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KINETICS OF SOLVENT EXTRACTION OF ZIRCONIUM (IV) FROM CHLORIDE MEDIUM BY D2EPHA IN KEROSENE USING THE LEWIS CELL TECHNIQUE: A COMPARISON WITH SINGLE DROP TECHNIQUE

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The kinetics of the title system has been investigated by the Lewis cell technique for 1-day ageing of the aqueous phase and compared with those obtained from the single drop technique. The mass transfer flux equations for Zr(IV) have been derived for three aqueous acidities of 0.10, 1 and 5 mol dm^{-3} HCl, respectively, as: J (kmol $m^{-2}s^{-1}$ = $10^{-5.53\pm0.04}$ $(1+0.00038[Zr(IV)]^{-1})^{-1}$ $[H^+][H_2A_2]_{(o)}$ $(1+0.70[Cl^-])$, J (kmol $m^{-2}s^{-1}$) = $10^{-5.80\pm0.02}$ $1+0.004$ $[Zr(IV)]^{-1})^{-1}$ $[H_2A_2]_{(o)}$ $[Cl^-]$ and J (kmol m^{-2} s^{-1}) = $10^{-6.58\pm0.03}$ $(1+0.0038$ $[Zr(IV)]^{-1})^{-1}[H]^+[H_2A_2]_{(o)}[Cl^-]$. The values of E_a (kJ mol^{-1}) in kinetic and diffusion regimes are 92 & 14.7 and 84 & 15 for 1 and 5 mol dm^{-3} HCl systems, respectively. For 0.10 mol dm^{-3} HCl system, E_a value cannot be measured for kinetic regime but its value is 15 kJ mol^{-1} in diffusion regime. At intermediate controlled regime (Zr(IV) ≈ 3 mmol dm^{-3}), E_a value varies from 11, 12 and 12 kJ mol^{-1} to 42, 105 and 108 kJ mol^{-1} respectively, for 0.10, 1 and 5 mol dm^{-3} HCl systems on varying the temperature from 318 K to 288 K.On the basis of these data, the mechanisms of extraction in different conditions have been suggested.

Key words: Extraction kinetics, Zr(IV), D2EHPA, Lewis cell, Kerosene HCl interface.

Introduction

The extraction equilibria of the Zr(IV)-Cl- D2EHPA-kerosene system has been reported (Biswas and Hayat 2002a). Extraction equilibria have been found to be complicated by the slow change in the composition of the extractable aqueous Zr(IV) species on ageing and variation of the aqueous phase acidity. It is found that the equilibrium data for 1-day ageing can be justified well if the existences of $[Zr_8(OH)_{20}(H_2O)_{24}Cl_{12}]$ in 0.10 mol dm^{-3} HCl medium (Singhal *et al* 1996), $Zr_4(OH)_8(H_2O)_{16}Cl_6]^{2+}$ in 1 mol dm^{-3} HCl medium (Singhal *et al* 1996) and $Zr(H_2O)_5Cl_3]^+$ in 5 mol dm^{-3} HCl medium (Hannane *et al* 1990) are considered to take part in extraction equilibration reactions. On ageing for 30 days, the above species take up 1, 2 and 3 chloride ions, respectively. For 1-day ageing, the extraction equilibrium constants have been estimated to be $10^{-5.0}$, $10^{-4.1}$ and $10^{-3.4}$ for 0.10, 1 and 5 mol dm^{-3} HCl systems, respectively.

In another paper (Biswas and Hayat 2002b), the kinetics of the titled system of 1-day ageing has been measured by the single drop technique. The rate constants have been measured to be $10^{-5.37}$, $10^{-5.77}$ and $10^{-6.62}$ for 0.1, 1 and 5 mol dm^{-3} HCl systems, respectively. According to Danesi and Chiarizia (1980), a change in the experimental technique and concentration condition may alter a kinetic regime to a diffusional regime or mixed control or vice versa. Now, it is established that not only the concentration term, but also the temperature condition alters the mechanism of extraction (Hughes and Biswas 1991 and 1993; Biswas and Begum 2000; Biswas and Mondal 2003). This paper discusses the kinetics of the Zr(IV) extraction from Cl^- medium of 1-day ageing by D2EHPA in kerosene by the lewis cell technique to compare these results with those obtained from the single drop technique.

Experimental

Reagents. D2EHPA was procured from BDH (98% purity) and used as such. As a source of Zr(IV), octahydrated zirconyl chloride (M.C and Bell, 98%) was used. Kerosene was bought from the local market and redistilled to collect the fraction distilling over 220-260°C. It was mostly colorless and aliphatic in nature. All other chemicals were of reagent grade and used without further purification.

*Analytical.*The concentration of Zr(IV) in the aqueous phase was estimated by the EDTA-pyrocatechol violet method (Charlot 1964) at 590 nm using a WPA S104 spectrophotometer. For pH adjustment of the aqueous solution of Zr(IV) required in the above method (pH5.2), a Mettler Toledo 320 pH meter was used.

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*Cell and technique.*The construction of Lewis cell and operating technique are given elsewhere (Biswas *et al* 1998). Identical aliquots (100 cm^{-3}) of aqueous and organic solutions (pre-thermostated) can be taken in the cell without much disturbing the interface and allowed for mass transfer for a definite time. An electrical stirrer having two 1 cm blades stirs the organic phase clockwise and a magnetic stirrer (2 cm long capsule) is used to rotate the lower aqueous phase in the same direction. The speed of stirrer is controlled at 2.5 Hz maximum without disturbing the interface. After 2-6 min, 2 cm^3 aqueous phase is taken out for analysis. The amount of Zr(IV) transferred into the organic phase can be estimated from the analysis and varies within ± 1%. All experiments have been carried out at (30 ± 1)°C, otherwise stated. The interfacial area in all experiments is kept at 3.37 x 10^{-3} m^2, otherwise mentioned, which can be altered by setting circular plastic rings within the cell where interface is formed.

*Preparation of the aqueous solution.*The aqueous solution containing definite amounts of Zr(IV), Cl^- and H^+ are prepared and aged exactly for 24 h before being used in the cell for extraction rate study.

Notations, abbreviations and subscripts.

a,b,c,d, = orders with respect to Zr(IV), H^+, H_2A_2 and $C1^-$ concentrations, respectively

$^{a}a_{Zr(IV)}$ = amount of Zr(IV) transferred in 100 cm^3 organic phase through interface A in time t, kmol

A = interfacial area through which mass transfer occurs, m^2

A = frequency factor

D = diffusion coefficient, m^2s^{-1}

D2EHPA = di-2-ethylhexylphosphoric acid

E_a = activation energy, kJ mol^{-1}

h = higher temperature or concentration region

H_2A_2 = dimeric D2EHPA

J = mass transfer flux, kmol $m^{-2}s^{-1}$

k_f = forward extraction rate constant, unit depends on the concentration region Zr(IV) and $C1^-$

K_{Zr} = proportionality constant of Zr(IV) concentration term in rate equation, mol dm^{-3}

K_{cl} = proportionality constant of Cl^- concentration term in rate equation, dm^3 mol^{-1}

l = lower temperature or concentration region

r = radius of diffusing species

t = time allowed for mass transfer through interface, S

T = temperature, K

x = concentration change of Zr(IV) in the aqueous phase, mg dm^{-3}

η = coefficient of viscocity, cp

(ini) = initial

(o) = organic phase

*Treatment of experimental data.*If *x* (mg dm^{-3}) is the concentration change of Zr(IV) in the aqueous phase, then (*x* /10) mg of Zr(IV) will be transferred into the organic phase provided 100 cm^3 each phase being used in the cell. If t (s) represents the time during which mass transfer occurs, the mass transfer flux of Zr(IV) can be calculated from the following relationship:

$$J(\text{kmol m}^{-2}\text{s}^{-1}) = (10^{-7}\, x) / (91.22 \times 3.368 \times t) \quad \ldots\ldots (1)$$

In a single-run, the *x* vs t plots generally hold almost straight line relationship during the initial periods of extraction. After the lapse of some period, the rate of variation of *x* with t is decreased. Since for small t-values, the values fo *x* are very small, the calculated J-values are not found to be reproducible. So J-values have been calculated for Δ t = (4-0) x 60s, (8-4) x 60s, (12-8) x 60s, (16-12) x 60s, and (20-16) x 60s and the averages of J-values have always been taken.

At a constant temperature and phase agitaion, J is related to concentration terms as:

$$J = k_f [Zr(IV)]^a [H^+]^b [H_2A_2]^c{}_{(o)} [Cl^-]^d \quad \ldots\ldots (2)$$

Which can be rewritten as:

$$\log J = \log k_f + a \log[Zr(IV)] + b \log[H^+] + c \log[H_2A_2]_{(o)} + d \log [Cl^-] \quad \ldots\ldots (3)$$

Equation (3) can be used to evaluate the reaction orders (a, b, c and d) and rate constant (k_f). If the concentrations of the variables are kept constant, the slop of logJ vs log[4^{th} variable] will give the reaction order with respect to the fourth variable concentration. From the intercept of the plots, the value of k_f can be evaluated. The temperature dependence data can be treated by the Arrhenius theory.

Results and Discussion

Fig 1 shows the variation of the amount of mass transfer, $a^o{}_{Zr(IV)}$ and flux on the interfacial area used in the Lewis cell. It is found that the mass transfer amount is directly proportional to interfacial area, but the flux is independent of interfacial area. In subsequent experiments, the cell with interfacial area of 3.37 x 10^{-3} m^2 has been used.

The variation of mass flux of Zr(IV) on its concentration for 1 mol dm^{-3} HCl system ([Cl^-] = 3 mol dm^{-3}) is shown in Fig 2. The experimental points do not fall on a straight line. The solid line is theoretical and represented by:

$$\log J = -6.85 - \log (1+K_{Zr}[Zr(IV)]^{-1}) \quad \text{..... (4)}$$

Whereas, the dashed with doted line (asymptote at higher concentration region of Zr(IV) is:

$$\log J = \log k_f[H^+]^b [H_2A_2]^c_{(o)} [Cl^-]^d = -6.86 \quad \text{..... (5)}$$

and the dashed line (asymptote at lower concentration region of Zr(IV)is:

$$\log J = -6.85 - \log K_{Zr} + \log [Zr(IV)] \quad \text{..... (6)}$$

At the point of intersection of Eqs. (5) and (6), $-\log K_{Zr} + \log [Zr(IV)] = 0$, which gives K_{Zr} as 0.004.

Similar, plots for 0.10 and 5 mol dm^{-3} systems are given in Fig 3. For 0.10 mol dm^{-3} HCl system, the solid line is represented by:

$$\log J = -7.71 - \log (1 + 0.00038[Zr(IV)]^{-1}) \quad \text{..... (7)}$$

and for 5 mol dm^{-3} HCl system, the solid line represents:

$$\log J = -6.9 - \log (1 + 0.0038[Zr(IV)]^{-1}) \quad \text{..... (8)}$$

The dependences of mass transfer flux of Zr(IV) on the aqueous phase acidity at constant chloride concentration of either 3 (for [HCl]< 3 mol dm^{-3}) or 5 mol dm^{-3} (for [HCl] = 2-5 mol dm^{-3}) are shown in Fig 4. Similar results are obtained for all extractant concentrations used. Within 0.30 to 3 mol dm^{-3} HCl region ($[Cl^-]$ = 3 mol dm^{-3}), the flux is independent of H^+ concentration. But the flux is directly proportional to hydrogen ion concentration in its higher and lower concentration regions.

The variation of flux with the variation of extractant concentration is displayed in Fig 5. In all cases, straight lines of unity slopes are obtained. So, the rate of extraction is directly proportional to extractant concentration in the organic phase. The results of the chloride dependence study are given in Fig 6. For H^+ concentrations of 1 and 5 mol dm^{-3}, the rate of extraction is proportional to chloride ion concentration in the aqueous phase. For 0.10 mol dm^{-3} H^+ concentration, the flux varies according to the following relationship:

$$\log J = \log k_f (1 + 0.00038[Zr(IV)]^{-1})^{-1}[H^+][H_2A_2]_{(o)} + \log (1 + K_{cl}[Cl^-]) \quad \text{..... (9)}$$

Where, K_{cl} is estimated to be 0.70 by the curve-fitting method. From the intercepts of the straight lines, asymptotes or tangents in Fig 2-6, the values of rate constant (k_f) at 303 K have been evaluated (Table1). The average values of $\log k_f$ are $10^{-5.53\pm0.04}$, $10^{-5.80\pm0.02}$ and $10^{-6.58\pm0.03}$ for 0.10, 1 and 5 mol dm^{-3} HCl systems, respectively. Units of k_f depend on the concentration regions of H^+, Zr(IV) and Cl^-.

Fig 7, shows the temperature dependence of flux in the intermediate control regime of Zr(IV) concentration. In all acidities of the aqueous phase, Arrhenius plots are not straight lines. In lower temperature region under investigations, the E_a values are over 100 kJ mol^{-1} for 1 and 5 mol dm^{-3} HCl systems, whereas, it is about 42 kJ mol^{-1} for 0.10 mol dm^{-3} HCl system. These values in the higher temperature region of investigation are 12, 11 and 11 kJ mol^{-1}, respectively. Fig 8 shows the Arrhenius plots in the kinetic and diffusion regions of Zr(IV) concentration. At all aqueous acidities, E_a values are about 14.5 kJ mol^{-1} in diffusion regime and over 80 kJ mol^{-1} in the kinetic regime.

From the above experimental results, the mass transfer flux of Zr(IV) for 5, 1 and 0.10 mol dm^{-3} HCl systems derived from the Lewis cell technique at 303 K, respectively, are as follow:

$$J = 10^{-6.58\pm0.03}(1 + 0.0038[Zr(IV)]^{-1})^{-1}[H^+][H_2A_2]_{(o)}[Cl^-] \quad \text{.....(10)}$$

$$J = 10^{-5.80\pm0.02}(1 + 0.004[Zr(IV)]^{-1})^{-1}[H_2A_2]_{(o)}[Cl^-] \quad \text{..... (11)}$$

and

$$J = 10^{-5.53\pm0.04}(1+0.00038[Zr(IV)]^{-1})^{-1}[H^+][H_2A_2]_{(o)}(1+0.7[Cl^-]) \quad \text{.....(12)}$$

In comparison to followings, respectively, derived from the single drop technique (Biswas and Hayat 2002a):

$$J = 10^{-6.62\pm0.13}(1+ 0.0056[Zr(IV)]^{-1})^{-1}[H^+][H_2A_2]_{(o)}[Cl^-] \quad \text{.....(13)}$$

$$J = 10^{-5.77\pm0.14}(1+ 0.0056[Zr(IV)]^{-1})^{-1}[H_2A_2]_{(o)}[Cl^-] \quad \text{.....(14)}$$

and

$$J = 10^{-5.37\pm0.10}(1+ 0.00036[Zr(IV)]^{-1})^{-1}[H_2A_2]_{(o)}(1+0.69[Cl^-]) \quad \text{....(15)}$$

It is therefore, seen that the rate measurements by the single drop technique and the Lewis cell operated at 2.5 Hz yield almost similar flux equations, with only appreciable change in the value of K_{Zr} (0.0056 from single drop technique and ~0.004 from Lewis cell). The valus of the rate constants are almost unchanged within the vairance limit. So, it is concluded that the single drop (falling) technique and the Lewis cell operated at 2.5 Hz possess comparable hydrodynamic condition to yield similar flux equations. The values of E_a measured from both techniques mentioned are also comparable. Hence the mechanism of extraction in different aqueous acidity regions given from the results obtained by the single falling drop technique (Biswas and Hayat 2002b) will also be applicable to the results obtained by the Lewis cell technique.

In all acidity regions under investigation, the rate of extraction is independent of Zr(IV) concentration in its higher concentration regions and so, the extraction processes are diffusion controlled at higher Zr(IV) concentration regions of Zr(IV). But in the lower concentration region of Zr(IV), the rate is directly proportional to Zr(IV) concentration

and so the processes are chemically controlled. It is found that within Zr(IV) concentration of ~ 4-30 mmol dm^{-3} for 1 and 5 mol dm^{-3} HCl systems and of ~ 0.4 - 3 mmol dm^{-3} for 0.10 mol dm^{-3} HCl system are intermediate controlled. So, the most results collected in this paper are for intermediate controlled process. For 0.10 mol dm^{-3} HCl system, not only the concentration region of Zr(IV) but also the concentration region of Cl^- determine the regimes. At low concentration region of Cl^-, the process will be diffusion controlled and in the higher concentration region of Cl^-, the process will be chemically controlled.

In diffusion controlled processes, the flux equations lack Zr(IV) concentration term and the diffusion of $H_3A_2^+$ (formed by the association of proton with H_2A_2) from the interface to the aqueous film of the interface is the slowest step. This is supported by low E_a value(~ 14 kJ mol^{-1}). It is known that for diffusion controlled process the activation energy is below 20 kJ mol^{-1} (Habashi 1968). In lower concentration region of Zr(IV), the following chemical reactions have been suggested for 0.10, 1 and 5 mol dm^{-3} HCl system, respectively, as rate determining (Biswas and Hayat 2002b):

$$[Zr_8(OH)_{20}(H_2O)_{24}Cl_{12}]_{(i)} + H_3A_2Cl_{(i)} \rightarrow [Zr_8(OH)_{20}(H_2O)_{24}Cl_{13}]^- [H_3A_2]^+_{(i)} \quad \text{.....(16)}$$

$$[Zr_4(OH)_8(H_2O)_{16}Cl_7]^+_{(i)} + H_2A_{2(i)} \rightarrow [Zr_4(OH)_8(H_2O)_{16}Cl_7.H_2A_2]^+_{(i)} \quad \text{.....(17)}$$

$$[Zr(H_2O)_5Cl_3]^+_{(i)} + H_3A_2Cl_{(i)} \xrightarrow{-H_2O} [Zr(H_2O)_4Cl_4.H_3A_2]^+ \quad \text{..... (18)}$$

These chemically rate determining steps are supported by the high E_a values (> 80 kJ mol^{-1}) since for chemically controlled process the E_a value is over 50 kJ mol^{-1} (Habashi 1968).

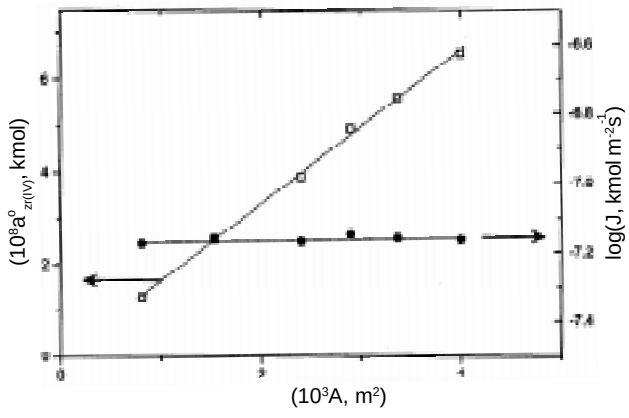

Fig 1. Dependence of the amount of mass transfer and flux on interfacial area. $[Zr(IV)]_{(ini)}$ = 3.60 mmol dm^{-3}, [HCl] = 1 mol dm^{-3} ; $[H_2A_2]_{(o)}$ = 0.03 mol dm^{-3} ; $[Cl^-]$ = 3 mol dm^{-3}; Δt = 240 s ; ageing time = 1-day; temp. = (30±1)°C.

