is decrease in cyanide and phenol concentration but plants with higher concentration become green sick and died after 5 days. In the case of cyanide removal, 37% removal was obtained at higher concentration but for lower concentration, the plants became green sick after 11 days and died and showed 69% removal. Similarly, in the case of phenol removal, 31% removal was obtained at higher concentration but for lower concentration, the plants became green sick after 11 days and died and showed 62% removal.

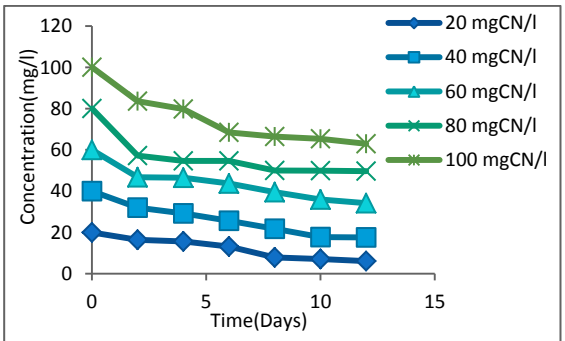

Fig.3 Variation in concentration of cyanide with time

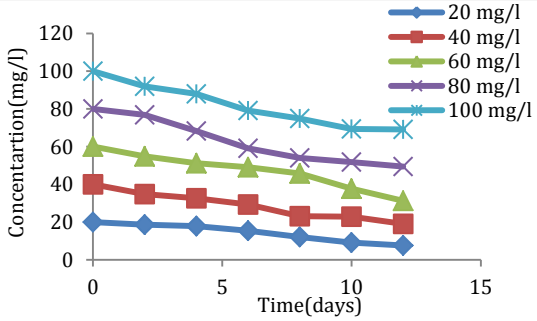

Fig.4 Variation in concentation of phenol with time

C. Biomass Growth

Figure 5 and 6 depicts the variation in percentage growth of biomass of *E.Crassipes* at different concentration of cyanide and phenol respectively. There are six plants in which the experiment has been conducted and the biomass of each plant is different for different cyanide and phenol concentration of 0, 20, 40, 60, 80,100 mg/l respectively. At higher concentration the biomass of the plants reduced slightly and after 4 days the plants get green sick and died. But at lower concentration the biomass of the plants reduced at lower rate and died after 11 days. The percentage growth in biomass of plants at higher concentration is greatly reduced but the percentage growth in biomass of plants at lower concentration is reduced but in lesser extent. But there is positive growth is obtained in biomass of plants for control plants. Due to the variation in the initial weight of the plants the percentage change in biomass cannot be increasing with the increasing concentration.

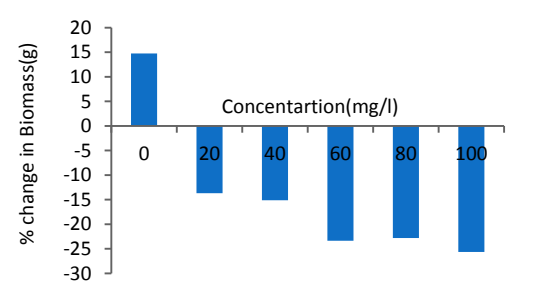

Fig.5 % change in biomass of E.Crassipes at different concentration of cyanide

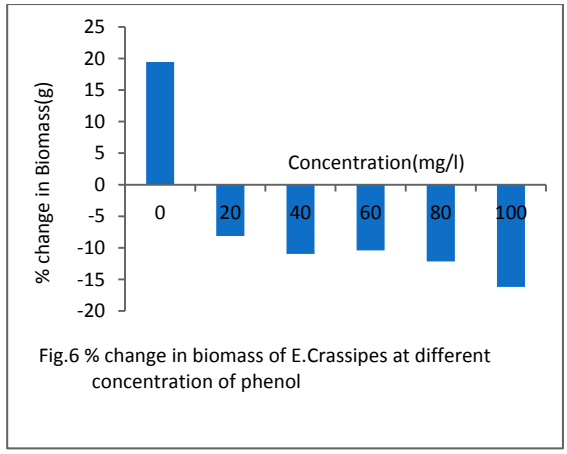

Fig.6 % change in biomass of E.Crassipes at different concentration of phenol

D. Water Use Efficiency

Figure 7 and 8 shows the variation in water use efficiency of *E.Crassipes* plants at different concentration. For control plants, there is positive change in water use efficiency but for the solution with cyanide concentration there is negative change in Water use efficiency of plants. This negative change is obtained because of the lesser survival of plants after 2-4 days. As the plants became green sick, the water use efficiency of plants decreases.

