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EFFECT OF GLUCOSE AND LACTOSE ON UPTAKE OF PHENOL BY *LEMNA MINOR*

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ABSTRACT

Previous researches have demonstrated that presence of some carbohydrates in the culture medium will cause callus induction and frond regeneration in *Lemna minor*, and it is expected that presence of carbohydrates will affect the uptake of organics by this plant. However, limited studies have investigated the uptake of organics by this plant. The aim of this study was to clarify the effect of conventional carbohydrates such as glucose and lactose on uptake of phenol by *Lemna- minor*. Experiments were carried out in the presence and absence of glucose and lactose in the growth solution. The growth solution was fresh water collected from river and phenol was added to it. The initial concentrations of phenol were 20, 50 and 100 mg/L and density of *Lemna minor* (fresh weight) were 50, 100 and 150 g/m^2. The plants were contacted with growth solutions for 4 weeks. Control bottles (without plants) were examined with the same manner. The bottles were placed under white cool light with 12h photo periods at room temperature. Every four days sample was taken and the concentration of phenol was determined by spectrophotometer. Uptake of phenol by *lemna-minor* increased with increasing the contact time and decreased by increasing phenol concentration. In the presence of glucose and lactose in the growth solutions, uptake of phenol was decreased. Phenol uptake was dependent to plant density and when *Lemna minor* completely covered the surface of the water, uptake decreased.

In the presence of glucose and lactose, uptake of phenol by *Lemna minor* was decreased. Uptake of phenol by lemna minor was affected by density of plant.

Key words: Phenol, Lemna-minor, Glucose, Lactose, Organics uptake

INTRODUCTION

The use of aquatic and wetland plants, such as duckweed, to uptake pollutants in wastewater treatment is considered by researchers (Wang *et al.*, 2002). Some pollutants such as heavy metals may be toxic for duckweed (Khellaf and Zerdaoui, 2009). But some organic pollutants such as phenol has less toxic effect on Lemna-minor; EC50 of phenol for *lemna minor* is reported 50 mg/L (Barber, 1995). Phenolic compounds, because of their domestic, agricultural and industrial uses, are commonly found in surface waters. Lemna minor is a small floating plant that growths in surface waters. This plant contains flat, 2-5 mm, nearly symmetrical, green fronds (leaves). Fronds may occur singly, or grouped into colonies of 2-4 fronds. Lemna minor is indigenous to many freshwater habitats throughout the world. The primary mode of reproduction is asexual budding (Caux *et al.*, 1988).

Lemna gibba was used to study the toxicity and metabolism of phenol. The toxicities of phenol were described in terms of the effect of increasing concentrations on the vegetative reproduction of duckweed over a 7-day growth period. The

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respective EC_{10} and EC_{50}'s were 0.08 & 0.54 mM for phenol (Barber, 1995).

Glucose, lactose and other utilizable sugars inhibit flowering of Lemna minor. Glucose supplementation of the growth medium increased the invitro activity of glucose-6-P dehydrogenase in Lemna minor. The reasons of the flowering inhibition discussed in few papers (Posner, 1971). The addition of 0.5% (w/v) glucose to the culture medium resulted in an increase of APR levels in plant roots (mRNA , protein and activity) comparable to those of plants kept under normal light conditions. Treatment of roots with D-Sorbitol or D-mannitol did not increase APR activity (Hesse *et al.*, 2003).

Basal media, plant growth regulator type and concentration, sucrose and light were examined for their effects on duckweed (lemna gibba) frond proliferation, callus induction and growth and frond regeneration. This study showed that three percent sucrose was best for callus induction and growth (Moon and Stomp, 1997). The callus of Lemna minor (duckweed) is capable of heterotrophic assimilation of galactose and beta-lactose, whereas the intact plant is not. Sucrose-grown callus adapted rapidly, uniformly, and reversibly to utilization of galactose or lactose in heterotrophic growth. Single-nutrient deficiencies of callus growth medium were corrected fully by milk whey; the lactose of milk whey was utilized by Lemna and callus grows well at pH 4.0-6.5 and tolerates 0.05% (5.5 mM) lactic acid at pH 5.0 with slowed growth (Frick and Morley , 1995).