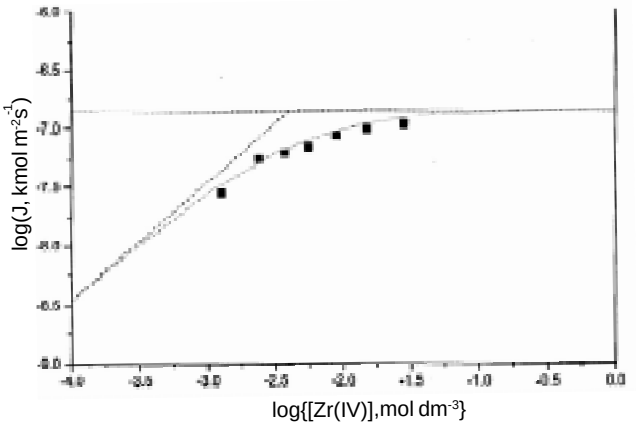

Fig 2. Dependence of the mass flux of Zr(IV) on its concentration in the intermediate acidity region of the aqueous phase. [HCl] = 1 mol dm^{-3} ; $[H_2A_2]_{(o)}$ = 0.03 mol dm^{-3} ; $[Cl^-]$ = 3 mol dm^{-3} ; Δt = 240 s ; ageing time =1-day ; temp. = (30±1)°C.

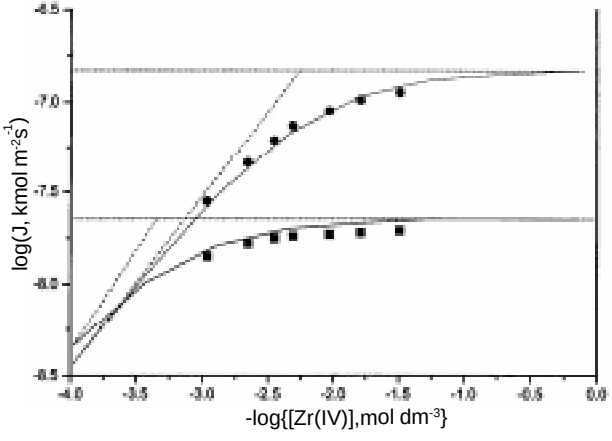

Fig 3. Dependence of the mass flux of Zr(IV) concentration in lower and higher concentration regions of hydrochloric acid in the aqueous phase. [HCl] = 0.1 mol dm^{-3} ;(■) and 5 mol dm^{-3} (●) ; $[H_2A_2]_{(o)}$ = 0.02 mol dm^{-3} ; $[Cl^-]$ = 3 mol dm^{-3} (●); and 5 mol dm^{-3} (■) ; Δt = 240 s ; ageing time = 1-day ; temp. = (30±1)°C.

Within Zr(IV) concentration of 4-30 mmol dm^{-3} for 5 and 1 mol dm^{-3} HCl system and of 0.4 - 3 mmol dm^{-3} for 0.10 mol dm^{-3} HCl system, the intermediate controlled mechanism is changed with the variation of temperature. The intermediate controlled process becomes diffusion controlled at higher temperature region and chemically controlled at lower temperature region. This is because of the fact that the rate of a chemical reaction is governed by Arrhenius equation, whereas, the rate of diffusion is governed by Stokes-Einstein equation (Habashi 1968). Arrehnius equation ($k_f = Ae^{-Ea/Rt}$) states that if temperature is doubled, the rate of reaction is increased 100 folds but Stokes-Einstein equation ($D = RT / 2\pi\eta rN$) indicates that if tempera-

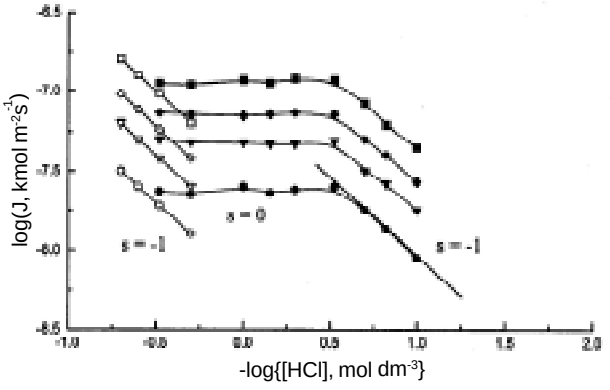

Fig.4. Dependence of Zr(IV) transfer flux on aqueous acidity. $[Zr(IV)]_{(ini)}$ = 3.60 mmol dm^{-3}; $[H_2A_2]_{(o)}$ = 0.01 mol dm^{-3} (○, ●); 0.02 mol dm^{-3} (▽, ▼); 0.03 mol dm^{-3} (◇, ◆); 0.05 mol dm^{-3} (□, ■); $[Cl^-]$ = 3 mol dm^{-3} (●, ▼, ◆, ■); 5 mol dm^{-3} (○, ▽, ◇, □); ageing time = 1-day; temp. = (30±1)°C.

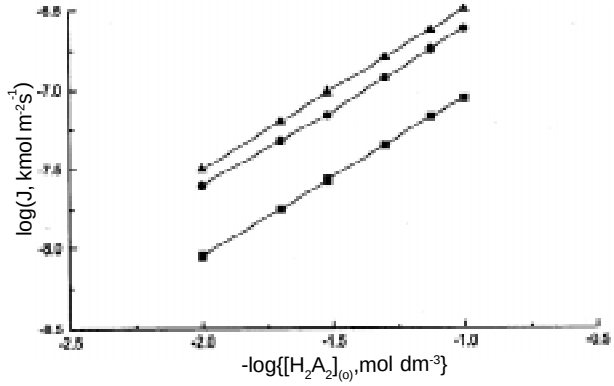

Fig.5. Dependence of flux on extractant concentration in the organic phase. $[Zr(IV)]_{(ini)}$ = 3.60 mmol dm^{-3}; [HCl] = 0.1 mol dm^{-3} (■) ;1 mol dm^{-3} (●) ; 5 mol dm^{-3} (▲); $[Cl^-]$ = 3 mol dm^{-3} (■, ●) ; 5 mol dm^{-3} (▲) ; ageing time = 1-day; temp. = (30±1)°C.

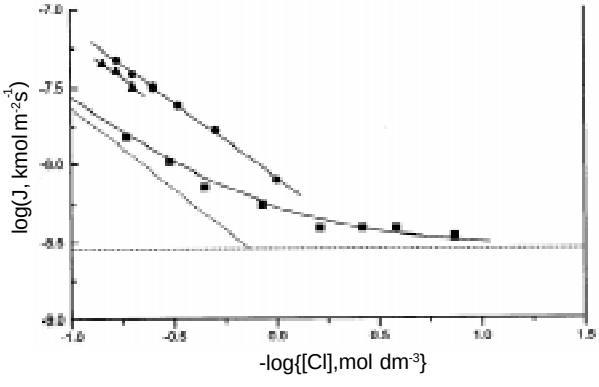

Fig.6. Dependence of flux on chloride ion concentration in the aqueous phase. $[Zr(IV)]_{(ini)}$ = 3.60 mmol dm^{-3}; [HCl] = 0.1 mol dm^{-3} (■), 1 mol dm^{-3}; (●), 5 mol dm^{-3} ; (▲), $[H_2A_2]_{(o)}$ = 0.01 mol dm^{-3} ; ageing time = 1 day ; temp. = (30±1)°C.

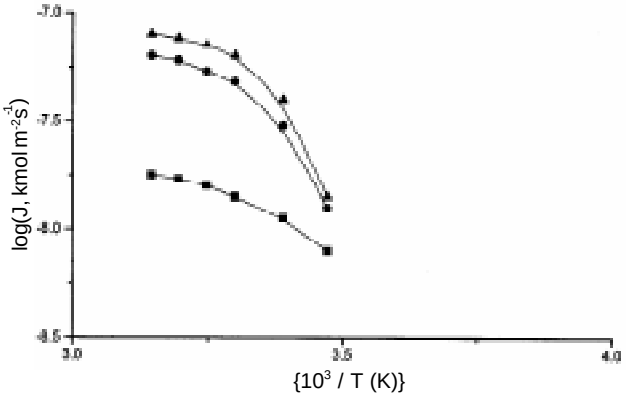

Fig.7. Dependence of flux on temperature in the intermediate control regime. $[H_2A_2]_{(o)}$ = 0.02 mol dm^{-3} ; ageing time = 1-day; (■), [HCl] = 0.1 mol dm^{-3}; $[Cl^-]$ = 3 mol dm^{-3} $[Zr(IV)]_{(ini)}$ = 1 mmol dm^{-3}; $E_a{}^1$ = 42 kJ mol^{-1}; $E_a{}^h$ = 11kJ mol^{-1}; (●), [HCl] = 1 mol dm^{-3}; $[Cl^-]$ = 3 mol dm^{-3}; $[Zr(IV)]_{(ini)}$ = 3.36 mmol dm^{-3} ; $E_a{}^1$ = 105 kJ mol^{-1}, $E_a{}^h$ = 12 kJ mol^{-1}; (▲), [HCl] = 5 mol dm^{-3}; $[Cl^-]$ = 5 mol dm^{-3}; $[Zr(IV)]_{(ini)}$ = 3.36 mmol dm^{-3}; $E_a{}^1$ = 108 kJ mol^{-1}; $E_a{}^h$ = 12 kJ mol^{-1}.

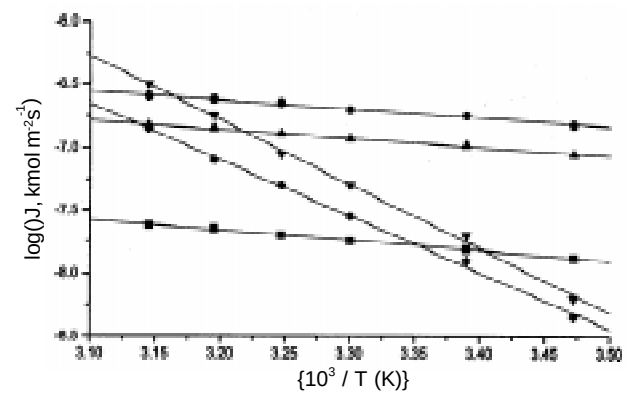

Fig.8. Dependence of flux on temperature kinetic and diffusion regimes. $[H_2A_2]_{(o)}$ = 0.02 mol dm^{-3}; ageing time = 1-day. Diffusion regime: (■); [HCl] = 0.1 mol dm^{-3}; $[Cl^-]$ = 3 mol dm^{-3}; $[Zr(IV)]_{(ini)}$ = 3.2 mmol dm^{-3}; E_a = 14 kJ mol^{-1}; (●), [HCl] = 1 mol dm^{-3}; $[Cl^-]$ = 3 mol dm^{-3}; $[Zr(IV)]_{(ini)}$ = 32 mmol dm^{-3}; E_a = 14.7 kJ mol^{-1}; (▲), [HCl] = 5 mol dm^{-3}; $[Cl^-]$ = 5 mol dm^{-3}; $[Zr(IV)]_{(ini)}$ = 32 mmol dm^{-3}; E_a = 15 kJ mol^{-1}. Kinetic regime: (▼), [HCl] = 1 mol dm^{-3}; $[Cl^-]$ = 3 mol dm^{-3}; $[Zr(IV)]_{(ini)}$ = 1 mmol dm^{-3}; E_a = 92 kJ mol^{-1}; (◆), [HCl] = 5 mol dm^{-3}; $[Cl^-]$ = 5 mol dm^{-3}; $[Zr(IV)]_{(ini)}$ = 1 mmol dm^{-3}; E_a = 84 kJ mol^{-1}.

ture is doubled, the rate of diffusion is doubled. An intermediate controlled process at a particular temperature may be converted to a diffusion controller process solely on rising temperature because of the fact that on increasing temperature, chemical rate is increased much faster than the diffusion rate and so the chemical reaction remains no longer as rate controlling. Conversely, an intermediate controlled process may be converted to a chemically controlled process on decreas-

Table 1
Evaluation of k_f at 303 K for 1-day ageing using Lewis cell technique

Fig. no.	[Zr(IV)], mol dm^{-3}	[H^+], mol dm^{-3}	[Cl^-], mol dm^{-3}	[H_2A_2]$_{(o)}$ mol dm^{-3}	Intercept,1	logk_f	Average logk_f	S.D
(i) For 0.1 mol dm^{-3} HCl system								
3	-	0.1	3.0	0.02	-4.40[1]	-5.61	-5.53	0.04
	-	0.1	3.0	0.02	-7.7[h]	-5.50		
4	0.0036	-	3.0	0.01	-7.08	-5.53		
	0.0036	-	3.0	0.02	-6.60	-5.53		
	0.0036	-	3.0	0.03	-6.60	-5.53		
	0.0036	-	3.0	0.05	-6.45	-5.60		
5	0.0036	0.1	3.0	-	-6.06	-5.51		
6	0.0036	0.1	-	0.01	-8.55[1]	-5.51		
					-8.69[h]	-5.49		
(ii) For 1 mol dm^{-3} HCl system								
2	-	1.0	3.0	0.03	-4.46[1]	-5.82	-5.80	0.02
	-	1.0	3.0	0.03	-6.85[h]	-5.81		
4	0.0036	-	3.0	0.01	-7.60	-5.77		
	0.0036	-	3.0	0.02	-7.32	-5.79		
	0.0036	-	3.0	0.03	-7.16	-5.81		
	0.0036	-	3.0	0.04	-6.39	-5.80		
5	0.0036	1.0	3.0	-	-6.61	-5.78		
6	0.0036	1.0	-	0.01	-8.10	-5.79		
(iii) For 5 mol dm^{-3} HCl system								
3	-	5.0	5.0	0.02	-4.48[1]	-6.60	-6.58	0.03
	-	5.0	5.0	0.02	-6.90[h]	-6.60		
4	0.0036	-	5.0	0.01	-8.10	-6.49		
	0.0036	-	5.0	0.02	-7.90	-6.59		
	0.0036	-	5.0	0.03	-7.72	-6.58		
	0.0036	-	5.0	0.05	-7.50	-6.59		
5	0.0036	5.0	5.0	-	-5.50	-6.59		
6	0.0036	5.0	-	0.01	-8.20	-6.59		

ing the temperature because of the fact that on decreasing temperature, chemical rate is decreased much faster than the diffusion rate and so the diffusion remains no longer as rate controlling.

Conclusion

The rate measurements made by the Lewis cell technique operated at 2.5 Hz give almost identical results with those obtained from the single drop technique and so, the contribution of hydrodynamics to mass flux in both techniques mentioned will be of the same order.

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SYNTHESIS OF 5, 7-DIHYDROXY-6, 8-DI-C-PRENYL-4′-*O*-PRENYL-FLAVANONE

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The prenylated flavanone **(9)** has been synthesized from phloroacetophenone **(1)**. All the new products have been characterized by the spectral data and microanalysis.

Key words: Synthesis, Chalcone, Flavanone

Introduction

Flavones and their derivatives are naturally occurring and have a variety of biological properties, such as antibacterial (Mitscher *et al* 1993), antifungal (Conn 1981) and antitumour activity (Mizabuchi and Sato 1984). A large number of natural products including flavonoids are being reported in the literature every year and their structures need to be confirmed by synthesis. This paper reports the synthesis of 5,7-dihydroxy-6, 8-di-C-prenyl-4′-*O*-prenylflavanone **(9)** from phloroacetophenone **(1),** which may be used as a synthetic marker. Phloroacetophenone on treatment with methoxy-methyl chloride using K_2CO_3 and acetone afforded 2-hydroxy-4, 6-di (methoxymethoxy) acetophenone **(2)** (Hossain 1999), which on nuclear prenylation using well-cooled solution of KOH and prenyl bromide gave three products viz 2-hydroxy-4, 6-di (methoxymethoxy)-3-C-prenylacetophenone **(3),** 2-hydroxy-4, 6-di (methoxymethoxy)-5-C-prenylacetophenone **(4)** (Hossain and Islam 1993), and 2-hydroxy-4,6-di (methoxymethoxy)-3, 5-di-C- prenylaceto-phenone **(5)** and several other minor products. Similarly *O*-prenylation of p-hydroxybenzaldehyde using K_2CO_3/acetone/prenyl bromide gave 4-*O*-prenylbenzaldehyde **(6)**. Alkaline condensation of 2-hydroxy-4, 6-di(methoxy-methoxy)-3, 5-di-C-prenylac-etophenone **(5)** and 4-*O*-prenylbenzaldehyde **(6)** yielded 2′-hydroxy-4′, 6′-di (methoxymethoxy)-3′, 5′-di-C-prenyl-4-*O*-prenylchalcone **(7).** Compound **(7)** on treatment with NaOAc/EtOH furnished 5,7-di (methoxymethoxy)-6, 8-di-C-prenyl-4′-*O*-prenylflavanone **(8),** which upon demethoxymethylation afforded 5,7-dihydroxy-6, 8-di-C-prenyl-4′-*O*-prenylfla-vanone **(9).**

Experimental

Melting points were determined using an electrothermal melting point apparatus (Gallenkamp) and are uncorrected. IR spectra were recorded (KBr discs) on a Pye-Unicam SP3-300 IR spectrophotometer (ν_{max} in cm^{-1}), ^{1}H-NMR spectra on a Varian 300 MHz instrument in $CDCl_3$ with TMS as an internal standard (chemical shifts in δ, ppm) and UV spectra on Milton-Roy UV-visible spectrophotometer Ultrospeck in methanol (λ_{max} in nm). TLC was performed using silica gel 60G. Mass spectra were recorded on Time of Flight (GC-MS TOF). Satisfactory elemental analyses were obtained for all the compounds and structures are in accord with the UV, IR and ^{1}H-NMR data.

*Methoxymethylation of phloroacetophenone **(1)***. A mixture of phloroacetophenone (**1**, 10 g) in dry acetone (100 ml), methoxymethyl chloride (5.67 g) and anhydrous potassium carbonate (40 g) was refluxed for about 3 h. The progress of the reaction mixture was examined by TLC. On completion of the reaction acetone was distilled off and water was added and the mixture was then extracted with ether washed with water and dried over anhydrous Na_2SO_4. The organic layer was evaporated to dryness. The ether extract on silica gel column chromatography (mesh 60-120) using petrol (40-60°), petrol-benzene (4:1), petrol-benzene (4:3) and increasing quantities of benzene as eluent gave the major compound **(2)** and several other minor compounds. Compound **(2)** was purified from column and by preparative TLC over silica gel GF_{254} using benzene-petrol (25:1) as developing solvent. It was crystallized from petrol as colorless crystals (4.09g) R_f 0.69 (benzene-petrol; 25:1); m.p 80-81°C; IR: 3450, 2874, 1654, 1610, 1600, 1476, 1365, 1275, 1234, 1190, 1156, 1064, 1034, 966 984, 934, 886; 1H-NMR: 2.45 (s,1H,1-$COCH_3$), 3.45 (s,6H,-CH_2OCH_3x2), 5.55 (s,4H,-CH_2O-CH_3x2), 6.45 (s, 1H,H-3), 6.67 (s, 1H H-5), 12.76 (s, 1H, -OH).

*Nuclear prenylation of 2-hydroxy-4,6-di (methoxymethoxy) acetophenone **(2)***. 2-Hydroxy-4,6-di-(methoxymethyleneoxy) acetophenone (**2**, 1g) was added to a well cooled solution of KOH) (2 g) in absolute methanol (30 ml)

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and the whole solution was cooled to 0°C. Prenyl bromide (0.5 g) was added and the reaction mixture allowed to stand at room temperature for 24 h with constant stirring. The reaction mixture was diluted with water and acidified with cold diluted HC1. The mixture was then extracted with ethyl acetate. The ethyl acetate extract was dried over anhydrous Na_2SO_4 and concentrated. It was then subjected to column chromatography over silica gel (mesh 60-120) and eluted successively with petrol-benzene (5:1), petrol-benzene (1:3), petrol-benzene (1:5) and compounds **(3), (4)** and **(5)** were obtained.