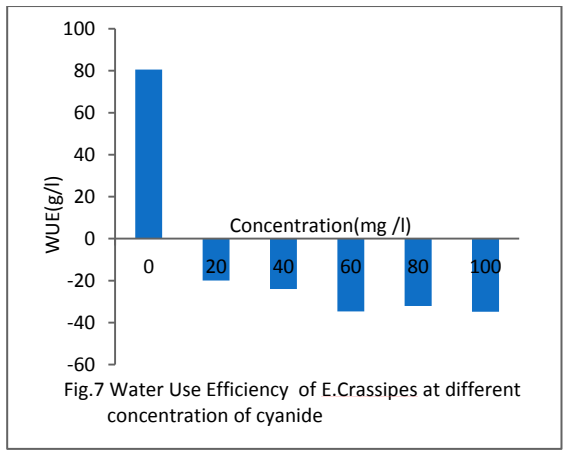

Fig.7 Water Use Efficiency of E.Crassipes at different concentration of cyanide

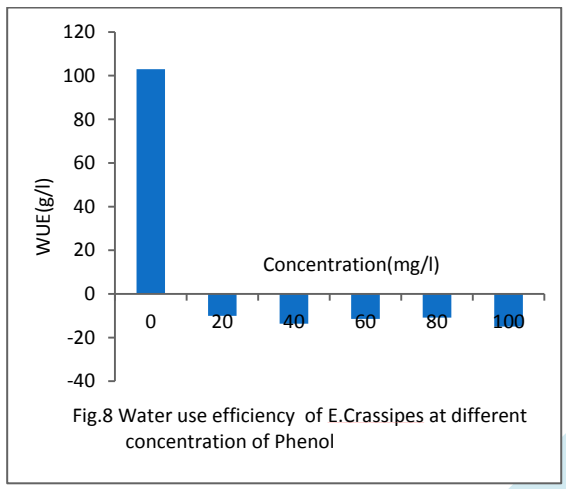

Fig.8 Water use efficiency of E.Crassipes at different concentration of Phenol

E. Metabolic Changes in *E.Crassipes*

The metabolic properties of *E.Crassipes* are changes with concentration. The properties were examined before and after the exposure of plants at different concentration of cyanide and phenol [20]. Carbohydrate content in plants reduces in greater rate i.e 52% for cyanide and the starch content in plants reduces in greater rate i.e 58 % for phenol. The greater reduction in chlorophyll and carbohydrate content has been observed in cyanide exposed plants then phenol and the greater reduction in protein and starch content have been observed in phenol exposed plants than cyanide.

I. Chlorophyll content in plants

Figure 9 and 10 shows the variation in chlorophyll content of *E.Crassipes* at different concentration in the case of cyanide and phenol removal respectively [20]. Due to the variation in the sickness of plants, the leaves of the plants became variably green sick. At last, there is increasing concentration of chlorophyll at high concentration of phenol.