Lemna gibba (duckweed) was used as a bioassay organism to test the allelochemical effects of Salicylic Acid (SA), Ferulic Acid (FA). Growth rates (K), Dry Weight (DW), and total Chlorophyll (CHL) production were measured after seven days of growth. The bioassay procedure used 50 mL of E medium with and with out Sucrose in 125 mL Erlen-meyer flasks plus the selected concentration of allelochemical. At concentration of 50 Micro molar and greater, SA caused inhibition of K and DW production in Lemna gibba. FA inhibited the DW and CHL production at 100 Micro molar when the compound was in Emedium containing Sucrose (Ramirez toro *et al.* ,1988). Addition of some compounds to growth media may enhance the organic uptake of Lemna minor, for example, addition of small volumes of acetone to media (%1 Acetone) was observed to enhance contaminant uptake rate. These results demonstrated that contaminant uptake was significantly faster in acetone amended systems than in no-solvent amended systems (Sanz and Ullrich,1989).

There is lack of research regarding whether presence of carbohydrate (Glucose and Lactose) can affect the uptake of organics by plants or not.

The aim of this study was to clearing the uptake of phenol by *Lemna-minor* in the presence and absence of carbohydrates (glucose and lactose).

MATERIALS AND METHODS

Duckweed (*Lemna minor*) was collected from waterways near Mazandaran Uinv. Med. Sci., Sari- Iran and maintained in 10-L tanks under fluorescent lighting in the laboratory and fed with Hoagland's media according to Standard Methods. All plants used for examination of phenol uptake were taken from a singular, uniform plant stock culture at the same point in time. Therefore plant metabolic processes were considered to be uniform. Growth solution was taken from rivers that can support duckweed growth. Predetermined Fresh Weight (FW) of selected plants were contacted with growth solutions in the bottles. Phenol in different concentrations was added to all bottles.

The experiments were conducted in two modes: in the presence and absence of glucose and lactose. Control bottles containing growth solution with glucose, lactose and phenol but without plants were prepared to quantify the lost of phenol in bottles.

Initial concentrations of phenol in growth solution in the bottles were 20, 50 and 100 mg/L. All glucose and lactose solutions were being prepared freshly on first day of each batch. Concentration of glucose and lactose in the growth solutions were 100mg/L. Fresh weights of *Lemna minor* were added to bottles containing growth solution, phenol, glucose or lactose. Fresh weights of *Lemna minor* added to bottles separately were 0.1, 0.2 and 0.3 grams. All bottles were continuously illuminated

under cool white light, in the laboratory room temperature with 12h photo periods. Every four days, samples were taken from bottles by syringe and filtrate with 0.45 micron membrane filter for both sample and control bottles. The concentration of residual phenol in samples was determined spectrophotometrically. The absorbance of the colored complex of phenol with 4-aminoantipyrine was read at 510nm. Fig. 1 shows the experimental setup.

RESULTS

Uptake of Phenol with concentration of 20 mg/L by Lemna minor in different densities including 50, 100 and 150 g FW/ m^2 in growth media is shown in Fig. 2. This figure shows that in the presence of 100 ppm glucose, uptake of phenol by lemna minor has been reduced for all plant densities. The maximum phenol uptake by lemna in the presence of glucose after 24 days exposure time was reached to 66%.