*2-Hydroxy-4,6-di (methoxymethoxy)-3-C-prenylacetophenone **(3)**:* It was crystallized from ethanol as white needles (140 mg), m.p. 34°C, R_f 0.64 (benzene). It gave positive alcoholic ferric chloride test. UV: 229, 265, 285 nm; IR: 3476, 1645, 1605, 1595, 1472, 1424, 1370, 1365,1363,1205,1145 1105, 1040, 980, 945, 910, 835 cm^{-1}; ^{1}H-NMR: 1.72 [s, 6H, $>C(CH_3)_2$], 2.45 (s, 3H,-$COCH_3$), 3.53 (m, 8H,-C<u>H</u>$_2$-CH and -CH_2OCH_3), 5.25 (t, 1H,-CH_2-C<u>H</u>), 5.54 (s, 4H,-CH_2OCH_3), 6.68 (s,1H,H-5), 12.43 (s, 1H,-OH).

*2-Hydroxy-4,6-di (methoxymethoxy)-5-C-prenylacetophenone **(4)**.* It was crystallized from petrol as white needles (140 mg), m.p. 48°C, R_f 0.58 (benzene). It gave positive alcoholic ferric chloride test. UV: 229,268, 280 nm; IR: 3476, 1645, 1605, 1595, 1472, 1424, 1370, 1365, 1363, 1205, 1145,1105, 1040, 980, 945, 910, 835 cm^{-1},^{1}H-NMR: 1.75 [s, 6H, $>C(CH_3)_2$], 2.43 (s, 3H,-$COCH_3$), 3.55 (m, 8H,-C<u>H</u>$_2$-CH and - CH_2OCH_3), 5.30 (t, 1H,-CH_2-C<u>H</u>), 5.55 (s, 4H,-CH_2OCH_3), 6.45 (s, 1H, H-3).

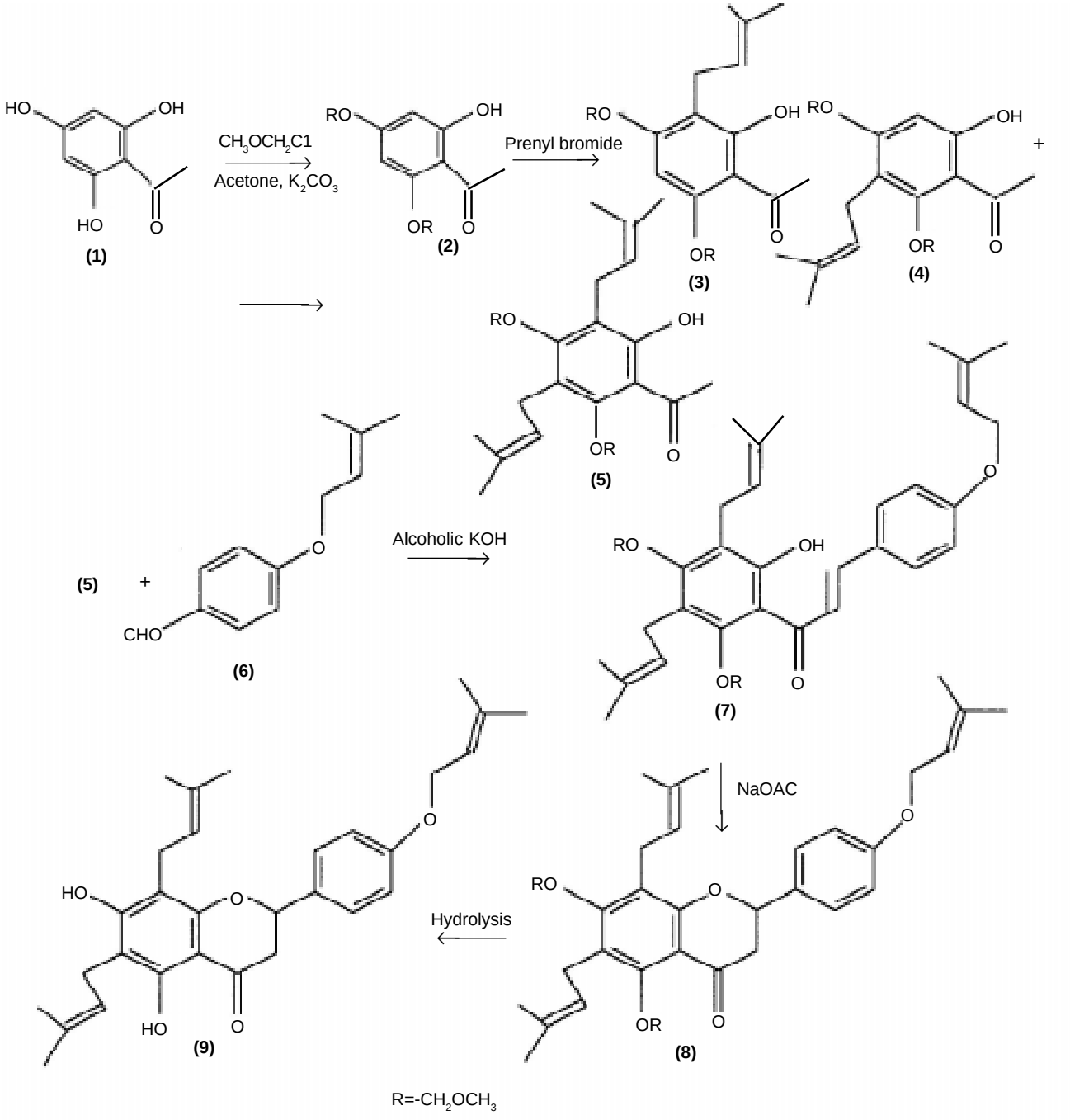
*2-Hydroxy-4,6-di (methoxymethoxy)-3,5-di-C-prenylacetophenone **(5)**.* It was a viscous liquid (675 mg) and was not crystallized from any solvent. UV: 225, 243, 288 nm; IR: 3455, 1645, 1600, 1590, 1420, 1375, 1365, 1325, 1240, 1205, 1100, 1050, 985, 910, 835, 725 cm^{-1}; ^{1}H-NMR: 1.70 [s, 12H,$>C(CH_3)_2$x2], 2.48 (s, 3H,-$COCH_3$), 3.52 (m, 8H, -C<u>H</u>$_2$-CH and -CH_2OCH_3), 5.22 (t, 1H,-CH_2-C<u>H</u>), 5.51 (s, 4H,-CH_2OCH_3), 12.10 (s, 1H,-OH).

*4-O-Prenylbenzaldehyde **(6)**.*A mixture of *p*-hydroxybenzaldehyde (1.20g), prenylbromide (1.46g) and K_2CO_3 (7g) in acetone (40ml) was refluxed for about 3 h and the progress of the reaction mixture was examined by TLC. After cooling, acetone was distilled off and water was added to the residue. The mixture was then extracted with ether, washed with water and dried over anhydrous Na_2SO_4. The organic layer was evaporated to dryness and the crude residue was passed through a small dry silica gel column. The product was crystallized from dilute alcohol and gave white needles (0.39g); m.p.52°C; (M^+,190); R_f 0.58 (benzene); UV: 229, 265; IR: 1760, 1645, 1605, 1410, 1372, 1368;^{1}H-NMR:1.75[s, 6H,$>C(CH_3)_2$], 4.51 (d, 2H, J 7Hz, -O-CH_2-CH), 5.52 (t, 1H, J 7Hz,-O-CH_2-CH), 6.43 (d, 2H, J 9Hz, H-2 and H-3), 6.98 (d, 2H, J 9Hz, H-5 and H-6), 9.78 (s, 1H,-CHO); [Found: C, 75.8; H, 7.4, $C_{12}H_{14}O_2$ requires: C, 75.4; H, 7.7%].

*2′-Hydroxy-4′,6′-di (methoxymethoxy)-3′, 5′-di-C-prenyl-4-O-prenylchalcone **(7)**.*A mixture of 2-hydroxy-4, 6-di (methoxymethoxy)-3, 5-di-C-prenylacetone (**5**, 1.87g) and 4-*O*-prenylbenzaldehyde (**6**,0.95g) in ethanolic KOH (50%, 20 ml) was kept at room temperature for 80 h, diluted with ice-cold water and acidified with diluted HC1. It was extracted with ether (100 ml). The ether extract was washed with water, dried over anhydrous Na_2SO_4 and ether was evaporated to dryness. The mixture was purified by preparative TLC over silica gel 60G using benzene as developing solvent. The product was crystallized from petrol as yellow crystals (0.78g); m.p. 112°C, (M^+,564); R: 0.71 (benzene-acetone; 5:2); UV: 235, 255, 265, 378; IR: 3470, 1760, 1645, 1605, 1590, 1478, 1370, 1362, 1040; ^{1}H-NMR: 1.72 [s, 6H, $>C(CH_3)_2$], 1.53 [s, 12H, $> C(CH_3)_2$x2], 3.41 (s,6H,-CH_2-OCH_3x2), 3.51 (d, 2H, J 7Hz,-CH_2-CHx2), 4.55 (d, 2H, J 7Hz, -O-CH_2-CH), 5.25-5.53 (m, 7H, -CH_2-OCH_3x2, -CH_2-CHx2,-O-CH_2-CH), 6.58 (d, 2H, J 9Hz, H-2 and H-3), 6.99 (d, 2H, J 9Hz, H-5 and H-6), 7,45 (d, 1H, J 9Hz,4 H-α), 8.01 (d, 1H, J 9Hz, H-β), 13.01 (s, 1H,-OH), [Found: C, 72.3; H, 7.8, $C_{34}H_{44}O_7$ requires: C, 72.6;H, 7.4%].

*5,7-Di(methoxymethoxy)-6,8-di-C-prenyl-4′-O-prenylflavanone **(8)**.* To a solution of **(7)** (1.78g) in ethanol (30 ml), sodium acetate (1.8g) was added. The reaction mixture was left at room temperature for 3 days. It was diluted with water and extracted with ethyl acetate. The ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated. The product was purified by preparative TLC over silica gel GF_{254} using benzene as developing solvent. It was crystallized from xylene as colorless crystals (0.82g); m.p. 129°C;(M^+,564); UV: 230, 245, 365, IR: 2944, 2845, 1645, 1600, 1590, 1540, 1410, 1370, 1360, 1255, 1040; ^{1}H-NMR: 1.72[s,6H,$>C(CH_3)_2$], 1.53 [s,12H,$> C(CH_3)_2$x2],2.92 (d, 2H, J 9Hz, H-3), 3.41 (s, 6H, -CH_2-OCH_3x2), 3.51 (d, 2H, J 7Hz,-CH_2-CHx2), 4.55 (d, 2H, J 7Hz, -O-CH_2-CH) 5.25-5.53 (m, 8H, H-2,-CH_2-OCH_3x2,-CH_2-CHx2),6.58 (d, 2H, J 9Hz, H-2 and H-3), 6.99 (d, 2H, J 9Hz, H-5 and H-6), 7,45 (d, 1H, J 9Hz, H-α),8.01 (d, 1H, J 9Hz, H-β), 13.01 (s, 1H, -OH), [Found : C, 72.4; H, 7.8, $C_{34}H_{44}O_7$ requires:C, 72.7; H, 7.4%].

*5,7-Dihydroxy-6, 8-di C-prenyl-4′-O-prenylflavanone **(9)**.* To a solution of the above methoxymethoxylated flavanone (**8**, 1g) in methanol (30 ml), HC1 (3N, 50ml) was added and boiled on a water bath for 15min. The reaction mixture



Scheme I

was diluted with water (150ml) and extracted with ethyl acetate. The ethyl acetate extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated. TLC examination of the residue showed several spots and the major product was purified by preparative TLC using ethyl acetate-benzene (10:7) as developing solvent. It was crystallized from petrol as white crystals (0.25g), m.p. 177°C, (M^+,476); UV: 225, 244, 265, 355; IR: 3520, 2945, 2810, 1645, 1605,1600, 1510, 1470, 1344, 1340; ^{1}H-NMR: 1.72 [s, 6H,>C$(CH_3)_2$], 1.53 [s, 12H, > C $(CH_3)_2$x2], 2.92 (d, 2H, J 9Hz, H-3), 3.41 (s, 6H, -CH_2-OCH_3x2), 3.51 (d, 2H, J 7Hz, -CH_2-CHx2), 4.55 (d, 2H, J 7Hz, -O-CH_2-CH), 5.25-5.53 (m, 8H, H-2, -CH_2-OCH_3x2,-CH_2 -CH x2), 6.58 (d, 2H, J 9Hz, H-2 and H-3), 6.99 (d, 2H, J 9Hz, H-5 and H-6), 7,45 (d, 1H, J 9Hz, H-α), 8.01 (d, 1H, J 9Hz, H-β), 13.01 (s, 1H, -OH), [Found : C, 75.6; H, 7.6, $C_{30}H_{36}O_5$ requires: C, 72.7; H, 7.4%].

Results and Discussion

The compound **(1)** was subjected to methoxymethylation (methoxymethyl chloride/K_2CO_3/acetone) to give compound **(2)** the formation of which was ascertained by spectral studies and elemental analysis. Infrared spectrum of **(2)** showed the absorption frequencies at 3450 and 1654 cm^{-1} indicating the presence of hydroxy and ketonic group in conjugation. In ^{1}H-NMR spectrum, a singlet at δ 2.45 indicated the presence of methyl protons of acetyl group. Two singlets at δ 3.45 and δ 5.55 indicated the presence of six protons of two -OCH_3 group and four protons of two-CH_2 group, respectively which confirmed that the methoxymethylation has taken place. Compound **(3)**, **(4)** and **(5)** were obtained by the nuclear prenylation (cool methanolic KOH/prenyl bromide) of **(2)** and the formation of which agreed with the data of spectral and elemental analysis. The ^{1}H-NMR spectrum of the prenylated compound **(7)** indicated the presence of C-prenyl unit. A sharp singlet at δ 1.70 revealed the presence of gem-dimethyl group and the presence of -CH_2- and -CH protons attached to the aromatic ring was indicated by a multiplet at δ 3.52 and a triplet at δ 5.22, respectively. Similarly *O*-prenylation of *p*-hydroxybenzaldehyde using prenyl bromide/K_2CO_3/acetone gave compound **(6)**. The compound **(5)** on a cross-aldol condensation with **(6)** in the presence of 50% ethanolic KOH afforded the compound **(8)** after dehydration of the initial aldol product. The characteristic IR absorption frequencies at 1645 cm^{-1} showed the presence of conjugated ketonic group and the absorption peaks at 1600 and 1590 cm^{-1} indicated the presence of unsymmetric ethylenic double bond and aromatic rings, respectively. The singlet for methyl protons of acetyl group disappeared while two new doublets at δ 7.45 and δ 8.01 appeared showing the presence of two vinylic protons (α and β protons; i.e cis isomer). The elemental analysis for C and H showed satisfactory results (within ± 0.4%). The NaOAc/EtOH treatment of **(7)** gave the corresponding flavanone **(8)**. Finally the compound **(9)** was obtained from **(8)** by demethoxymethylation (MeOH/3N HC1). The IR absorption frequencies at 3520 and 1645 cm^{-1} showed the presence of -OH (phenolic) group and ketonic group and the absorption peaks at 1605 and 1600 cm^{-1} indicated the presence of unsymmetric ethylenic double bond and the aromatic rings, respectively. One singlets at δ 13.01 indicated the presence of -OH protons which confirmed the completion demethoxymethylation. The B-ring protons have their usual chemical shift value.

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Effect of pH and Concentration on the Removal of Magnesium from Magnesium Chloride Solution by Bentonite

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By virtue of their well-known cation exchange capacities of clays, particularly bentonites are important minerals to be used as adsorbent of various undesirable ions drained out as industrial waste. One of such pollutant is magnesium containing waste. The removal of magnesium from magnesium containing solutions is found to be dependent both on pH of the solution and the concentration of magnesium ions present in the solution. Using magnesium chloride solution of 50 mg/lit and 100 mg/lit concentration and bentonite (North West Frontier Province), it was found that adsorption equilibrium established within 6 h .

Key word**:** Bentonite, Adsorption, Freundlick equation

Introduction

Industries such as medicine, toiletry, printing ink, refractories, rubber insulating material and some other chemical industries extensively use magnesium compounds in their manufacturing processes. In sewerages of these industries the concentration of the magnesium ions is generally above the permissible limit (50 ppm), which causes deleterious effects to human health. Clays have been used for the adsorption of organic molecules (Jhonston and Cardile 1987) inorganic metallic ions (Diez 1980) and dyestuff, in waste water. In the present study a Pakistani bentonite is used for the removal of magnesium ions from magnesium chloride solution.

Bentonites are essentially hydrous aluminium silicates, with magnesium or iron, alkalies or alkaline earth metals (Grim 1962). They are composed of small crystalline particles of one or more clay minerals. The structure of bentonite is composed of a single silica tetrahedron sheet and a single alumina octahedral sheet combined in a unit in such a way that the corners of silica tetrahedron and the layer of octahedral sheet form a common layer. All the corners of silica tetrahedrons point in the same direction and towards the centre of unit (Mitra *et al* 1979). Cleavage between sheets leads to platelike particles and leave these layers unsaturated.

The ion exchange ability of bentonite is probably due to, *i)* Broken bonds due to sub-division of the giant crystal. *ii*) Disordered structure containing ions of incorrect valence. Where, there are unsaturated bonds or electrical charges, their counter ions can be adsorbed. This effect would be dependent on particle size (Worral *et al* 1958). The second cause of charged clay particles is the structural disorder due to isomorphism substitution of Al^{3+} for Si^{4+} and Mg^{2+} for Al^{3+}, giving rise to inherent negative charges. This is independent of particle size (Whittaker 1939). In addition, some anions may be adsorbed by the replacement of exposed hydroxyl ions and because of the structural arrangement of some of the anions with tetrahedral units (Foster 1951). This absorption behaviour of bentonite was observed to be influenced by various factors such as contact time, solution concentration (Miyazaki 1996), temperature (Shakila *et al* 1998) and pH (Compton *et al* 1994).

The present work has been undertaken with a view to investigate the adsorption characteristics of bentonite for the adsorption of magnesium from magnesium chloride solution and the effect of concentration of magnesium, pH of the salt solution on the extent of adsorption of magnesium.

Experimental

In present studies, bentonite from Peshawar area was used. Snow's method is used to determine the surface area (Iqbal 2001). The chemical analysis of the bentonite and other characteristics are given in Table 1. The method is described as below.