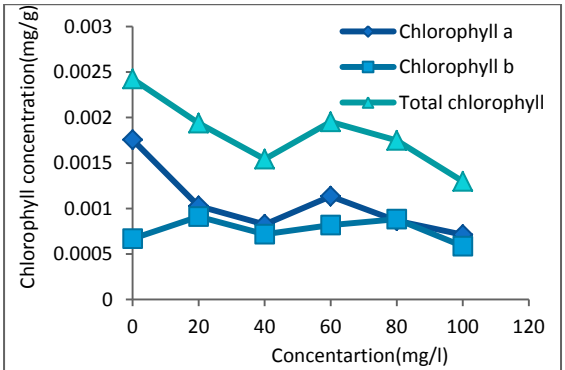

Fig.9 Chlorophyll concentration of plant at different concentration of Cyanide

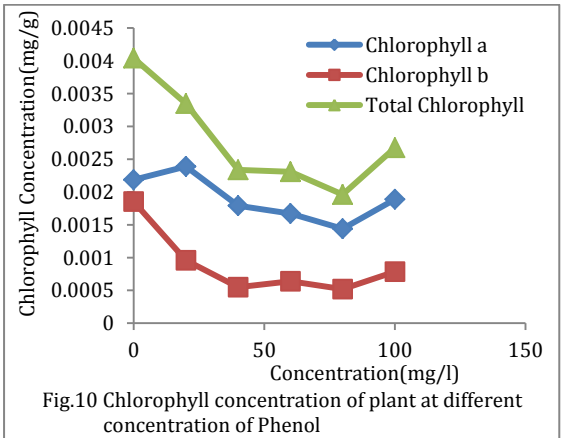

Fig.10 Chlorophyll concentration of plant at different concentration of Phenol

II. Protein content in plants

Figure 11 and 12 shows the variation in protein content of *E.Crassipes* at different concentration in the case of cyanide and phenol removal [12].

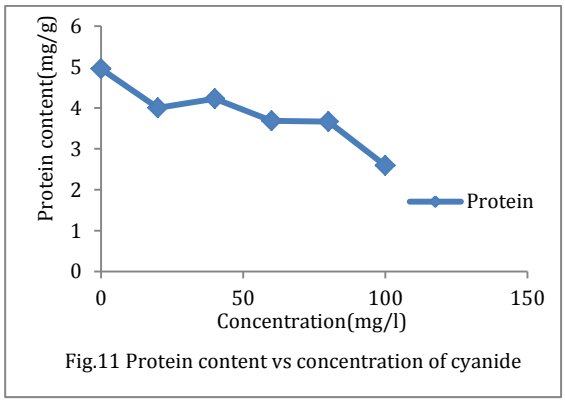

Fig.11 Protein content vs concentration of cyanide

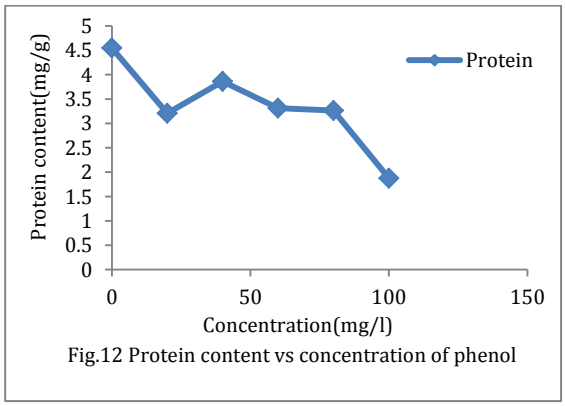

Fig.12 Protein content vs concentration of phenol

III. Carbohydrate content in plants

Figure 13 and 14 shows the variation in carbohydrate content of *E.Crassipes* at different concentration in the case of cyanide and phenol removal respectively[13].