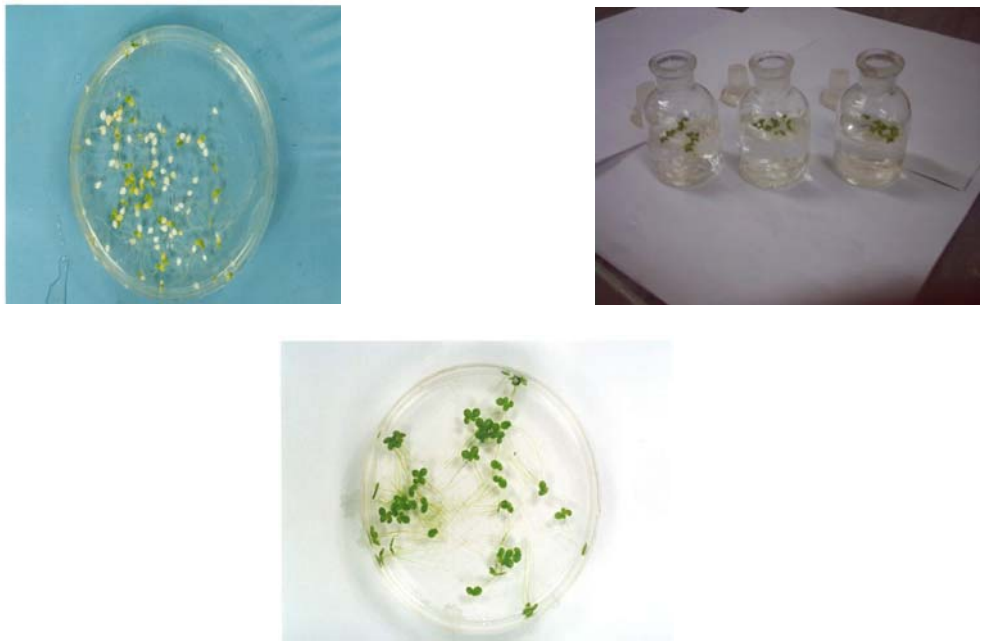

Fig.1: Experimental setup used in this study

As the figure shows, phenol removal was increased with increasing the plant density, but there is not very difference between plant density of 100 and 150 g/m^2. Uptake of phenol by *Lemna minor* has been completed after 24 days.

Fig. 3 shows phenol removal by Lemna minor in different densities in the presence of 100ppm Lactose. The uptake of phenol is affected in very little amount. As the graph shows, in two modes both containing and lacking Lactose, phenol uptake by lemna is nearly equal. Also the graph shows that effect of Lactose on reduction of phenol uptake by Lemna minor is very smaller than effect of Glucose. By increasing the time of contact and the amount of lemna phenol uptake was increased.

With increasing phenol concentration, the removal efficiency is decreased (Fig.4). Presence of glucose reduced phenol uptake by *Lemna minor* just like as discussed for 20 mg/L phenol. Also it can be seen from Fig.4 that for plant density of 100 and 150 grams per square meter, phenol uptake was similar.

Figure 5 shows uptake of 100 ppm phenol by lemna minor in different densities in the presence and absence of lactose. As it can be seen from the figure, phenol uptakes were decreased in all bottles. Uptakes of phenol in the presence of lactose were dramatically decreased. There was not any phenol lost in the control bottles during the period of experiments.

DISCUSSION

Degradation of organic material is enhanced by Lemna minor through both additional oxygen supply and additional surface for bacterial growth. A dense cover of Lemna on the water surface was suggested to inhibit both oxygen entering the water by diffusion from the air and photosynthetic production of oxygen by phytoplankton because of the poor light penetration (Cowgill, 1991). Moreover there is a difference in the way oxygen is diffused into water by Lemna, where the Lemna roots and lower frond surfaces provide oxygen at a "microsite level", in this situation oxidation of functional groups in organic compounds is more complete (Day and Saunders, 2004). Plant Density (PD) (g FW plant / m^2 media surface) is an important parameter. At high densities, Lemna minor covers fully the surface of the water and causes a screen effect, such that diffusion of atmospheric oxygen into the medium is limited. Therefore the density of Lemna on the surface of water can affect on removal rate of organics (Wang and Williams, 1990). The effect of mat density on duckweed (*Lemna minor*) growth was studied under controlled conditions. The results revealed a maximal biomass growth rate of 88 g/ m^2 at an optimal initial mat density of 45 g /m^2 (Frédéric *et al*., 2006). The reduction in phenol uptake by *lemna minor* in the presence of glucose may be due to inhibitory effect of glucose on flowering of *lemna minor* (Posner, 1971) or it can be attributed to conjugation reaction between glucose and phenol in the solution.