Determination of surface area of bentionite. Potassium iodide (KI) 71.3 g and 7.5 g of iodine (I_2) crystals were dissolved in a small quantity of distilled water and the volume made up to 125 ml. About 1 g of washed bentonite was taken in an iodine flask and 5 ml of iodine solution was added. It was allowed to stand for 2 h. Then 45 ml of distilled water was added and flask was gently spiralled for 1 min. It was kept for 1 h, 20 ml of the supernant solution was taken in titration flask and two drops of starch solution was added. A

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Table 1
Chemical and physical properties of bentonite

Representative chemical composition	
SiO_2	57.80%
Al_2O_3	20.80%
Fe_2O_3	3.00%
CaO	4.20%
MgO	3.00%
Na_2O	10.00%
K_2O	0.05%
Loss on ignition	10.00%
pH value (10% slurry)	7.00%
Brightness	57.00%
Specific surface area	200 m^2/g
Mean particle size (80%)	20microns

titration was made against standard sodium thiosulphate ($Na_2S_2O_3$) solution by noting the end point on the disappearance of colour. The surface area in m^2/g was calculated using the formula [12.5x (24-V)], where V is the volume of sodium thiosulphate used in titration.

ii. Determination of equilibrium time of adsorption of magnesium on bentonite clay. Finely ground bentonite 0.5 g was taken in different flasks, 50 ml of magnesium solution (50 mg / lit, and 100 mg / lit) was added and closed tightly by stopper. Then these flasks were placed on a water bath with magnetic stirrer, for various duration of time, i.e. 0.5, 1, 2, 3, 4, and 6 h. The solution was stirred at a constant temperature 25°C. The slurry was then filtered; 2 ml of filtrate was titrated against standard EDTA solution (0.01M) in the presence of 1 ml buffer solution (pH=10) and a few drops of Eriochrome black T (EBT) indicator. The end point was noted when colour changed from wine-red to blue. The quantity of magnesium ions adsorbed was calculated by the difference in concentration of blank solution and sample solution for different time periods and equilibrium time was determined by plotting the time (hours) versus extent of adsorption of magnesium μg/g.

iii. Determination of adsorption isotherm of magnesium on bentonite. Magnesium chloride solution of different concentration, i.e., 20, 40, 60, 80 and 100 mg/lit were prepared. Then 50 ml solution of each concentration was transferred to conical flasks containing 0.5 g bentonite. The flasks were tightly closed by means of cork and stirred for 6 h at a constant temperature. The slurry was then filtered and 20 ml from the clear filtrate were titirated against standard EDTA (0.01 M) solution in the presence of 2 ml buffer solution (pH=10) and a few drops of EBT indicator. The end point was noted when the colour changed from wine-red to blue. The quantity of magnesium adsorbed was calculated by taking the difference between the blank concentration and sample concentration using the following formula.

$$\text{Apparent adsorption } (\mu g/g) = \frac{(V_1+V_2)\times M\times \text{At.Wt. of Mg}\times 100}{\text{Wt. of clay (g)}}$$

Where,

Wt. = Weight of clay (g)
M = Molarity of EDTA solution
V_1 = Volume of EDTA solution used for 50 ml blank magnesium salt solution.
V_2 = Volume of EDTA solution for 50 ml magnesium salt solution containing clay sample.

Determination of the effect of pH on adsorption isotherm on clay. The pH of magnesium solution (20, 40, 60, 80 and 100 mg/lit) was adjusted (7-8) by the addition of 0.1 N NaOH in the pH range of 7-8. The determination of adsorption isotherm at pH range 7-8 was also performed for each concentration, pH of magnesium chloride solution was also adjusted in the range of 3-4 by gradual addition of 0.1 N HCl. The same procedure was adopted for the determination of adsorption isotherm at intermediate pH. Blank determination was also performed for each concentration to study the effect of pH on the adsorption of magnesium on bentonite.

Results and Discussion

The surface area of bentonite determines by using Snow's iodine adsorption method comes out to be 200 m^2/g at pH7 under ambient conditions. The surface area is much higher (about twice) than ordinary China clays (90 m^2/g), which is an indication of its effectiveness for adsorption of metal ions.

Effect of time on adsorption of magnesium from two different concetrations (50 mg/lit, 100 mg/lit) of magnesium chlorides of bentonite is shown in Fig 1. It is evident that equilibrium is established within 6 h in both cases. Apparently, concentration of the solution seemed to have no effect on the equilibrium time. However, the amount of magnesium ions adsorbed from the concentrated solution is higher as compared to dilute solution.

Fig 2 depicts the effect of pH of adsorbent solution on the quantity of magnesium ion adsorption. In strong acidic solution (pH 2-3) almost a straight line is obtained. However, the amount adsorbed is small. It appeared at low pH, the magne-

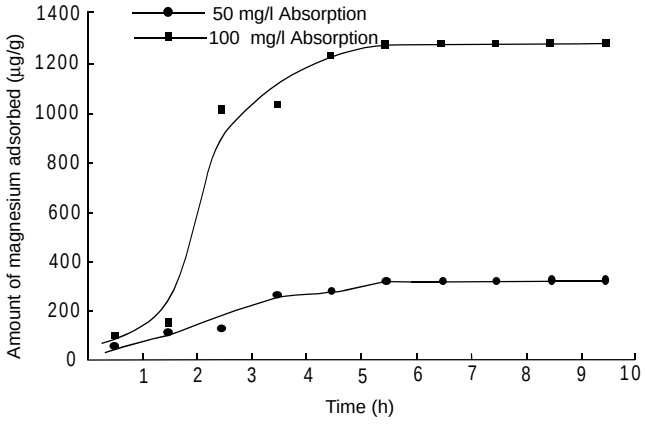

Fig 1. Adsorption of magnesium from magnesium chloride solution at different interval clay dosage = 0.5 g.

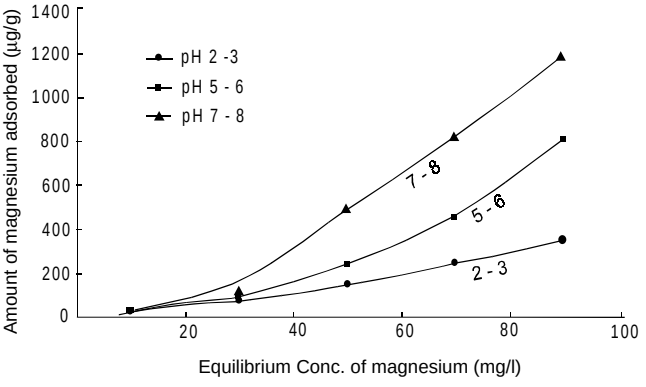

Fig 2. Adsorption of magnesium from magnesium chloride solution at different pH ranges clay dosage = 0.5 g.

sium ions have less opportunity to come in contact with the negatively charged surface, which preferred H^+ ions that are easily available from the medium and consequently reduce the number of active sites. Similar behaviour has been reported (Iqbal *et al* 2001) while studying the adsorption of Mg^{2+} on China clay. However, the quantity of the adsorption on bentonite is 3-4 times higher than China clay, which can be explained by taking into account the much larger surface area of the bentonite used in the present study. It is observed that as the pH of the solution changes to higher values, there is a marked increase in the adsorption of magnesium ions. The highest adsorption of magnesium ions was obtained at 7-8 pH. One of the contributory factors for the higher adsorption at 7-8 pH can be solubility of $MgCl_{2,}$ which is less soluble at this pH range.

Generally adsorption phenomenon of solute from a solution is explained by using Freundlick equation (Barrow 1966), which in logarithmic from as:

$$Ln(x/m) = lnk+1/n\ lnc$$

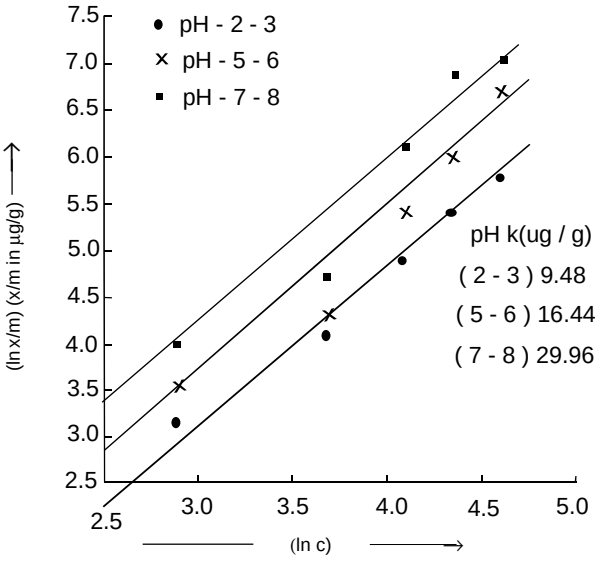

Fig 3. Freundlich isotherm of magnesium from magnesium chloride solution at different pH ranges clay dosage 0.5 gm

Where "x" is the amount of solute adsorbed and "n" is the amount of adsorbent. "c" is the equilibrium concentration and "k" is the adsorption capacity.

Adsorption data obtained in the present work are plotted in Fig 3. Three adsorption isotherms with almost same values of slope were obtained, which indicates that mode of adsorption is similar throughout the pH range investigated. It is evident from the plot that the value of adsorption capacity constant (k) increases with the increase in pH value of the solution. Adsorption capacity constant has minimum value of 9.48 at pH range (2-3), which increases to 16.44 for pH range (5-6) while the maximum value 29.96 was obtained for the pH range (7- 8). These values are in accordance with earlier observation that there is an enhanced adsorption of magnesium ion at higher pH value.

Conclusion

Pakistan has huge deposits of bentonite, which can be used for the removal of magnesium ions to minimize the pollution level. Present investigation indicates that bentonites have the advantage of higher surface area and better adsorption capacities than China clay. Removal of magnesium ion was found to be pH dependent. pH range seemed to be the best for efficient removal of magnesium ions.

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TRAFFIC NOISE IN LAHORE CITY, PART I: ROAD TRAFFIC NOISE

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Traffic noise survey was conducted at 15 sites in the different residential and commercial areas of Lahore city and at each survey site, noise data were collected from 0900 to 1700 h. The data collected have been analyzed for the recorded range, L_{A99}, L_{A90}, L_{A50}, L_{A10} and L_{A1} and approximate vales of L_{Aeq8h} were evaluated for each survey site. The results are discussed with reference to some criteria for community annoyance and ways and means to limit high-level traffic noise are suggested.

Key words**:** Environmental pollution, Noise pollution, Traffic noise, Lahore city.

Introduction

Road traffic noise is the most widespread source of noise in all countries and the most prevalent cause of annoyance and interference. Traffic noise surveys conducted in Karachi by Shaikh *et al* (1987 and 1997) and Hyderabad by Shaikh and Shaikh (2000) shows that in (i) Karachi with the exception of a few occasional peaks, the levels of traffic noise levels vary in the range of 61 to 97 dB(A), with L_{A90}, L_{A50} and L_{A10} values in the range of 70.1 - 78.4, 79.6 - 84.5 and 85.6 - 90.8 dB(A), respectively and (ii) Hyderabad in the range of 57.1 - 101.9 dB(A), with L_{A99}, L_{A90}, L_{A50}, L_{A10} and L_{A1} values in the range of 60.4 - 73.3, 66.2 - 79.6, 75.2 - 82.8, 85.0 - 90.9 and 89.1 - 99.0 dB(A), respectively and L_{Aeq12h} values in the range of 81.2 - 86.9 dB(A). These levels are excessively high and much above the community annoyance limits recommended by the International Standards Organization (ISO) and some other individual countries. Roadside dwellers and traders are constantly exposed to such a high level noise for about more than 12 h a day.

The result of another survey (Ahmad 1992; Ahmad 1994) in Karachi, Lahore, Faisalabad, Hyderabad and Sukkur shows that the levels of traffic noise in these cities vary in the range of 72 - 95, 74 - 90, 70 - 92, 60 - 90 and 60 - 85 dB(A), respectively. However, the methodology used by these surveys, such as (i) most of the readings reportedly taken in dB (ii) distance of the meter from the nearest line of flow of vehicles (iii) time weighting (iv) fewer readings (v) average values generally based on minimum and maximum readings and (vi) incorrect range of values raises questions about the credibility of the results and inferences to made thereof (Shaikh and Shaikh 2000; Shaikh 2003). The Environmental Protection Department, Lahore, has reported traffic noise levels for six places in Lahore, in the range of 26 - 121 dB (not dB(A) (EPD 1996). For Village Bath, Lahore, traffic noise levels have been reported in the range of 26 - 50 dB (not dB(A), which is unimaginable and may have been occasioned by technical problem in the measuring equipment (e.g. battery voltage drop). EPA's measurement of traffic noise levels with the handheld device inclined at about 45 degree was irregular and rendered the results unreliable. More standard measurement procedures were employed in the surveys carried out by Shaikh *et al* (1987, 1997 and 2001).

Therefore, in order to have detailed assessment of prevailing road traffic noise in different areas and localities, traffic noise survey was conducted at 15 sites on busy roads with heavy traffic density in the residential and commercial areas of Lahore city. Due to the absence of proper regulatory laws to limit highlevel traffic noise in Pakistan, the results are discussed with reference to the community annoyance criteria, recommended by ISO and some other individual countries. Some suggestions for limiting highlevel traffic noise are also discussed.

Materials and Methods

Measuring instruments and techniques. The measuring instrument consisted of a Sound Level Meter. The meter was regularly calibrated against an acoustic calibrator and checked before and after each series of measurements. During all the measurements, the meter was kept at 1.5 m above the ground level and at a distance of about 5 m from the edge of the nearest

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line of flow of vehicles (ASA 1984; Hassel and Zaveri 1988) and about 1-2 m from the façade (ISO - 1996 (1982), PSI - 4005 (1997); however, in some cases due to existing road situations, measurements were made at kerbsides. Traffic noise data was recorded in dB(A) with time constant 'fast'.

At each survey site, noise data were collected from 0900 - 1700 h in every 10 m. In each set, ten readings were recorded in a period of about 2 m and repeated after intervals of 8 m. In each measuring mode between the intervals, the noise level was worked out as the average value of 10 readings. Also the maximum and minimum values in each measuring mode were recorded. The data collected have been analyzed for L_{A99}, L_{A90}, L_{A50}, L_{A10} and L_{A1} and approximate values of L_{Aeq8h} for each survey site are evaluated by using the following relationship (May 1971):

$$L_{Aeq} = L_{A50} + (L_{A10} - L_{A90})^2 /56$$

Preferred Speech Interference Levels (PSIL) for each survey site have been evaluated by using the relationship between PSIL and dB(A) (May 1971):

PSIL = dB(A) - 7

Results and Discussion

The results evaluated for the recorded range and percentile values for the 15 survey sites are given in Table 1. Fig 1, 2 and 3 show the diurnal variation, statistical distribution and cumulative distribution, respectively of road traffic noise recorded at Shalimar Chowk from 0900 to 1700 h. The result shows that in Lahore, the levels of road traffic noise vary in the range of 60.4 - 97.3 dB(A), with L_{A99}, L_{A90}, L_{A50}, L_{A10} and L_{A1} in the range of 63.1 - 66.3, 68.3 - 74.1, 74.8 - 82.4, 84.3 - 87.5 and 89.6 - 94.1dB(A), respectively and estimated L_{Aeq8h} values 82.4 - 85.4 dB(A). The evaluated PSIL are found to be in the range of 58.3 - 77.5 dB for about 80% of the daytime.

The road traffic noise levels reported for 22 sites in Lahore by Ahmad (1992 and 1994) show that in Lahore city traffic noise levels vary in the range of 74 - 90 dB(A) and average values in the range of 77 - 85 dB(A) and traffic noise level at these sites fluctuates in the range of 3 - 11 dB(A), which is unimaginable.

For community annoyance for cities with business, trade and administration, like Lahore (i) ISO - 1996 (1982) suggests maximum limit of 55 - 65, 50 - 60 and 40 - 50 dB(A) L_{Aeq} for daytime, evening time and night, respectively (ii) World Health Organization (WHO 1980) allows 55 dB(A) L_{Aeq} (iii) for urban residential areas with high background noise levels, Denmark (1982) allows 50 dB(A) L_{Aeq} for daytime (iv) for areas which are primarily residential, Germany allows 55 dB(A) L_{Aeq} for daytime and 40 dB(A) L_{Aeq} for night (Federal Republic of Germany 1974). Earlier surveys on road traffic noise nuisance show that more than 50% of the population, surveyed were annoyed at about 68 dB(A) in Paris (Aurbee 1971), 60 dB(A) L_{Aeq} in London (Longdom 1976) and 56 dB(A) L_{Aeq} in Stockholm (Fog and Jonsson 1968). For non-occupational noise exposure, Walsh-Healy noise rules (Anon 1969) allows 75 dB(A) L_{Aeq} for 8h a day or 80 dB(A)

Table 1
Road traffic noise levels at fifteen survey sites in Lahore city

S. no.	Place	Recorded range dB(A)	L_{A99} dB(A)	L_{A90} dB(A)	L_{A50} dB(A)	L_{A10} dB(A)	L_{A1} dB(A)	L_{Aeq8h} dB(A)
1.	Ferozpur road	60.8 - 95.6	63.2	73.5	80.7	87.3	92.7	83.9
2.	Centre point, gulberg	62.1 - 92.6	63.7	70.1	78.8	85.1	89.6	82.8
3.	Yateem Khana road	64.6 - 96.9	66.7	74.1	81.9	86.8	93.2	84.8
4.	Secretariat chock	62.6 - 92.4	65.5	71.9	81.2	86.1	90.6	84.8
5.	Mall road	64.7 - 93.5	64.7	73.3	81.3	86.8	91.5	84.5
6.	Shalimar chock	62.3 - 93.3	64.4	70.9	80.8	86.9	92.7	85.4
7.	Shah Alam chock	61.5 - 95.5	64.2	77.6	80.4	85.6	91.6	83.4
8.	Bhatti gate	63.2 - 97.3	65.4	72.1	81.2	87.5	94.1	85.4
9.	Chock-Sadder cantt.	62.3 - 91.7	63.6	70.6	79.6	85.9	91.7	83.6
10.	China chock	61.2 - 92.3	62.8	68.3	78.9	85.7	91.5	83.4
11.	Mazzang chock	62.3 - 91.8	64.4	70.3	78.9	84.4	89.8	82.5
12.	Chock chooburi	62.5 - 92.8	65.4	72.1	82.4	87.2	92.0	83.7
13.	Chock Ghari Soohahu	63.4 - 92.2	66.3	72.2	80.1	85.8	92.7	83.3
14.	Minar-e-Pakistan	60.4 - 95.5	64.2	71.5	78.9	86.6	92.6	83.7
15.	Model town	61.2 - 95.3	63.1	70.1	79.3	84.3	92.7	82.4

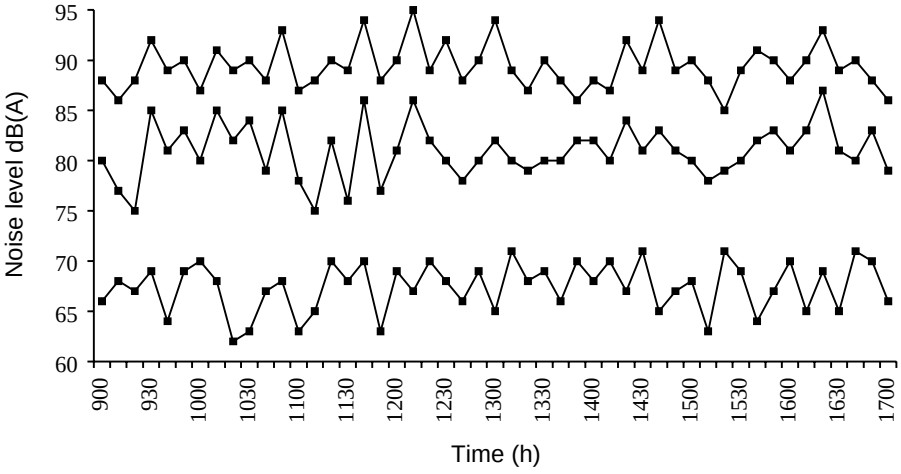

Fig 1. Diurnal variations in road traffic noise levels recorded at Shalimar Chowk from 0900-1700 h. Upper, lower and middle curves show the maximum, minimum and average values recorded in each measuring mode of 2 min duration between each sampling interval.

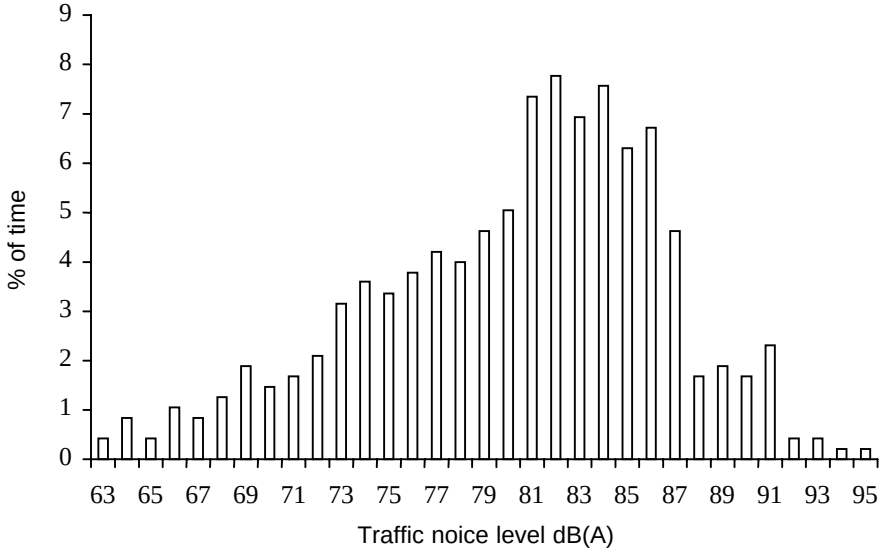

Fig 2. Statistical distribution of road traffic noise recorded at Shalimar chowk from 0900-1700 h.