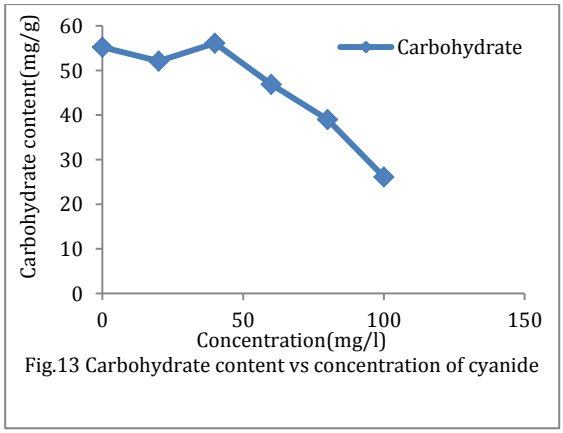

Fig.13 Carbohydrate content vs concentration of cyanide

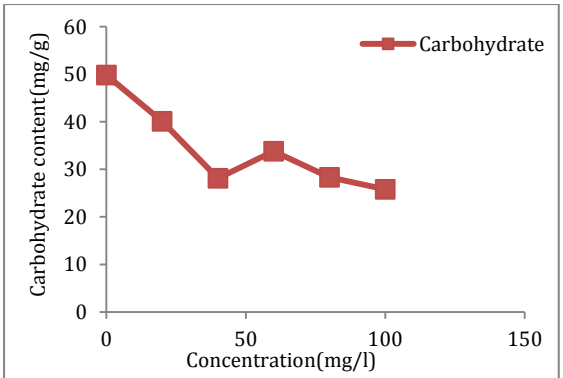

Fig.14 Carbohydrate content vs concentration of phenol

IV. Starch Content in plants

Figure 15 and 16 shows the variation in starch content of *E.Crassipes* at different concentration in the case of cyanide and phenol removal respectively [13].

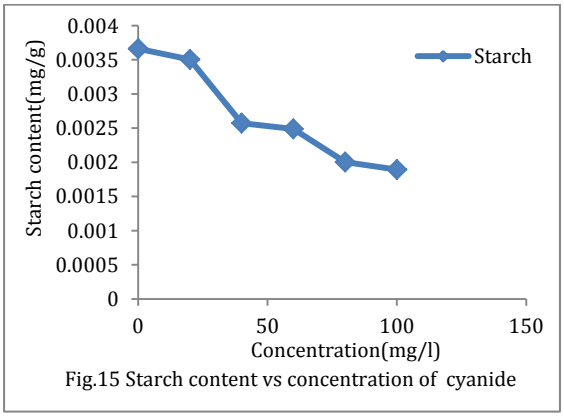

Fig.15 Starch content vs concentration of cyanide

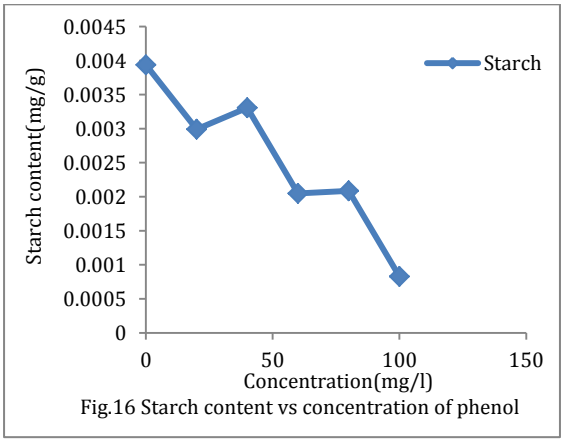

Fig.16 Starch content vs concentration of phenol

F. Effect of initial concentration

Figure 17 and 18 represents the variation in percentage removal of cyanide and phenol with increasing concentration of cyanide and phenol respectively. The initial concentration of cyanide and phenol were taken as 0,20,40,60,80 and 100 mg/l. It was observed that the percentage removal of cyanide and phenol was decreased by increasing initial concentration. The percentage removal of cyanide decreases from 69% to 37% and the percentage removal of phenol decreases from 62% to 31%. Further experiment can be done on the higher concentration of cyanide and phenol after 100 ppm.