The reason for equal phenol uptake in two modes may be due to metabolism of Lactose by Lemna minor. Some research shows Lamna minor can grow on Lactose medium and breakdown lactose heterotrophically (Frick and Morley , 1995). The reason for reduction in phenol uptake by increasing concentration is toxic effect of high concentration of phenol on Lemna.

Phenol uptake by PD= 100 were more than PD=150. The reason for this phenomenon may be full coverage of liquid surface by *lemna minor* in PD=150, in this case because of insufficient surface needed for reproduction of *Lemna minor*, growth is minimized. Also in the case of full coverage of Lemna, oxygen penetration from surface into bulk of water is minimized (Cowgill, 1991).

In the case of high concentration of phenol (100 mg/L), due to toxic effect of phenol on plant, uptake of phenol was low, reproduction of lemna was stopped. In the presence of lactose phenol

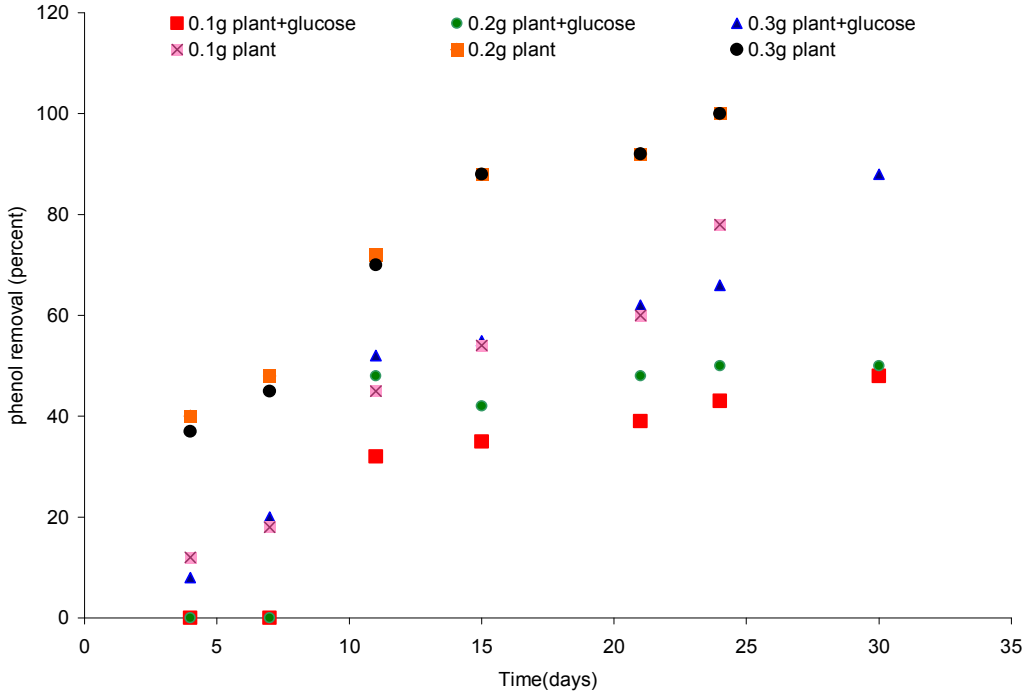

Fig. 2: Uptake of phenol (20mg/L) by different amount of Lemna minor in the presence and absence of glucose