L_{Aeq} for 4 h a day. For exterior noise in residential areas, Federal Highway Administration (Virginia Department of Highways 1972 and 1973) establishes a standard L_{A10} 70 dB(A) and US Department of Housing and Urban Development (HUD 1971) categorizes the site as unacceptable and discourages the construction of new buildings units, where exterior noise levels exceed 80 dB(A) L_{Aeq} for more than 1 h per 24 h or 75 dB(A) L_{Aeq} for 8 h per 24 h.

The results show that the L_{A90} values of noise levels at these survey sites exceed 68.3 dB(A), which are above the maximum permissible noise levels recommended for community annoyance in the urban residential areas. The L_{A50}, L_{A10} and evaluated L_{Aeq} 8 h values at these sides exceed 78.8, 87.5 and 82.4 dB(A), respectively, indicating that traffic noise levels in Lahore city are excessively high and much above the limits recommended for community annoyance and may result in adverse effects on roadside traders and dwellers, who are constantly exposed to such a high level non-occupational noise for a long duration. The PSIL values 58.3 - 77.5 evaluated above, show that for reliable face-to-face communication, between the speaker and listener at a distance of one metre, the speaker has to use 'raised' to 'shouting' voice (Webster 1968, 1969), which is discourteous. But due to poor education and lack of knowledge about the civic privileges and ill effects of high-level noise, no vigorous community action has been surfaced against highlevel traffic noise in the major cities in Pakistan.

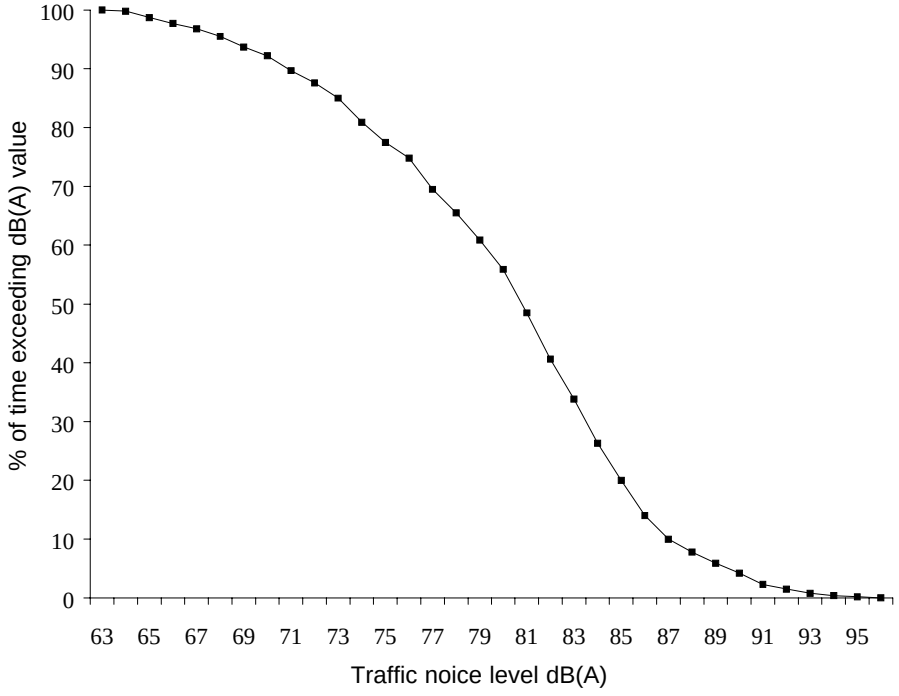

Fig 3. Cumulative distribution of road traffic noise recorded at Shalimar chowk from 900-1700 h.

As mentioned earlier (Shaikh and Shaikh 2000) the main reason of highlevel traffic noise in Pakistan is the absence of proper regulatory laws to limit highlevel traffic noise. The other reasons are poor model of vehicles, emission of high level noise from individual vehicles, use of defective silencers, use of pressure horns and other multi-tone devices, poor maintenance of vehicles, poor condition of vehicles, rash driving, etc.

The existing Motor Vehicle Rules (1969) in Pakistan may control emission of high-level noise from an individual vehicle to some extent, but due to some unknown reasons, these are not being implemented properly. The Pakistani standard (NEQS 1993) allows a limit of 85 dB(A) at a distance of 7.5 meters from the source, with no mention of type of vehicle and measuring technique, hence it may not be useful in controlling emission of highlevel from different type of vehicles. Therefore, in order to limit emission of highlevel noise from different type of vehicles, there is an urgent need to revise this standard. In this regard, as recommended earlier by one of the authors (Shaikh 2001), noise emission limits for different type of vehicles may be set as (i) 85 dB(A) for auto-rickshaws, buses and trucks, (ii) 82 dB(A) for motorcycles, mini-buses and mini-trucks and (iii) 80 dB(A) for cars and other light vehicles, which are very close to the motor vehicle noise emission limits recommended by the European Economic Community (EEC directives 1978, 1984a, 1984b), legislated and properly implemented. Eventually, one may hope to set Pakistani Standard in the light of type and engine capacity of the vehicles.

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PHYTOCHEMICAL ANALYSIS AND LAXATIVE ACTIVITY OF THE LEAF EXTRACTS OF *EUPHORBIA HETEROPHYLLA* LINN (EUPHORBIACEAE)

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A hot aqueous decoction of the leaves of *Euphorbia heterophylla* Linn (Euphorbiaceae) 1.24 kg gave on cooling and defatting with dichloromethane, an aqueous solution which on successively extracting with n-butanol and ethylacetate gave 25.89g and 1.31g of residue, respectively on removal of solvent. The semi-solid extract from the ethylacetate fraction on hydrolysis with dilute tetraoxosulphate (VI) acid gave a yellow powder which on acetylation gave colourless needle clusters identified as quercetin tetracetate. The butanolic fraction had laxative action and contained saponins, phenols, terpenes and diterpenes identified as phorbols but no anthraquinones. The residual aqueous solution contained mainly sugars identified as xylose, maltose, galactose, lactose and lactulose, which are bulkforming laxatives. The purgative action was found to be a joint action of both the phorbols in the butanol fraction and the bulk forming laxative sugars in the residual aqueous fraction.

Key words**:** *Euphorbia heterophylla*, Laxative activity, Tetraoxosulphate (VI).

Introduction

Hot aqueous decoctions of *E. heterophylla* leaves have been used for ages to produce purgation in the southeastern part of Nigeria (Gill 1992). The use is so common, that it became necessary to find out scientifically the chemical principles responsible for the purgation especially as some phorbols could function as co-carcinogens (Kinsella 1987; Shaofen *et al* 1991).

In the developing countries there are about 1 billion people living in extreme poverty and vast numbers suffering and dying for want of safe water and medicine, they have no alternative for primary healthcare (WHO 1995).

The aqueous extract of the leaves of *E. heterophylla* is used by the natives to produce purgation. Infact, according to a traditional herbal practitioner when the leaves are used to cook "yam porridge" purgation ensures within 3-4 h after consumption (Sevil *et al* 1993 and 1994).

Therefore, the objectives of this study is to ascertain the claim by the users of this herb and also to investigate the chemical principle(s) responsible for the laxative activity.

Experimental

Taxonomic identification of plant material. The plant material (leaves only) was collected at the University of Benin in March 2000 and identified by Prof. L S Gill of the Department of Botany.

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Preparation and extraction. The fresh leaves were collected, weighed and washed with distilled water. 1.24 kg of the fresh leaves of *E. heterophylla* was boiled with 1.5 litres of distilled water for 10 min and filtered then evaporated at 50˚C to a syrup liquid by using a rotary evaporator. The total aqueous decoction was first extracted successively with 3 x 200 ml and 100 ml of n-butanol. The residual aqueous extract was extracted with three aliquots parts of 200 ml of ethylacetate. The butanolic fraction was defatted with 200 ml of dichloromethane. The three fractions butanol, ethylacetate and dichloromethane were evaporated to dry- ness to give 25.902g, 1.312g and 0.858g, respectively. The three fractions were subjected to phytochemical studies.

Isolation. 0.470g of the yellow semisolid obtained on removal of the solvent from the ethylacetate fraction hydrolysed with 5ml of dilute tetraoxosulphate (VI) for 30 min. The precipitate obtained (designated FH) was re-dissolved in ethylacetate and subjected to thin layer chromatographic analysis using ethylacetate : n-hexane (9:1). ^{1}H-NMR, ^{13}C-NMR and IR spectrophotometric measurements were obtained.

Acetylation. 0.197g of compound FH was acetylated with 5ml acetic anhydride and 3 drops pyridine to give 90.312 mg of colorless needle clusters (designated FA). Compound F A was subjected to ^{1}H-NMR, ^{13}C-NMR and mass spectrophotometric analysis. The melting point was also determined with the kofler melting point apparatus.

Isolation and identification of the sugars in the extracts. The cold and residual aqueous fractions after the extraction

with n-butanol and ethylacetate were gave positive test for sugars; hence, the fractions were subjected to osazone formation.

Procedure. 2.082g of the fraction were reacted with 0.5 ml phenylhydrazine, 0.5ml glacial acetic acid and 1g of sodium acetate crystals. The solution was heated in a water bath at 100°C for 30 min. The yellow osazone crystallized out about 15 min after heating. It was filtered by suction through a heated buckner funnel. The hot filtrate was again heated and crystallization occurred on cooling. The shapes of the crystals were observed under the microscope. The process was repeated until a clear solution obtained and no crystallization took place.

Due to the variations and closeness of the melting of all osazones of the common sugars, the identification of the osazones was based primarily on its crystal form (Frederick and Bernard 1960).

Chemical investigation of the butanol fraction. The three extracts including total aqueous decoction, butanol and residual aqueous fractions were screened phytochemically and the results are shown in Table 1.

The butanol fraction 1.723g was dissolved in CH_3OH : H_2O mixture (17:3) to extract any phorbol esters. The water : methanol mixture was extracted with n-hexane to remove unwanted compounds such as triterpenoids and triglyceride (Rowan and Onwukaeme 2001). The hydroalcoholic layer was further partitioned against several aliquots of diethylether, sufficient water being added to produce two layers. The ether extract containing diterpenoid esters were combined; dried over anhydrous Na_2SO_4 and evaporated to dryness to give a residue 0.035 g. The phorbol esters in the diethylether layer were detected on TLC using antimony (III) chloride and 60% H_2SO_4, with a solvent system of chloroform: diethylether (95:5). The plates were developed in the oven for 5 min at 100°C. A single greyish color spot at R_f value of 0.96 confirmed the presence of diterpene (phorbol) esters in the butanol fraction.

Table 1
Phytochemical composition of the extracts of *E. heterophylla*

Phytochemical composition	Total aqueous decoction	Butanolic fraction	Residual aqueous fraction
Carbohydrates	+	-	+
Osazone formation	+	-	+
Saponins	+	+	-
Anthranquinones	-	-	-
Alkaloids	-	-	-
Flavonoids	+	-	-
Phenols	+	+	-
Terpene	+	+	-
Diterpenes	+	+	-

+ , indicates presence of compounds; - , indicates absence of compound.

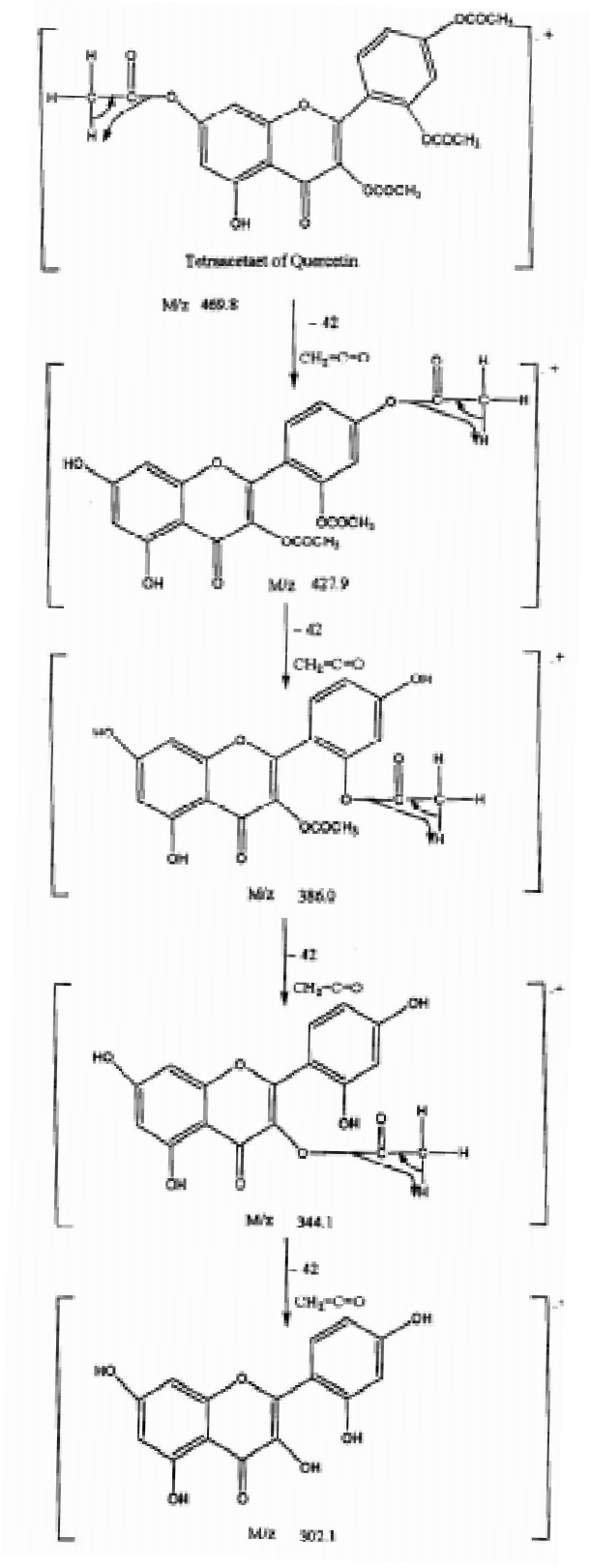
Laxative studies. The crude total aqueous decoction, butanol and the residual aqueous fractions were subjected to laxative tests using Lou's method (Lou 1949). Albino rats weighing between 115-240g were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmacy, University of Benin. They were kept in well ventilated rats cages (Lou's Cages) with free access to water and feeds (ad libitum) and left in this environment for 2 weeks to acclimatize.

Administration of the extract. The rats were divided into two (2) groups of 4 each. Group 1 for the test drug (*E. heterophylla*) while group II was for the control drug (*Cassia acutifolia* leaves-herb tea). The rats were food and water starved, fasted over the night preceeding the doses and each rat was placed singly into each compartment of the cage. The next day, the faeces were examined and the rat with wet faeces was excluded from the experiment. The weights of the rats were taken.

100, 200, 300, 400, 500, 600 and 700mg/kg doses of the aqueous decoction were given to the rat in each experiment. Similarly, rats of group II were given 100mg/kg to 700mg/kg of an aqueous extract of *C. acutifolia* as control. Immediately after dosing, the rats were given the mixture of food and water mixed together (10 parts of feed plus 7 parts of distilled water) and observed at every hour for 12 h for the excretion of wet faeces which were recognized by the wire adhesion method and also by physical examination (Latven *et al* 1951). At the end of the 12 h of observation the total number of wet faeces and of dry faeces were noted. The percentage of wet faeces was also calculated.

Results and Discussion

Compound FH. Melting point, 258°C; TLC, Ethylacetate : n-hexane (9:1); ^{1}H-NMR, (400 MHZ, DMSO-d_6), δ 2.45 (DMSO-d_6), 3.25 (H_2O from DMSO), 6.15 (1H, d, H-6), 6.42 (1H, d, H-8), 7.58 (1H, d, H-6), 8.0 (1H, dd, H-3′, H-5′), 9.38 (1H, OH at H-7), 10.8 (1H, OH at H-3) 12.6 (1H, strong intramolecular H-bonded, OH at C-5 to C=O at C-4); ^{13}C-NMR, (400 MHZ, $CDCl_3$,) signals at δ 146.0 (C-2), 136.4 (C-3), 176.2 (C-4), 159.6 (C-5), 98.8 (C-6), 165.3 (C-7), 95.0 (C-8), 160.0 (C-9) 154.0 (C-10, 123.5 (C-1′), 116.8 (C-2′),



Fragmentation pattern of compound FA

Scheme 1

145.0 (C-3′) 148.6 (C-4′), 116.9 (C-5′), 123.7 (C-6′), 40.6 (DMSO); IR, (cm^{-1}), 3400 (OH, intra molecular H-bonded), 2365.8 (due to inequalities in path length), 1695 (C=O), 1168.1, 1076.9 (C-O stretching), 997.8 (C=C), 821.6 (2 isolated aromatic C-H), 880 (1 isolated aromatic C-H).

Compound FA. Acetylation of 0.1975g of compound FH gave 90.31mg of FA as colorless needle clusters.

Melting point, 165°C; TLC, Ethylacetate R_f 0.63; ^{1}H-NMR, (400 MHZ, $CDCl_3$), 1.5 (H_2O from $CDCl_3$), 7.25 ($CDCl_3$), 2.38 (9H, s, 3 acetylmethyl protons at C-4′, C-2′, C-7), 2.42 (3H, s, 1 acetylmethyl proton at C-3), 6.85 (1H, d, H-6), 7.34 (1H, d, H-8), 7.36 (1H, d, H-6′), 7.7 (1H, dd, H-3′), 7.72 (1H, dd, H-5′); ^{13}C-NMR, (400 MHZ, $CDCl_3$), 20.5 (aliphatic CH_3 of O-CO-CH_3), 77.3 (chloroform-d_6), 150.5 (C-2), 167.9 (C-3), 170.1 (C-4, C=O), 167.9 (C-7), 167.87 (C-2′), 167.8 (C-4′), 144.5 (C-5), 124.1 (C-9), 126.6 (C-10), 127.9 (C-1′); MS (70eV) M/Z, 469.8 (6%), 427.9 M^+ (64%), 386.6 (90%), 344.1 (100%), 302.1 (90%), 273.2 (20%), 245.2 (14%), 217.2 (10%); IR (cm^{-1}), 3460 (free OH), 3099.1 (-$COCH_3$), 2335 inequalities in path length), 1690 (C=O), 1168.1, 1076.9 (C-O stretching), 997.8 (C=C), 821.6 (2 isolated aromatic C-H), 880 (1 isolated aromatic C-H).

The MS for compound FA showed the presence of M^+ at 469.8 formulated as $C_{21}H_{16}O_{10}$ quercetin tetracetate requires 470.3886. The sequential loss of a four 42 units in the fragmentation pattern of FA (peaks 469.8 to 427.9 to 386.0 to 344.1 and to 302.1) indicate the loss of four ketene units arising from four acetate groups (scheme 1). The ^{1}H-NMR showed the presence of a signal at δ 2.38 integrating for 9 protons corresponding to the 9 protons of the acetyl methyl groups located at the equivalent positions C-2′, C-7 and C-4′. The 3H signal at δ 2.42 is ascribed to the 3 protons of the acetylmethyl group at (C-3).