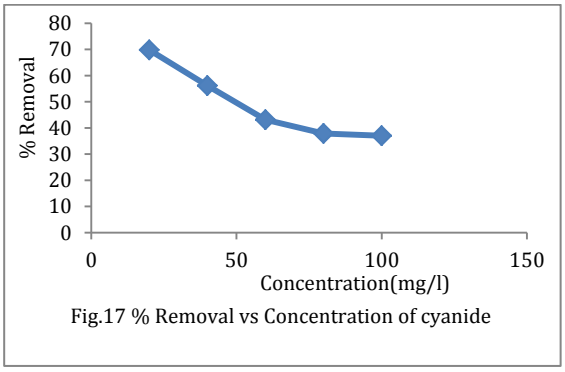

Fig.17 % Removal vs Concentration of cyanide

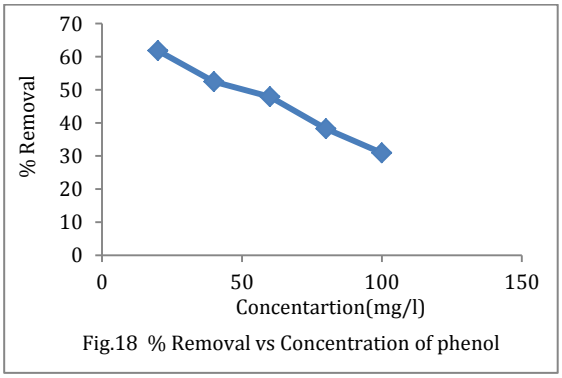

Fig.18 % Removal vs Concentration of phenol

G. Effect of pH

Figure 19 and 20 represents the variation in percentage removal of cyanide and phenol with increasing pH. The concentration of cyanide and phenol were taken as 100 mg/l at different pH of the solution which was varied from 3 to 11. It was observed that the percentage removal of cyanide and phenol was increased by increasing pH up to 7 but after it the percentage removal of cyanide and phenol was decreased. At pH 7, the maximum percentage removal of cyanide and phenol was observed i.e 37% and 33% respectively. As we know that cyanide and phenol are both easily soluble in aqueous solution at controlled pH.

It is well known fact that cyanide is a singularly charges anion which can form from the combination of one carbon and nitrogen atom by triple bond. Free cyanide and HCN is the most

toxic form. At pH 9.3-9.5, the free cyanide and HCN are in equilibrium, whereas at pH 11, most of the cyanide present in solution as free cyanide. But at pH 7, most of the cyanide present as HCN in the solution [29]. HCN is highly soluble in water. Phenol is also soluble in water due to the hydroxyl group present in phenol [30].

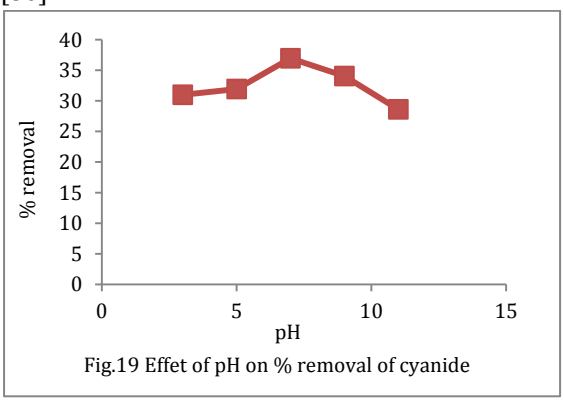

Fig.19 Effet of pH on % removal of cyanide

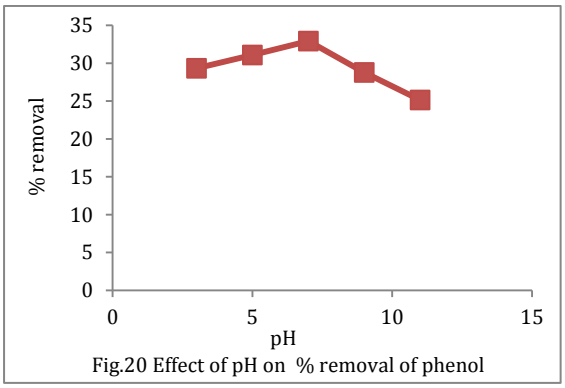

Fig.20 Effect of pH on % removal of phenol

H. ISOTHERM MODELLING

The Langmuir and temkin isotherm model are applied on the phytoremediation process to find out the best suited isotherm model for the equilibrium removal of cyanide and phenol from *E.Crassipes*. Lineraized form of these models which is given in table 1 and these models were applied on the experimental data.

The constants and correlation coefficient for Langmuir and Temkin isotherms was calculated and obtained results are tabulated in table 3.

Figure 21 and 22 shows the Langmuir isotherm for the removal of cyanide and phenol respectively. The maximum capacity i.e q_m was calculated by Langmuir isotherm and defined as the total capacity of plants for the removal of pollutants. The small value of Langmuir constant B described that the pollutants had high binding affinity for the plant. The adsorption model were used to identify the interaction of metal ions with the biomass of plant [9]. A dimensionless separation factor R_L is used for the Langmuir isotherm to depict the characteristics of biosorption [17].

$$R_L = \frac{1}{1+BC_i} \qquad (1.5)$$

Where B is the Langmuir constant and C_i is the initial concentration [8].