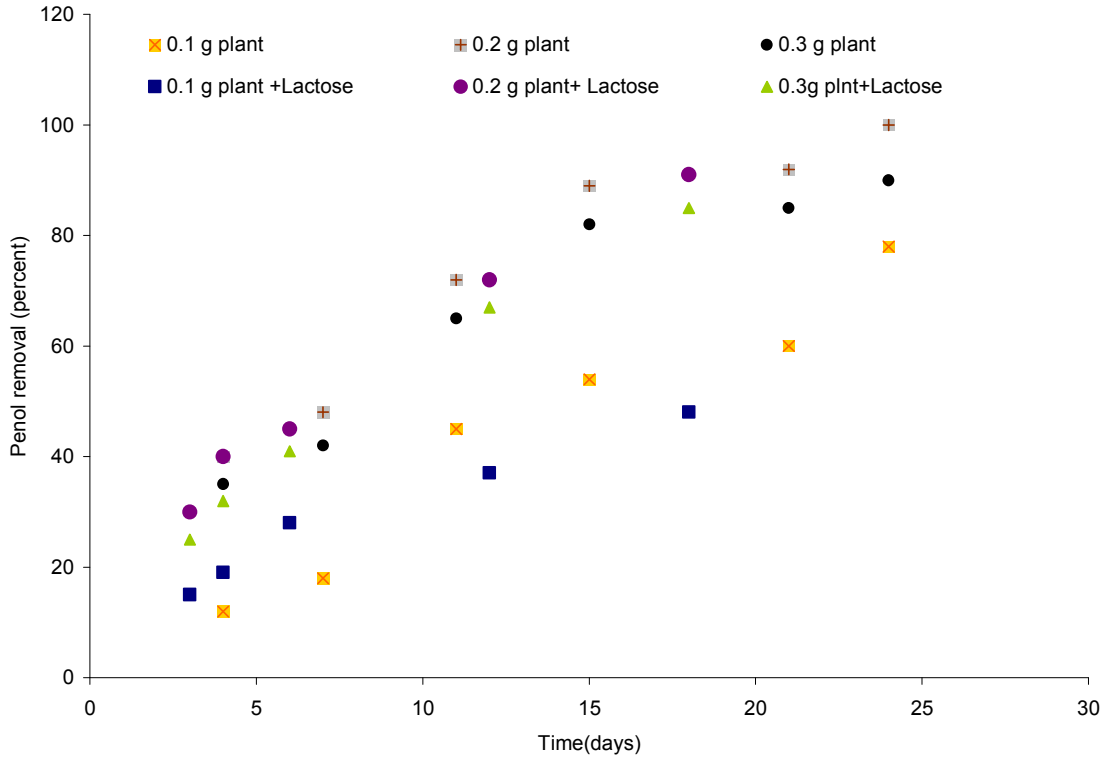

Fig. 3: Uptake of phenol (20mg/L) by different amounts of Lemna minor in the presence and absence of lactose