^{13}C-NMR spectrum revealed the presence of four ester carbonyl carbons resonating at δ 167.943, 167.865, 169.370 and 170.126. The ketonic carbonyl at C-4 appeared in the spectrum at δ 170.126. This is also evident in the ^{13}C-NMR spectrum of quercetin (FH). Both FH and FA gave positive tests for flavonoids.

These data with the melting point characterised compound FA as the tetracetate of quercetin and its precursor as quercetin by inference. The phytochemical screening of the three fractions showed the absence of alkaloids and anthraquinones (known laxative agent) but showed the presence of sugars, flavonoids and diterpene (phorbol esters) (Table 1). The total aqueous decoction and residual aqueous fractions tested positive for a variety of sugars found to be xylose, lactose, glucose, galactose, maltose and lactulose. The presence of

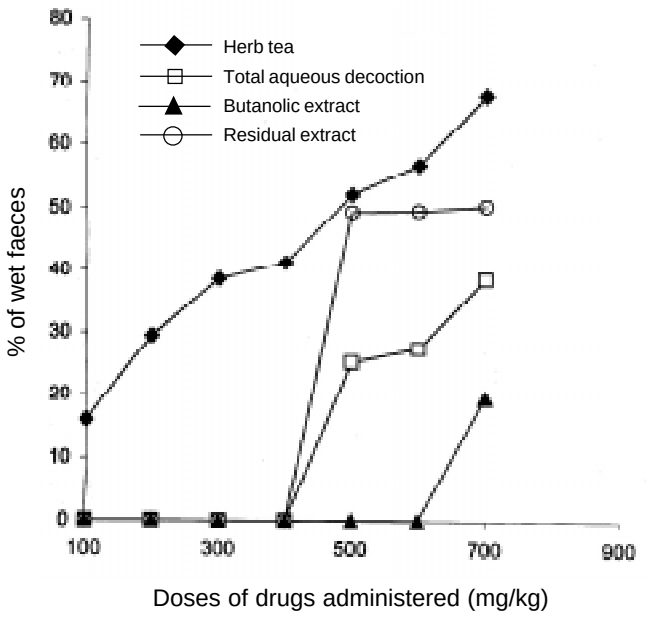


Fig 1. Graph of percentage of wet faeces produced by herb tea and fractions of *E. heterophylla.*

diterpenes (phorbol esters) was indicated in the total aqueous and butanolic fractions. The laxative studies showed a higher laxation by the residual aqueous fraction (49.3%) than that produced by the total aqueous fraction (25.2%) at doses of 500mg/kg body weight. The butanol fraction produced no laxative effect at a dose of 500 mg/kg. However, production of laxative effect at doses of 700mg/kg and above by the butanol fraction is attributable to the presence of small quantities of phorbol esters. At a dose of 700mg/kg, the laxative activity produced by the residual aqueous fraction, total aqueous decoction and the butanol fraction were 50.41%, 38.53% and 19.54%, respectively (Fig 1). Therefore, the laxative activity produced by the total aqueous decoction (38.5%) was due to the combined action of the sugars and the phorbol esters. For the butanol fraction, the 19.54% laxative effect produced at 700 mg/kg was probably due to the mild irritant action of the phorbol esters (Shaofen and Kiven 1991). On the other hand, the increased laxation produced by the residual aqueous fraction (50.41%) is due to the presence of the sugars namely xylose, glucose, lactose, lactulose and maltose, which are bulk-forming laxative by comparison with the laxative *Plantago* psyllium which is known to contain similar chemical compounds. Further studies will be carried out to isolate and characterize the phorbol esters.

Conclusion

The chemical principles responsible for the laxative activity of the leaves of *E. heterophylla* have been shown to be the sugars, xylose, lactose, galactose, glucose, lactulose and maltosc. The phorbol esters in the butanol fraction which showed complete absence of the sugars are partly responsible for the laxative activity.

Compound FH was isolated from the leaf of *E. heterophylla* and characterized as quercetin. FA (acetylated derivative of querectin) was synthesize and characterized to confirm the structure of FH.

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ULTRASONIC STUDIES ON SOME AQUEOUS SOLUTIONS OF CARBOHYDRATES AT THREE DIFFERENT TEMPERATURES

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Density, viscosity and ultrasonic velocity measurements have been performed by ultrasonic interferometer technique in some aqueous solutions of three carbohydrates (sucrose, D-glucose and β-D lactose) as a function of molality with different concentration of the order 0.1 to 0.5 mole/kg. At three different temperatures, via 303.15K, 313.15K and 323.15K and at atmospheric pressure, ultrasonic velocity, partial molar volumes, partial molar isentropic compressibility have been calculated and plotted against concentration. The velocity results confirm the conclusions that were originally derived from viscosity data by Einstein that the sugar molecules have a 'salvation envelope' attached with a layer of water molecules which decreases with thickness as the temperature of the solution rises. It has been observed that in such solutions it is quite legimate to look for the dispersion caused by viscosity of the solutions. The apparent molar volume occupied by solute molecules remains constant at one particular temperature, irrespective of the change in concentration of sucrose in water. The compressibility increases slowly as a function of concentration at constant temperature. The data revealed that the compressibility of these different solutions is related with three diamensional hydrogen bond water structure. It is governed by the stereochemistry of carbohydrate. By these studies an overview of the hydration characteristics and the effect of relative position of hydroxyl group within a carbohydrate molecule is given. By increasing the carbohydrate concentration in water the ultrasonic velocity increases, while there is no effect on compressibility of moles. For monomer the apparent molal compressibility depends on the hydration of the mole. The results agree with the previously obtained kinematics data of literature values.

Key words**:** Carbohydrates, Concentration, Density, Viscosity, Isentropic compressibility, Ultrasonic velocity, Apparent molar volume.

Introduction

The velocity of elastic waves in solution is defined by solute-solvent and solute-solute interactions which are determined by the chemical structure of the solute and solvent molecules. (Nobuo and Kyoshi 1974). However, a clear cut picture of results have been obtained by uncertainities about intrinsic volumes of solute molecules. Still acoustical methods have been developed, with only a minor contribution to the detailed description of the solute-solvent interactions of molecules in aqueous solutions. The successful applications of acoustical methods to the physicochemical investigation of solution became possible after the development of adequate theoretical approach and methods for the precise ultrasonic velocity measurements in small volume of liquid mixture.

Enormous literature is available on the physical and chemical nature of carbohydrates molecules and their structure, most of them are monomers and some are dimmer. Glucose is a dimmer, which is able to rotate about its linkage such that the hydrophobic surfaces were bound through intermolecular forces. These have much interaction for water molecules and it seems reasonable to expect the voids in volume associated with each solute molecule (Paul *et al* 1999). A group of physicts in USSR (Shel'nikov and Privie 1991) have worked on the bulk elastic properties of glucose, galacotose, lactose, sucrose and starch. From their data on ultrasonic velocity and the density of the solutions, the hydration number of carbohydrates in relation to the stereo chemistry have been a subject of study since a long time and due to their importance in our life (Blanshard and Mitchell 1979).

The compressibility of solution can be determined by the effect of solvent, solute and solution. (Roscoe 1952; Mark 1960). The effect of solute molecules can be separated into three parts, the compressibility of solute molecules, solute-solute interactions and the size of the molecule. If the concentration of the solution becomes very low, the second effect becomes negligible (Pryor and Roscoe 1954) measured the ultrasonic velocity of the solution of sucrose and sacchride from the temperatures of 20˚C to 80˚C and they showed qualitatively that the salvation envelop decreases in size with increasing temperatures, provided the sugar molecules could

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be regarded as incompressible but they did not approach to zero concentration.

It is a well-known fact that in solution of electrolytes, the water molecules are bound to the electrolytic ions that are compressed owing to the strong electric field within the ions to form a hard structure. From the literature (Arakawa and Sasaki 1972), it is seen from the adiabatic compressibility measurements, the degree of hydration can be evaluated. They assumed that the compressibility of bound water and of the ions itself is negligibly small. Shio (1958) has deduced a more general formula for the amount of bound water by taking into the account, the compressibility of bound water and of solute particles. This was applied to solutions of three carbohydrates (sucrose, β-D-lactose and glucose). Details of few parameters related with this structure study at three temperatures can be found here.

The partial molar volume V_ϕ. It is informative for a solution of the character of solute-solvent interactions. It is the sum of the intrinsic volumes of the solute and the volume contribution due to a solute-solvent interactions (Miller *et al* 1997)

V_ϕ = Vintrinsic + Vsolute + solvent

Isentropic partial molar compressibility β_o. This is a pressure derivative of partial molar volume V^o. It will directly reflect the carbohydrate+water interactions. If it is assumed that molecules of all carbohydrates are incompressible. The compressibility can be measured at constant temperature or at constant entropy. In order to determine the isothermal apparent molar compressibility, we have to know about its expansibility and heat capacity of the solution to calculate from the isentropic compressibility. The isentropic and isothermal compressibilities are different numerically, however when one is interested to use either of them. (Arakawa and Sasaki 1972).

The purpose of this study is to discuss the results of a physico-chemical study on three carbohydrates water mixtures with varying viscosity and density measured at three different temperatures. Ultrasonic velocity that potentially seems viable deals with our investigation of the thermodynamic and transport properties of these mixtures. This work is in continuation of our previous investigation by ultrasonic properties of liquids glycol and their mixtures.

Materials and Methods

All the carbohydrates (sucrose, D-glucose, β-D lactose) taken were made anhydrous. Carbohydrates were supplied by Koch Light Laboratories, France, (Analar grade) and dried in a vacuum oven at 333K before use. The low conductivity water was used for preparing the solution, which were made by passing distilled water through a cation anion resins. The specific conductance of water was $5x10^{-8}$ ohm/cm. All the solutions were prepared by weight percentage and the necessary corrections were applied for the water content in carbohydrates. The doubly distilled water was used for making the solution. All the samples were kept in glass stopper flasks. All the measurements were carried out at each temperature for five different concentrations of every carbohydrate. The same solutions were used for density, viscosity and isentropic compressibility measurements.

Sample preparation. The mixtures were prepared by mixing known mass of carbohydrates in pure water in an air tight ground stopper bottle taking due precautions for exposing them from the humid atmosphere. All the weights were taken on an Ohaus electronic balance accurate down to ±0.01 mg, the possible error in the mole fraction is estimated to be less than $\pm 2.5x10^{-4}$ mg.

Density measurements. Density of each solution was measured at 303.15K, 313.15K and 323.15K by using Pycnometer, (Limpkins double stem with volume 18 ml). The Pycnometer was calibrated with conductance water immediately after density measurement, the error in the density measurement was estimated to be $\pm 3x10^{-3}$ kg/m^3.

Viscosity measurements. The viscosity of each solute and solvent (sugar solution) was conducted independently by 'Happler's falling ball viscometer at various temperatures within an accuracy of ±1%. The apparatus is calibrated each time against the standard solutions before measurements of the viscosity. It is determined from the rate of fall of steel ball using the formula (1) (Weissberger A 1959).

Viscosity can be evaluated by Equation (1):

$\eta = KT (ds-dl)/dt$ ..... (1)

Where, K is the Boltzman's constant and T is the absolute temperature, ds and dl are the density of solid ball and density of liquid.

Compressibility and velocity measurements. By the interferometer technique of ultrasonic wavelength measurements, see for pictorial representation of diagram (Ahmad *et al* 2000) in which a conventional 300W of power output from Scholler (USLG-300) is an ultrasonic wave generator was employed for producing longitudinal stress waves. They excite the x cut crystal (Piezoelctric transducer) through a 50 ohm line in the presence of an air pressure of 50 Lbs/inch^2. On the top of the transducer is a water seal proof glass cell, is the liquid sample holder is placed for measuring the wavelength of the waves.

The interferometer can have a great advantage for good parallelism by micrometer position. The surface of the transducer

and the parallelism is established by the application of pulse echo method. The piezoelectric transducer is driven through loosely coupled coils by a source of constant frequency (2.0 MHz) and the current across the crystal which is indicated in milliampere meter. By moving the variable ganged capacitor in the master oscillator, the sharp resonant frequency is selected from the upper face of the transducer and are sent through liquid to the reflector, By means of the micrometer the separation between the reflector and transmitter can be varied while in turn it produces the phase change of the waves incident on the receiving plate of the micrometer. It produces a variation in the output current of the generator which repeates as the phase of the wave by odd multiple of λ in the liquid mixture. The principle is known as single round (Gamsey *et al* 1969), which involves the passage of repeated pulses of ultrasonic waves through the solution and measurement of wavelength of these radiations.

Frequency measurements. The frequency of the ultrasonic waves were measured by frequency counter TRIO- FC 754-A (Keenwood) Japan, with display time 1 sec averaging over a period of 10 sec with full scale deflection of 10Hz to 500MHz.

Temperature measurements. A glass thermometer with a least count of 0.01°C/div was used for liquid temperature measurements.

Calculation of ultrasonic parameters. *Ultrasonic velocity, V*: By varying the Screw Gauge head position up or down which is connected with reflector and immersed in liquid, the reflector is moved through each λ multiplying with the frequency of the waves, the ultrasonic velocity is calculated as:

$$V = (\lambda \text{ meter x frequency MHz}) \text{ m/sec} \quad \ldots\ldots (2)$$

Partial molar volume V_ϕ: After measuring the density of the liquid carbohydrates solutions at different molalities, the apparent partial molar volume can be obtained by using equation 3:

$$V_\phi = 1000(d-d_0)/mdd_0 + M_2/d^* \text{ cm}^3\text{/mole} \quad \ldots\ldots (3)$$

Where, m is the molalities of carbohydrates, M_2 is the molar mass of the carbohydrate, the partial molar volume is obtained by plotting the apparent partial molar volume against molality (Rod *et al* 2000). d*, density of solution and d_0 is density of water.

Isentropic compressibility coefficient β_s: They were measured by monitoring the speed of sound through a solution in an adiabatic and reversible way, which ensures that the compressibility is isentropic from this data, the compressibility coefficient β_s can be obtained, by equation 4:

$$\beta s = 1/u^2 \underline{\sim} - 1/V\ (dV/dP)_s \quad \ldots\ldots (4)$$

Where, 'u' is the speed of sound in liquid and 'd' is the density of liquid, 'V' is the volume and 'P' is the atmospheric pressure. The 'β_s' can be used to calculate the apparent partial molar compressibility, K_ϕ via the different method.

$$K_\phi = 1000(\beta_s-\beta_{so})/md + \beta_s V_\phi \text{ cm}^3\text{/mole/bar} \quad \ldots\ldots (5)$$

Where, (β_s and β_{so}) are the isentropic coefficient of compressibility of the solution and water respectively, 'm' is molality of the carbohydrate, 'd' is the density of solution and 'V_ϕ' is the apparent molar volume at that concentration. By definition the limiting value of the isentropic apparent molar compressibility $K_s(\phi)$ is equal to the isentropic partial molar compressibility:

$$K_2(\phi) = K_\phi(s) + mdK_\phi/dm \quad \ldots\ldots (6)$$

Results and Discussion

Analysis. When anhydrous sucrose or glucose is dissolved in water, the volume of the resulting solution is almost equal to the sum of the volumes of water plus solid sugar particles. The ratio of the volume of sugar particles to the volume of solution thus gives the fractional volume of the solution occupied by the sugar molecule. Since a water molecule is much smaller in size than a sugar molecule, it is reasonable to suppose the solution to behave as suspension of solid particles occupying fractional volume c in homogeneous liquid. The solid particles are regarded as compressible, since the compressibility of individual sugar molecules must be very much less than the pure water, so that the adiabatic compressibility of the solution should be:

$$\beta = \beta_o(1-c) \quad \ldots\ldots (7)$$

Where, β_o is the adiabatic compressibility of water. The viscosity of sugar solution can also be treated by regarding a solution as suspension of solid particles occupying a fractional volume c in a homogeneous liquid. Here again when ultrasonic waves are passed through this suspension, the dispersion of the waves takes place due to particles and the temperature of the solution rises and the viscosity decreases. The collision force of ultrasonic waves, is of the right order, but the decrease in viscosity with rise in temperature, is observed at high concentration. The observed values of heat of solution are always higher than calculated values. Assuming that the volume of sugar molecules is effectively increased by a layer of attached water molecules (Panday *et al* 1987).

We have chosen to measure isentropic compressibility through these molar measurements. The following points are worth mentioning in regard to its molecular structure.

- By increasing the carbohydrate concentrations in water

Table 1
Measured and calculated values of results of thermodynamic measurements at three temperatures (303.15K, 308.15K and 313.15K) for sucrose + water solutions

S. no.	Temp. in K	Conc. mole/kg	Density kg/m^3	Viscosity c poise	Velocity m/sec	Partial molar volume V^E cm^3/mole	Isentropic compressibility cm^3/mole/bar x10^4
1.	303.15	0.1	1028.1	1.0021	1483.50	126.33	-17.66
		0.2	1036.0	1.0191	1488.80	128.14	-17.33
		0.3	1040.0	1.0212	1493.20	123.33	-17.06
		0.4	1041.0	1.0342	1496.10	126.25	-16.92
		0.5	1043.1	1.0591	1499.00	125.75	-16.75
2.	313.15	0.1	1046.7	1.1179	1501.50	133.33	-16.66
		0.2	1045.5	1.1190	1507.33	139.12	-16.11
		0.3	1048.3	1.1221	1519.19	140.55	-15.33
		0.4	1057.0	1.1371	1522.44	141.25	-15.25
		0.5	1059.1	1.1443	1528.22	141.33	-15.07
3.	323.15	0.1	1055.4	1.0021	1495.55	142.11	-12.55
		0.2	1063.3	1.1221	1499.33	143.14	-11.03
		0.3	1109.3	1.0212	1517.11	144.52	-10.55
		0.4	1114.7	1.0342	1529.33	145.34	-10.15
		0.5	1119.5	1.2171	1533.40	146.77	-09.87

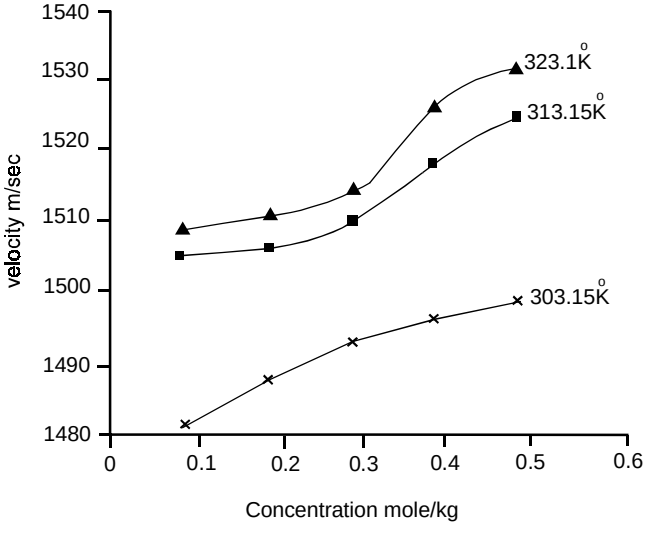

Fig 1. Variation of ultrasonic velocity with concentration at three different temperatures for sucrose + water.