Table 2:-Value of R_L and types of Langmuir isotherm

R_L value	Type of Langmuir isotherm
=0	irreversible
=1	linear
>1	unfavourable
0<R_L<1	favourable

The value of R_L is obtained in this study is tabulated in table 2. In this phytoremediation experiment the value of R_L lies between 0 to 1, this describes that the Langmuir isotherm is favourable for the experimental data.

Figure 23 and 24 shows the Temkin isotherm for the removal of cyanide and phenol respectively. The constants of Langmuir and Temkin isotherms were calculated from the slope and intercept of the graph.

The experimental data of phytoremediation of cyanide and phenol fit in the both Langmuir and temkin for *E.Crassipes* in the following order Langmuir>Temkin. The Langmuir isotherm provides better specific uptake than Temkin isotherm for cyanide and phenol.

I. Langmuir Isotherm

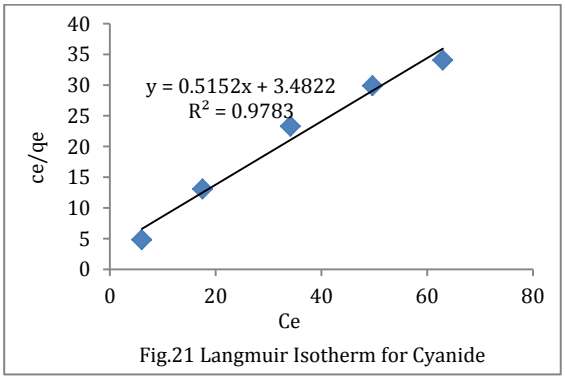

Fig.21 Langmuir Isotherm for Cyanide

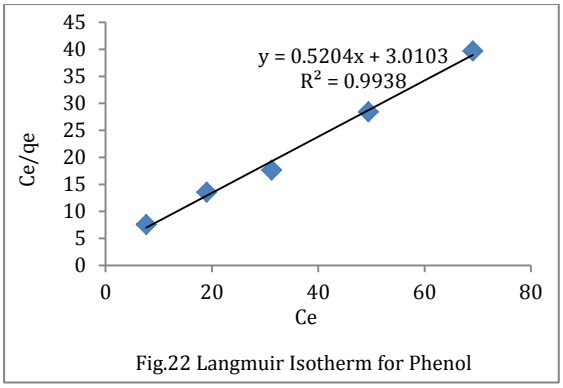

Fig.22 Langmuir Isotherm for Phenol

II. Temkin Isotherm

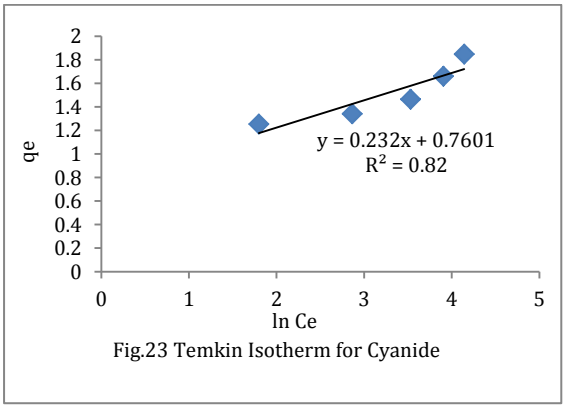

Fig.23 Temkin Isotherm for Cyanide

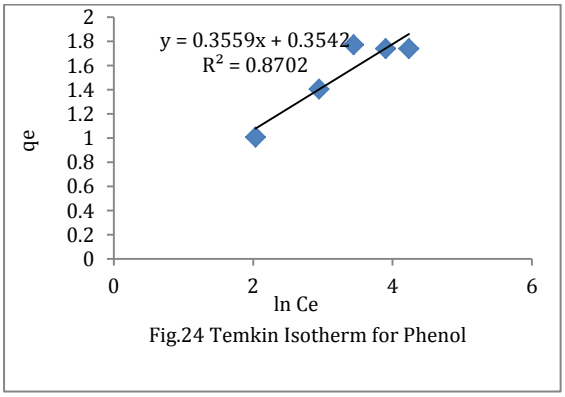

Fig.24 Temkin Isotherm for Phenol

III. Comparison of experimental data and different isotherms

Figure 25 and 26 explains the fluctuations generated between the experimental, Langmuir and Temkin data.