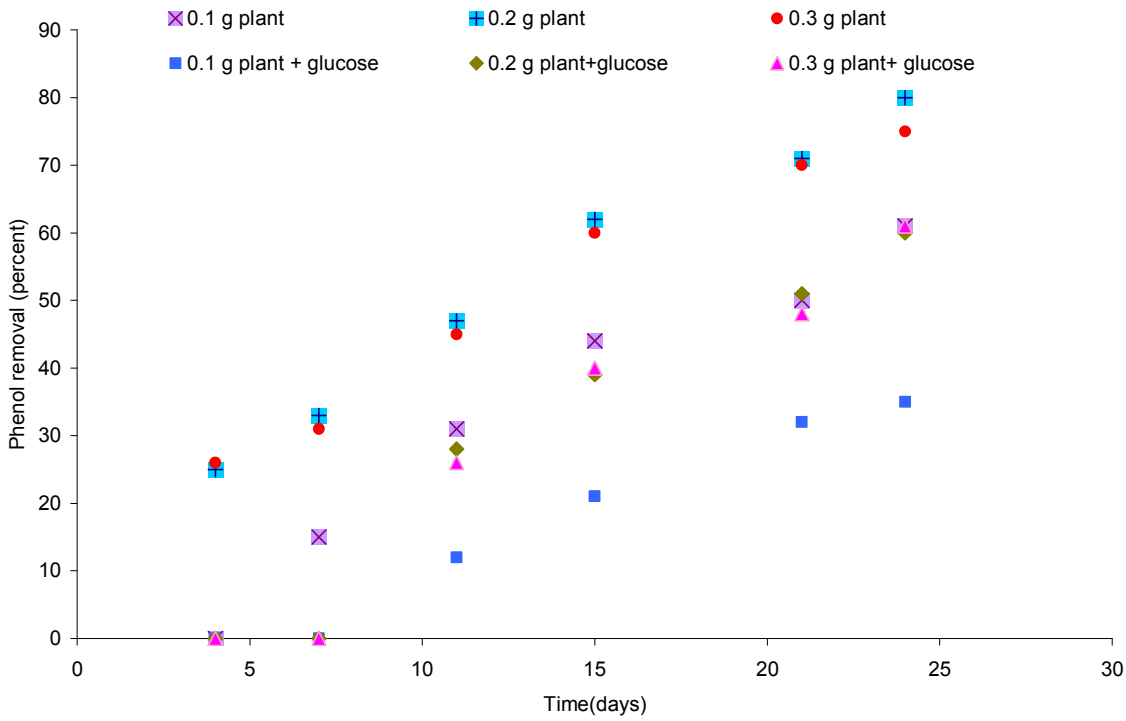

Fig. 4: Uptake of phenol (50mg/L) by different amounts of Lemna minor in the presence and absence of glucose

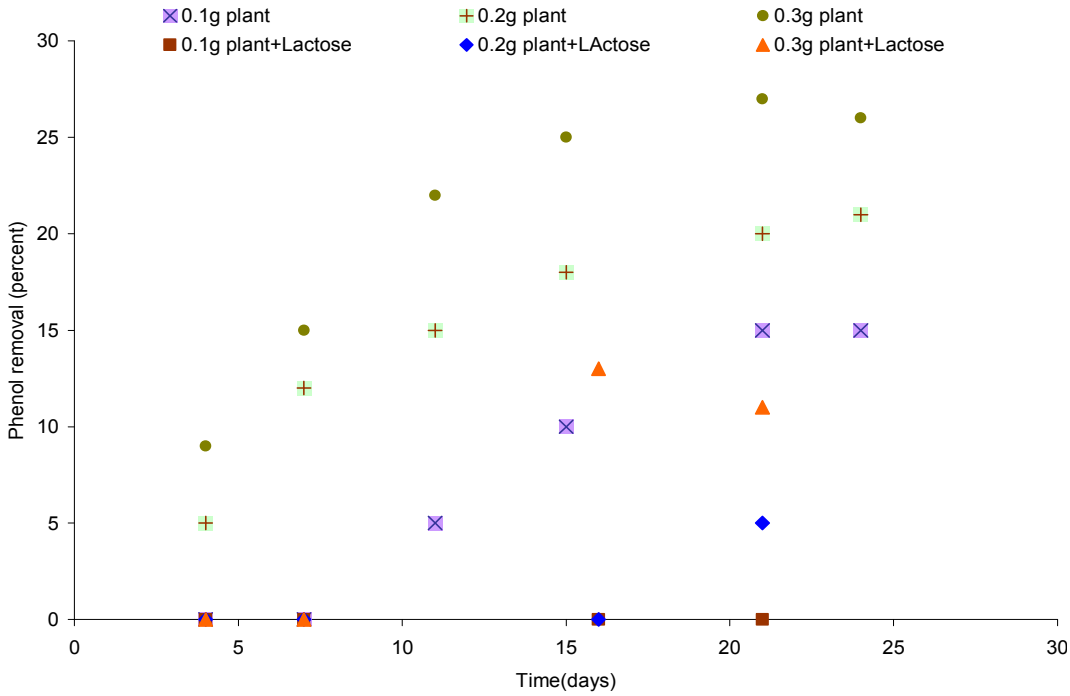

Fig.5: Uptake of phenol (100mg/L) by different amounts of Lemna minor in the presence and absence of lactose.

uptake more reduced and it can be seen that phenol removal is negligible (Fig.5).

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