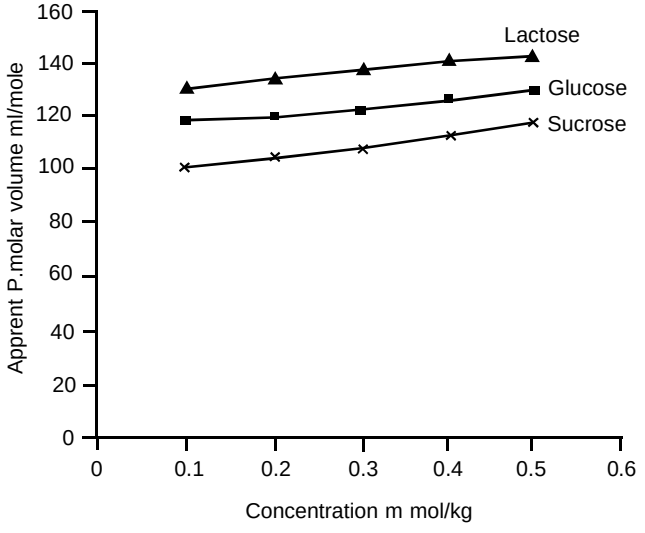

Fig 2. Apparent partial volumes as a function of concentrations of three different carbohydrates molecule at 303.15K.

the ultrasonic velocity increases, while there is slow rise in compressibility of moles at each temperature. See Fig 1, 2 and 3.

• Density is an important quantity in a sense it dictates how much free volume is available to each carbohydrate molecule in the system.

• The limiting values of isentropic compressibility directly gives insight into the compressibility of the hydration layer compared to that of pure water. The relative position of OH determines the hydration characteristics of such molecules.

• The molecular picture is that the distance between the oxygen and next nearest neighbor OH group within the carbohy-

Table 2
Measured and calculated values of results on thermodynamic measurements at three temperatures via 303.15K, 308.15K and 313.15K for glucose + water solutions

S. no.	Temp. in K	Conc. mole/kg	Density kg/m^3	Viscosity cp	Ultrasonic velocity m/sec	Partial molar volume V^E cm^3/mole	Isentropic compressibility cm^3/mole/bar x 10^4
1.	303.15	0.1	951.44	0.981	1505.00	126.33	-21.10
		0.2	953.20	0.987	1507.33	128.14	-21.30
		0.3	957.11	0.991	1512.20	123.33	-21.10
		0.4	959.55	0.998	1522.40	126.25	-20.44
		0.5	962.25	1.013	1528.20	125.75	-19.22
2.	313.15	0.1	988.30	1.022	1522.50	126.33	-19.00
		0.2	965.00	1.013	1497.00	128.14	-18.75
		0.3	967.30	1.008	1499.20	123.33	-18.55
		0.4	1008.00	1.005	1506.10	126.25	-18.44
		0.5	1015.10	1.091	1509.22	156.05	-18.33
3.	323.15	0.1	1015.10	1.087	1533.50	126.33	-18.22
		0.2	1019.50	1.097	1523.90	128.14	-18.13
		0.3	1025.30	1.118	1527.00	123.33	-18.10
		0.4	1033.00	1.125	1536.20	126.25	-18.02
		0.5	1037.30	1.132	1539.20	162.05	-18.00

The velocity of sound in distilled water = 1479 m/sec at 30°C; Formula wt of glucose (anhydrous)=342.32

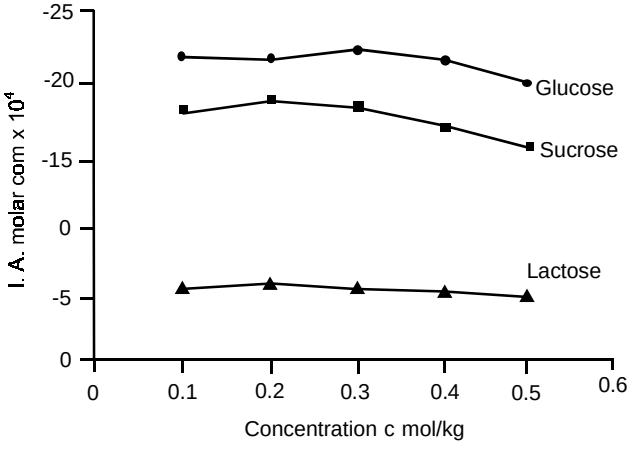

Fig 3. Isentropic apparent molar compressibility (negative values) as a function of concentrations of three different carbohydrates at 303.15K.

drate molecule is comparable with the oxygen distance between the water molecule.

• In this experimental study a molecular model resembles with that of Enistein model (Blanshard and Mitchell 1979), that a sugar molecule has a solvation envelope attached with a layer of water.

• Since glucose is a dimmer, which is able to rotate about its linkage such that hydrophilic surfaces were bound by intermolecular force and which increases the net hydrophilic character (Mark 1959).

• It is found through literature that hydration of carbohydrate is mostly dependent on the relative position of OH within the nearest neighbor in the bond structure. As we have evaluated in the above three carbohydrates, which have small partial molar volume due to extensive solute solvent interactions.

Conclusion

The following conclusions have been drawn from the above results on carbohydrates+water solutions within the limits of certain experimental errors:

• The sound velocity in such solutions depends upon concentration or viscosity. It increases with concentration of carbohydrates in water showing no complex formation which can occur in the above concentration region at one particular temperature. Density is particularly an important quantity to evaluate how much free volume is available to each molecule in the solution and there is a correspondence between free volume and density.

• At each temperature in the above three sugar solutions there is an increase in the partial molar volume which shows a formation of intermolecular hydrogen bonds (Miller *et al* 1997).

• The viscosity of sugar solutions can also be treated by regar-

Table 3

Measured and calculated values of results of thermodynamic measurements at three temperatures via 303.15K, 313.15K and 323.15K for β-D lactose + water solutions depending upon the constant values*

S. no.	Temp. in K	Conc. mole/kg	Density Kg/m^3	Viscosity cp	Ultrasonic velocity m/sec	Partial molar volume V^E cm^3/mole	Isentropic compressibility cm^3/mole/bar x 10^4
1.	303.15	0.1	1021.33	1.1121	1509.55	103.22	-6.00
		0.2	1015.22	1.1990	1512.11	104.14	-6.00
		0.3	1019.14	1.1198	1517.32	107.33	-5.70
		0.4	1022.50	1.2390	1529.11	106.22	-5.50
		0.5	1032.25	1.2419	1533.44	109.75	-5.22
2.	313.15	0.1	1061.25	1.2401	1523.00	110.00	-5.03
		0.2	1075.33	1.2231	1527.33	111.15	-4.97
		0.3	1077.00	1.3081	1521.11	114.55	-4.80
		0.4	1083.00	1.3750	1536.11	115.15	-4.72
		0.5	1085.00	1.3910	1549.22	116.05	-4.22
3.	323.15	0.1	1088.22	1.4050	1483.55	122.22	-4.06
		0.2	1092.05	1.4130	1553.99	126.14	-4.05
		0.3	1082.07	1.4280	1.564.12	128.55	-4.00
		0.4	1177.00	1.4340	14.96.10	130.15	-4.00
		0.5	1167.33	1.4310	1582.22	131.05	-3.90

*Molar compressibility of pure water $\beta_o = 8.17x10^{-4}$ cm^3/mole/bar; Formula weight of β-D lactose (anhydrous) = 342.32

ding a solution at suspension of solid particles occupying a fractional volume c in a homogenous liquid. (Roscoe 1952), when elastic waves were passed through the solution the heat energy is produced.

• The negative values of partial molar compressibilities of the three samples shows a type of linkage between their subunits at constant temperature or entropy, obvious from Fig 3. The negative values of compressibility suggests that when ions are introduced in to water they break the water structure by electro restriction force, the water around the ion is dense and less compressible.

• The amount of bound water in cc/g can be determined in by evaluation of compressibility.

• The limiting values of compressibility of the three solutions directly gives insight in the compressibilities of hydration layer compared to that of pure water i.e. $8.17x10^{-4}$ cm^3/mol/bar.

• The hydration number of such carbohydrate mixture depends on their composition (weight %) with water and temperature and detailed microscopic model (Uedair and Ikura 1989).

• Through this kinetic experiment we have tried to substantiate our results by measuring ultrasonic velocity at 303.15K 323.15K and 313.15K.

• These results show that OH radical plays an important role in the equilibrium and biochemical properties of solutions. The ultrasonic study seems to provide an adequate technique for the study of complex formation ion within these solutions.

• At infinite dilution the partial molar volume equal to the apparent partial volume (Gelma *et al* 1990).

• The above results are in good agreement with literature values (Paul *et al* 1999).

• Within the range of cm^3 mole/bar/concentration for each sample, the partial molar volume rises slowly for one temperature and at constant pressure as shown in Fig 2.

• Pressure derivative of partial molar volume will directly reflect carbohydrate + water interactions, if it is assumed that their molecules are incompressible.

• The viscosity of sugar solutions increases very slowly at low temperature. The solid particles occupying a fractional volume c in a homogenous liquid.

• The equilibrium composition of a carbohydrate in water is

strongly affected by pressure which was reported by (O'Connor *et al* 1983).

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Short Communication

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PULSE VOLTAMMETRIC DETERMINATION OF pK_a OF PARA-CHLOROANILINE

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Monochloroderivatives of aromatic amines are the degradation products of commonly used herbicides. The oxidation potential of monochloroanilines at a number of solid electrodes has been found to be dependent on the number of substituents in the ring system (Suatoni *et al* 1961). Voltammetric detection of these compounds and their separation in a mixture by liquid chromatography has been reported (Purnell and Warwick 1980; Hart *et al* 1981). Large amplitude pulse voltammetric experiments suggest that the oxidation reaction of 4-chloroaniline involves the elimination of the one electron as the initial oxidation reaction at the carbon paste anode. The experiments were performed by varying the values of rest potential, drop-time and sweep rate in large amplitude pulse voltammetry. The reasonably constant value of limiting current shows that 4-chloroaniline can be used as a standard for 1-electron oxidation for the investigation relating to the electrochemical studies of selected aromatic amines (Haque 2002). More recent work on these compounds relates to electrodegradation kinetics of *p*-chloroaniline at solid anodes (Casado *et al* 1994; Brillas *et al* 1995). The acidity and basicity of benzene and its derivatives including para-chloroaniline have been treated theoretically in terms of a new quantitative parameter (Feng *et al* 1995). Effect of potential on the adsorption of *p*-chloroaniline on silver electrodes has been studied using surface-enhanced Raman spectroscopy (Xu *et al* 1993). Haloaminobenzenes continue to be the focus of research (Freccero *et al* 2003). Recently their electrochemistry was reviewed (Haque 2003).

Normal pulse voltammetry has been used to determine pK_a of *p*-chloroaniline. The result is in close agreement with the value reported in literature. The procedure outlined in this work represents an example of a new method for the determination of pK_a of *p*-chloroaniline.

Preparation of buffers. Buffer solutions of the pH range 1.8-6.5, were adjusted to an ionic strength of 0.20 mol/dm^3 by addition of the required amount of sodium chloride. The buffer solutions used for electrochemical experiments in specific pH range and their total buffer concentrations were as follow: pH 1.8-3.1, phosphate (0.04 mol/dm^3); pH 3.7-5.7 acetate (0.04 mol/dm^3); pH 5.7-6.5, phosphate (0.04 mol/dm^3). pH was noted by using a digital pH meter and combination glass electrode (Rusling *et al* 1983).

Solutions of *p*-chloroaniline were prepared by dissolving the compound in buffers of pH 1.8-6.5, so that the final concentration of *p*-chloroaniline was $1x10^{-4}$ mol/dm^3. Purified nitrogen was bubbled through the solutions for five to ten minutes to remove oxygen and a nitrogen atmosphere was maintained above solutions during voltammetry.

Carbon paste electrode. The carbon paste electrode (CPE) was a disc of area 0.12×10^{-4} m^2 surrounded by a Teflon collar of diameter 0.3×10^{-2} m. The carbon paste was prepared from Fisher ACS grade graphite powder, grade no. 38 and nujol as described in literature (Adams 1969). The surface of this electrode was renewed prior to each scan.

Counter electrode. A platinum strip, A = 1×10^{-4} m^2, or a platinum wire was used as a counter electrode. Reference electrode (silver-silver chloride electrode): A low resistance silver-silver chloride saturated KCl, reference electrode was used for experiments in the aqueous system (Sawyer *et al* 1995). Voltammetry in a three electrode cell was done using conventional electrochemical instrumentation (Haque 1989).

The anodic normal pulse voltammetric behavior of para-chloroaniline was investigated at the carbon paste electrode over the pH range 1.8-6.5. The normal pulse voltammograms for *p*-chloroaniline in various buffers showed well formed plateaux (Haque 2002). At half the plateaux currents, E½ values were noted in various buffer solutions for the pH range 1.8-6.5. E½ vs. pH plot for $1x10^{-4}$ mol/dm^3 para-chloroaniline solutions in these buffers are shown in Fig 1. The first break in E½ vs. pH curve, in Fig 1, corresponds to the pKa value relating to deprotonation of the anilinium species. The value obtained 4.15 is the same as determined using spectrophotometry (Hart *et al* 1981). The first linear portion of E½ vs. pH plot shows a slope of -58 mV/pH. This value of the slope (Heyrovsky and Vavricka 1972) is consistent with the following equilibrium prior to charge transfer:

$$p\text{-}ClC_6H_4NH_3^+ \leftrightarrow p\text{-}ClC_6H_4NH_2 + H^+ \quad \text{.....(1)}$$

Our results thus establish that normal pulse voltammetry affords a reliable route to the determination of pKa value of the reaction noted as in equation (1) above. For comparison, it is mentioned that cyclic voltammetric oxidation of para-chloroaniline at glassy carbon electrode gave pKa value of 5.5 in Briton-Robinson buffers of pH 1.95-12.0 containing 50%

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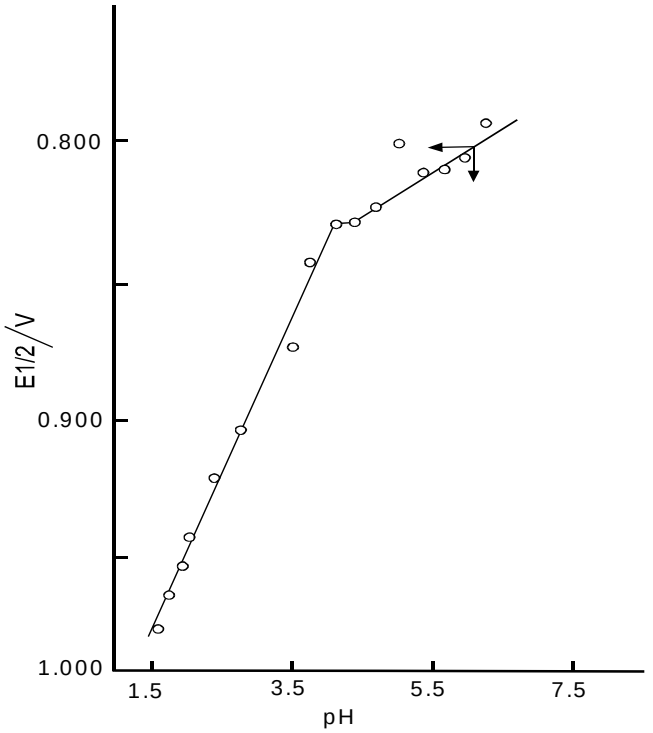

Fig 1. pH vs. half wave potential, E½ for the normal pulse voltammetric oxidation of $1x10^{-4}$ mol/dm^3 *p*-chloroaniline.

by volume of ethanol in water (Hart *et al* 1981). Cyclic voltammograms of $1x10^{-4}$ mol/dm^3 para-chloroaniline were also recorded in various buffer solutions over the pH range 1.8-6.5. The variation of Ep/2 with pH for the anodic peak of *p*-chloroaniline was linear with a break at pH equal to 4.65. This pKa value is higher than that determined by spectrophotometry (Hart *et al* 1981).

It is shown that in contrast to cyclic voltammetric method, normal pulse voltammetric method yields the correct value 4.15, for the pK_a of *p*-chloroaniline.

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COMPARATIVE ECOLOGICAL STUDY OF PHYTOPLANKTON. *PART II*. BAKAR AND PHOOSNA LAKES - PAKISTAN

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A comparative ecological survey of phytoplankton part II of Lake Bakar, district Sanghar and Lake Phoosna, district Badin was carried out during August, 1993 to July, 1996. A total of 122 species belonging to 45 genera of 15 families of 5 orders of class Chlorophyceae were recorded. 11 species were common in both Lakes. 94 species were present in Lake Bakar and 17 in Lake Phoosna. The study showed that the aquatic environment of Lake Bakar is qualitatively much better as compared to Lake Phoosna.

Key words: Phytoplankton, Bakar lake, Phoosna lake.

Introduction

Phytoplankton is an important group of algal flora. These are the producers of food in the food cycle of aquatic ecosystems, fixing energy by the process of photosynthesis. The phytoplankton are widely distributed and are an important component of various ecosystems like marine, rivers, ponds and streams etc. Algal flora is a good indicator of pollution (Patrick and Reimer 1966) and in the water bodies receiving animal, poultry and household waste.

Qualitative and quantitative determinations of phytoplankton are essential for determing the aquatic productivity as they are the chief source of food for aquatic animals including fishes. Bakar lake is subtropical (Blatter *et al* 1929; Mitcheal 1967) and is situated in desert area of Sindh at an altitude of 50 m, latitude 26° 06′ North, longitude 68° 10′ East. Its width is 2.5 km and length is 45km. According to Prescott (1961) referring to older, shallow lakes, highly productive for the eutrophic lake so Phoosna is a shallow eutrophic lake, situated in between 68° 55′ longitude (East) and 24° 50′ latitude (North) at a distance of 20 km from Badin, 5 km, towards north of Hyderabad Badin Road. It is a private owned fishing lake, spread over an area of 500 acres. The lake is shallow, about 2-3 meters deep. Since it is surrounded by agriculture land, consequently also receives leached plant nutrients. The present study was carried out as very little work has been done on the phytoplankton of lakes from Sindh blooms is Kinjhar Lake in summer season (Nazneen 1974).

The present work will give the comparative results of phytoplankton distribution in the Phoosna and Bakar Lakes, where different physico-chemical properties and other parameters have been taken into consideration to study the phytoplankton flora.

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Materials and Methods

Phytoplankton were collected monthly from August, 1993 to July, 1996 between 11 a.m. to 3 p.m. with the help of boat using phytoplankton net of 5-10 mm mesh. Water samples were collected using water sampler (Nansen bottle) for studying physico-chemical features using standard methods (APHA 1985) and for quantitative studies of phytoplankton. Samples were preserved in 4% formalin. The species composition was determined by Utremohal method (Lund *et al* 1958). The micro algae (Ultra nannoplankton) were not counted as Gorham *et al* (1974), considered these algae comparatively unimportant in high productive lakes. The association of (Ultra nannoplankton) with phyto and tychoplankton so easily collected with the algal net and secondly in polythene bags crush the algal material easily found ultra nannoplankton. Phytoplankton identification and counts were done using inverted light microscope olymphus Japan (20X, 40X objective and eyepiece 10X) and identified with the help of available literature (Husted 1930; Majeed 1935; Smith 1950; Prescott 1961; Patrick and Reimer 1966; Tiffany and Briton 1971; Vinyard 1979; Akiyama and Yamagishi 1981; Leghari *et al* 1997).

Results and Discussion

According to the results of the comparative ecological studies of phytoplankton of Bakar and Phoosna Lakes, qualitative measurement done for the production of phytoplankton is shown in Table 1. Lake Bakar is clearly very productive as

compared to Lake Phoosna. Out of 122 species identified from these Lakes 94 were present in Bakar Lake, 17 in Phoosna Lake and 11 were common in both the Lakes. This clearly indicates 5.5 times richness of Bakar Lake in phytoplankton species. A look at the percentage reveals that 77% of the species are present in Lake Bakar and 13.93% in Lake Phoosna and 9% are common in both Lakes. This productivity of Lake Bakar can be attributed to the amount of dissolved oxygen which is an indicator that the phytoplanktons take in CO_2 for photosynthesis and give out O_2.