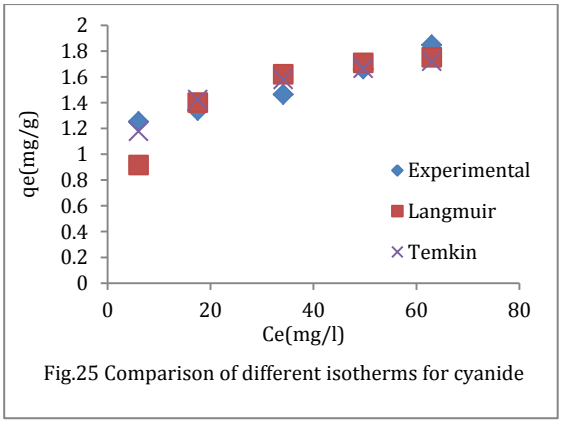

Fig.25 Comparison of different isotherms for cyanide

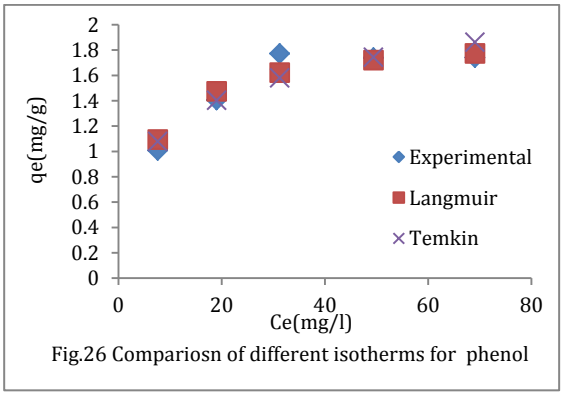

Fig.26 Compariosn of different isotherms for phenol

Table 3:- Constant and correlation coefficient of Langmuir and Temkin isotherm for phytoremediation of phenol and cyanide on *E.Crassipes*

Model	Parameters	Cyanide	Phenol
Langmuir Isotherm	R^2	0.9783	0.9938
	q_m	0.2871	0.3322
	B	0.1479	0.1728
	R_L	0.2525	0.2243
Temkin Isotherm	R^2	0.82	0.8702
	B	0.7601	0.3559
	A	1.356	2.70531

Conclusions

E.Crassipes showed a moderate rate of cyanide and phenol removal at higher concentrations for minimum time. These plants can be used as the biosorbent for the removal of cyanide and phenol from industrial wastewater. These plants has high biomass production with good root development at low expenditure, hence these plants are easily suitable for use in wastewater treatment process. The percentage removal of cyanide and phenol has been effected by different initial concentration and pH. The Langmuir and Temkin isotherms model constant were used to compare the removal capacity at different experimental data. The result proves that the Langmuir and Temkin isotherms were

well fitted in the experimental data. The toxicity of pollutants to plants at high concentration is increases and hence the metabolic properties of plants were decreases at high concentration of cyanide and phenol, These plants can be used as a remediator for those wastewater ponds where these are easily available and can survived in suitable environmental condition. The non-acceptable growth of *E.Crassipes* plants cannot be controlled and this causes a serious problem for the wastewater regions. But the availability and removal percentage through these plants can overcome the shortcomings and improve the other key points of other removal methods.

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At this moment of accomplishment, first of all I pay homage to my guide, Mr. C.B Majumder. This work would not have been possible without his guidance, support and encouragement. It is also a pleasure to mention my good friend, Mr. Shantam Rastogi who are always there when I really needed. Finally, and most importantly, I would like to thank my family for their faith in me and allowing me to be as ambitious as I wanted.

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