Total hardness was measured by the amount of $CaCO_3$ present in the Lake waters. The result reveals that Phoosna Lake has a minimum of 400 ppm hardness and Bakar Lake has 100 ppm while maximum was 516 ppm and 180 ppm present in Phoosna and Bakar Lakes, respectively (Table 2). Blue green algae was in high ratio in Phoosna Lake due to its hardness and the present studies indicate that phytoplankton is drastically low in this Lake.

It is also indicated in Table 2 that salinity level in Phoosna Lake is very high, maximum 3.8 ppt as compared to Lake Bakar which is 0.7 ppt. This is one of the reasons why the phytoplankton species are scanty in Phoosna Lake and flourish in Lake Bakar. Due to high salinity level, it was also observed that cells of the plants are damaged and deformed in Phoosna Lake. The T.D.S. ratio (330 ppm) is higher in Lake Bakar as compared to Phoosna Lake and this results in high productivity level of this Lake. Due to T.D.S. support the phytoplankton production increases and phytoplankton is the favourite food of fish which nourish on it. It has also been observed that the taste of the fish is very delicious, the reason being the ample and high quality of phytoplankton species present in Lake Bakar.

The orthophosphate content is very low (0.002 µg/l) in Lake Bakar due to which phytoplankton species flourish in this water. Moreover, due to greater depth of Lake Bakar (7.8 m) the temperature of its water does not rise as compared to Phoosna Lake which is shallow and therefore, the temperature rises more quickly in this Lake. Hence, the low temperature species also survive in the Bakar Lake.

It was observed during the studies conducted that there is proper inlet for water in the Lake Phoosna but there is no outlet. Due to stagnant water mostly the species recorded were the hard water ones. A perusal of Table 2 clearly indicates that variations in the physico-chemical properties of Lake have adverse effect on the fish of the Lake.

Lake Bakar on the other hand has proper inlet and outlet for water. The Nara Canal derived from this Lake serves to irrigate the agricultural lands. Due to this inlet and outlet

Table 1

The occurrence of various unicellular phytoplankton species during August, 1993 to July, 1996 in Bakar and Phoosna Lakes

Species	Baker lake	Phoosna lake
Division: Chlorophyta		
Class: Chlorophyceae		
Order: Chlorococcales		
Family: Oocystaceae		
1. *Ankistrodesmus convolutes* Corda	c	c
2. *A. falcatus* (Corda) Ralfs	c	c
3. *A. falcatus* var. *stipitatus* (Chod) Lemm	c	c
4. *Chlorella vulgaris* Beyerinck	p	a
5. *Chodatella baltonica* Scherefell	p	a
6. *C. chodatii* (Ber.) leg. var. *chodatii*	a	p
7. *Closteriopsis longissima* var. *tropical* W. & W.	p	a
8. *Excentrosphaera virdis* Moore	p	a
9. *Gloeotaenium loitelsbergerianum* Hansgirg	p	a
10. *Kirchneriella lunaris* (Kirch) Moebius	p	a
11. *Nephrocytium obesum* West & West	p	a
12. *Oocystis borgei* Snow	p	a
13. *O. crassa* (West & West) Nordstedt	p	a
14. *O. elliptica* W. West	p	a
15. *O. eremosphaeria* Smith	p	a
16. *O. gigas* Archer	p	a
17. *O. lacustris* Chodat	p	a
18. *O. marsonii* Lemm.	p	a
19. *O. parva* West & West	p	a
20. *O. pyriformis* Prescott	p	a
21. *O. solitaria* Wittrock & Nordstedt	p	a
22. *Planktosphaeria gelatinosa* Smith	p	a
23. *Scotiella antacotica* Fritsch	a	p
24. *Tetraedron asymmetricum* Prescott.	p	a
25. *T. caudatum* (Corda) Hansgirg	p	a
26. *T. muticum* (A. Broun) Hansgirg	c	c
27. *T. miticum* f. *punctulatum* Toni	a	p
28. *T. regulare* var. *incus forma* major Pres.	p	a
29. *T. regulare* var. *torsum* (Turner) Braun.	p	a
30. *T. trigonum* (Naegeli) Hansgirg	p	a
31. *T. tumidulum* (Reinsch) Hansgirg	p	a
32. *T. victoriae* Woloszynska	p	a
33. *Trochiscia hirta* West.	p	a
34. *T. granulata* (Reinsch) Hansgirg	c	c
35. *T. obtusa* (Reinsch) Hansgirg	p	a
36. *T. reticularis* (Reinsch) Hansgirg	p	a
37. *Westella botryoides* (West) Wildemann.	p	a
Family: Dictyosphaeriaceae		
38. *Dictyosphaerium pulchellum* Wood	p	a
39. *Dimorphococcus lunatus* A. Braun	p	a
Family: Characiaceae		
40. *Characium obtusum* A. Braun	c	c
41. *C. ambiguum* Hermann	a	p
42. *C. sphaericum* Naeg.	a	p
Family: Micractiniaceae		
43. *Golenkinia paucispina* West & West.	p	a

(Cont'd....)

(Table 1 Cont'd)

Species	Bakar lake	Phoosna lake
Family: Coelastraceae		
44. *Coelastrum astrodeum* var. *astrodeum*	a	p
45. *C. cambricum* Archer	p	a
46. *C. microporum* Naegeli A. Braun	p	a
47. *C. scarbrum* Reinsch	p	a
48. *C. sphaericum* Naegeli	p	a
Family: Hydrodictyaceae		
49. *Pediastrum boryanum* (Turp.) Men.	p	a
50. *P. tetras* (Ehr.) Ralfs	p	a
Family: Scenedesmaceae		
51. *Actinastrum hantzschii* var. *elongatum* Smith	a	p
52. *Crucigenia apiculata* (Lemm) Schmidle.	p	a
53. *C. irregularis* Wille	a	p
54. *C. quadrata* Morren	p	a
55. *C. rectangularis* (A. Broun) Gay	c	c
56. *C. tetrapedia* (Kirch) W & West	c	c
57. *Scenedesmus arcuatus* Lemm	c	c
58. *S. arcuatus* var. *arcuatus* K. Oshima	a	p
59. *S. arcuatus* var. *platidisca* Smith	p	a
60. *S. bijuga* (Turp.) Lagerheim	p	a
61. *S. ecornis* var. *deciformis* f. *obiciturus* Uherkovich	p	a
62. *S. hystrix* Legerheim	p	a
63. *S. quadricauda* (Turp.) Breb.	c	c
64. *S. quadricauda* var. *parvus* Smith	p	a
65. *Tetrallantos lagerheimii* Teiling	a	p
Order: Tetrasporales		
Family: Palmellaceae		
66. *Gloeocystis ampla* Kuetz. Legerheim	p	a
67. *G. major* Gerneck ex Lemm.	p	a
68. *G. vesiculosa* Naegeli	p	a
69. *Gloiococcus mucosus* West	p	a
70. *Palmella miniata* Naegeli	p	a
71. *Sphaerocystis schroeteri* Chodat	p	a
Family: Cocomaxaceae		
72. *Elakatothrix viridis* (Snow) Printz	a	p
Family: Tetrasporaceae		
73. *Schizochlamys compacta* Prescott	p	a
74. *S. gelatinosa* A. Braun	p	a
75. *Tetraspora cylindrical* (Wahl.) Agardh.	p	a
Order: Ulotrichales		
Family: Ulotrichaceae		
76. *Geminella crenulatocollis* Prescott	a	p
77. *G. ordinata* (W. & West) Heering	a	p
Order: Volvocales		
Family: Chlamydomonadaceae		
78. *Chlamydomonas ehrenbergii* Gorosh Pascher	p	a
79. *C. epiphytica* G. M. Smith	p	a
80. *C. polyperenoideum* Prescott	p	a
81. *C. pseudopertyi* Pascher	p	a
Family: Volvoaceae		
82. *Eudorina elegans* Ehr.	p	a

(Cont'd....)

(Table 1 Cont'd)

Species	Bakar lake	Phoosna lake
83. *Pandorina morum* Bory	p	a
84. *Volvox aureus* Ehr.	p	a
Order: Zygnematales		
Family: Desmidiaceae/Closterieae		
85. *Closterium acerosum* var. *elongatum* Breb.	a	p
86. *Cl. Dianae* Ehr.	p	a
87. *Cl. Leibleinii* Kuetz.	p	a
88. *Cl. Praelongum* var. *porosum* Kr.	p	a
89. *Cl. Ralfsii* var. *hybridium* Rabenk.	p	a
Sub-Family: Cosmarieae		
90. *Arthrodesmus incus* var. *extensus* Anderson	p	a
91. *Cosmarium contractum* Kirchner	c	c
92. *C. contractum* var. *ellipsoideum* W. & West	p	a
93. *C. crenatum* Ralfs	a	p
94. *C. excavatum* Nordstedt	p	a
95. *C. gibberulum* Lutkem.	p	a
96. *C. granatum* Breb	p	a
97. *C. margaritatum* (Lundell) Roy et Bisset	p	a
98. *C. maximum* (Borgesen) W. West	p	a
99. *C. moniliforme* f. *pandriforme* Heimerdl	p	a
100. *C. moniliforme* var. *limneticum* W. & West	p	a
101. *C. pachydermum* Lund	p	a
102. *C. subtumidum* Nordst.	p	a
103. *C. sulcatum* Nordstedt.	p	a
104. *C. tinctum* var. *tinctum* Ralfs.	p	a
105. *C. turpinii* Breb.	p	a
106. *C. turpinii* Breb var. *intermedium* n.v.	p	a
107. *Cosmocladium constrictum* Archer	p	a
108. *Euastrum binale* (Turpin) Her.	p	a
109. *E. dubium* var. *tropicum* (W. & West) Krieger	p	a
110. *E. pectinatum* Breb.	p	a
111. *Micrasterias pinnatifida* Ralfs	p	a
112. *Penium polymorphum* Perly	p	a
113. *P. simplex Nov.* sp.	p	a
114. *Pleurotaenium ehrenbergii* (Berb.) Bory	p	a
115. *Staurastrum brevispinum* Breb.	p	a
116. *S. gracile* Ralfs	a	p
117. *S. inflexum* Breb.	p	a
118. *S. hexacerum* (Ehr.) Wittr.	p	a
119. *S. hexacerum* (Ehr.) *forma pentagona*	p	a
120. *S. iotanum* var. *longatum* Hirano.	p	a
121. *S. orbiculare* var. *depressum* Roy it Bisset	a	p
122. *S. orbiculare* var. *ralfsii* West & West	p	a
a = absent	17 (13.93%)	94 (77.10%)
p = present	94 (77.10%)	17 (13.93%)
c = common/present	11 (9.02%)	11 (9.02%)
Total present	105	28

Division	Class	Order	Family	Genera	Species
1	1	5	15	45	122

a, Absent; p, Present; c, Common/present.

Table 2
Physico-chemical properties of Lakes Bakar and Phoosna

S. no.	Parameters	Bakar lake		Phoosna lake	
		Min	Max	Min	Max
1.	Soil temperature°C	11.000	26.000	12.0000	24.500
2.	Air temperature°C	17.000	39.000	18.0000	38.000
3.	Water surface temperature°C	15.000	31.300	20.0000	30.000
4.	Water bottom temperature°C	16.000	30.300	18.0000	30.000
5.	pH	7.700	8.900	8.2000	8.500
6.	Turbidity in NTU Range on 100	0.300	6.800	5.0000	45.000
7.	T.D.S (ppm)	220.000	330.000	160.0000	170.000
8.	Conductivity (m.Ohms x^{10})	40.000	103.000	40.0000	58.000
9.	Salinity (NaCl ppt)	0.100	0.700	3.0000	3.800
10.	Humidity (%)	33.000	77.000	31.0000	49.000
11.	Light transparency by Secchi disc (meter)	1.200	3.600	19.0000	43.000 (inch)
12.	Dissolved oxygen (mg/1)	5.000	11.500	5.0000	9.500
13.	Saturation (%)	40.500	91.000	40.5000	75.0000
14.	CO_2 (ppm)	15.000	77.000	(no free)	50.0000
15.	Ammonia nitrogen (NH_3 N_2 ppm)	0.020	0.110	0.0200	0.0400
16.	Density (30°Cg/v)	1.002	1.005	1.0044	1.0058
17.	Water color (Numbers)	12.000	17.000	14.0000	17.0000
18.	Orthophosphate (µg/1)	0.002	0.200	0.0300	0.2450
19.	Total hardness ($CaCO_3$ ppm)	100.000	180.000	400.0000	516.0000
20.	Ca^{++} Hardness (ppm)	60.000	120.000	200.0000	258.0000
21.	Mg Hardness (ppm)	40.000	80.000	200.0000	258.0000
22.	$CaCl_2$ Hardness (ppm)	66.600	109.900	222.0000	286.3800
23.	Mg Cl_2 (ppm)	57.000	109.300	190.0000	245.1000
24.	Grain Per Gallon (GPC)	6.400	10.440	23.2000	29.9300
25.	Refractive index (30°C)	1.330	1.330	1.3325	1.3328
26.	Total depth of lake (meter)	3.000	7.800	2.0000	3.0000
27.	Wave (inch)	2.000	18.000	2.0000	5.0000
28.	Taste	Tastless	Tastless	Tastless	Tastless
29.	Odour	Odourless	Fishy	Odourless	Fishy
30.	Wind	Cold	Dry hot	Cold	Dry hot
31.	Day	Cloudy	Clear	Cloudy	Clear
32.	Weather	Fogy	Dry clear	Fogy	Dry clear
33.	Wind in winter	North to South		North to South	
34.	Wind in summer	South to North		South to North	
35.	Water	Shallow near bank	Deep at centre	Shallow	Shallow
36.	Soil	Sandy on bank	Muddy in bottom	Muddy	Muddy
37.	Zone	Subtropical		Subtropical	

of water the species recorded are both soft and hard water species. The physico-chemical properties of the Lake clearly indicate that Lake Bakar is quite productive as compared to Lake Phoosna. The size and weight of the fish increases tremendously within one year which is more than 3 kgs. The fish species locally called Kurro and Dumbro attain the weight of 18 kgs which was recorded from this Lake. This clearly indicates that the ecological conditions of Bakar Lake are much better and suitable for the fish and other fauna.

The physico-chemical features of lake water are presented in Table 2. Dickman (1969) stated that lakes which act as temporary impoundments to the flow of water from inlet and outlet are unusual in species population because of the major role of flushing in regulating their primary productivity. Bakar Lake also act as a temporary impoundment and it appears that flushing may be a major cause of observed irregularities. Physical, chemical and biological features are strongly conditioned by surface level fluctuations, due to flooding and dewatering (Thornton *et al* 1990).

Table 3
Seasonal variation of unicellular phytoplankton of Lake Bakar

S.no	Species	Summer J-A	Autumn S-N	Winter D-F	Spring M-M
Division: Chlorophyta					
Class: Chlorophyceae					
Order: Chlorococcales					
Family: Oocystaceae					
1.	*Ankistrodesmus convolutes* Corda	c	c	c	c
2.	*A. falcatus* (Corda) Ralfs	c	c	c	c
3.	*A. falcatus* var. *stipitatus* (Chod) Lemm	c	c	c	c
4.	*Chlorella vulgaris* Beyerinck	a	r	c	c
5.	*Chodatella baltonica* Scherfell	a	vr	r	vr
6.	*C. chodatii* (Ber.) Leg. var. *chodatii*	a	a	a	a
7.	*Closteriopsis longissima* var. *tropical* W. & W.	a	a	vr	r
8.	*Excentrosphaera virdis* Moore	a	a	vr	vr
9.	*Gloeotaenium loitelsbergerianum* Hansgirg	a	a	vr	vr
10.	*Kirchneriella lunaris* (Kirch) Moebius	vc	c	c	c
11.	*Nephrocytium obesum* West & West	r	r	c	r
12.	*Oocystis borgei* Snow	c	c	c	c
13.	*O. crassa* (West & West) Nordstedt	r	r	c	r
14.	*O. elliptica* W. West	a	r	r	a
15.	*O. eremosphaeria* Smith	vr	vr	r	r
16.	*O. gigas* Archer	a	a	vr	vr
17.	*O. lacustris* Chodat	a	a	vr	a
18.	*O. marsonii* Lemm.	a	a	vr	a
19.	*O. parva* West & West	a	a	vr	r
20.	*O. pyriformis* Prescott	a	a	vr	vr
21.	*O. solitaria* Wittrock & Nordstedt	a	a	vr	a
22.	*Planktosphaeria gelatinosa* Smith	vr	r	r	r
23.	*Scotiella antacotica* Fritsch	a	a	a	a
24.	*Tetraedron asymmetricum* Prescott.	a	a	vr	vr
25.	*T. caudatum* (Corda) Hansgirg	a	a	vr	a
26.	*T. muticum* (A. Broun) Hansgirg	c	c	c	c
27.	*T. miticum* f. *punctulatum* Toni	a	a	a	a
28.	*T. regulare* var. *incus forma* major Pres.	a	a	vr	a
29.	*T. regulare* var. *torsum* (Turner) Braun.	vr	r	c	r
30.	*T. trigonum* (Naegeli) Hansgirg	c	c	c	c
31.	*T. tumidulum* (Reinsch) Hansgirg	a	a	a	vr
32.	*T. victroiae* Woloszynska	a	a	vr	r
33.	*Trochiscia hirta* West.	a	a	c	c
34.	*T. granulata* (Reinsch) Hansgirg	c	c	c	c
35.	*T. obtusa* (Reinsch) Hansgirg	a	a	vr	a
36.	*T. reticularis* (Reinsch) Hansgirg	a	a	vr	a
37.	*Westella botryoides* (West) Wildemann.	a	a	a	vr
Family: Dictyosphaeriaceae					
38.	*Dictyosphaerium pulchellum* Wood	a	vr	vr	r
39.	*Dimorphococcus lunatus* A. Braun	c	vr	c	vc
Family: Characiaceae					
40.	*Characium obtusum* A. Braun	c	c	c	c

(Cont'd....)

(Table 3 Cont'd....)

S.no	Species	Summer J-A	Autumn S-N	Winter D-F	Spring M-M
41.	*C. ambiguum* Hermann	a	a	a	a
42.	*C. sphaericum* Naeg.	a	a	a	a
Family: Micractiniaceae					
43.	*Golenkinia paucispina* West & West	a	a	vr	r
Family: Coelastraceae					
44.	*Coelastrum astrodeum* var. *astrodeum*	a	a	a	a
45.	*C. cambricum* Archer	a	vr	c	r
46.	*C. microporum* Naegeli A. Braun	vr	vr	c	r
47.	*C. scarbrum* Reinsch	a	a	vr	vr
48.	*C. sphaericum* Naegeli	a	a	vr	r
Family: Hydrodictyaceae					
49.	*Pediastrum boryanum* (Turp.) Men.	r	r	r	r
50.	*P. tetras* (Ehr.) Ralfs	vr	r	r	c
Family: Scenedesmaceae					
51.	*Actinastrum hantzschii* var. *elongatum* Smith	a	a	a	a
52.	*Crucigenia apiculata* (Lemm) Schmidle.	a	vr	vr	r
53.	*C. irregularis* Wille	a	a	a	a
54.	*C. quadrata* Morren	a	vr	r	a
55.	*C. rectangularis* (A. Braun) Gay	c	c	c	c
56.	*C. tetrapedia* (Kirch) W & West	c	c	c	c
57.	*Scenedesmus arcuatus* Lemm	c	c	c	c
58.	*S. arcuatus* var. *arcuatus* K. Oshima	a	a	a	a
59.	*S. arcuatus* var. *platidisca* Smith	r	r	r	r
60.	*S. bijuga* (Turp.) Lagerheim	r	r	r	r
61.	*S.ecronis* var. *deciformis* f. *obicitutus* Uherkovich	a	a	vr	a
62.	*S. hystrix* Legerheim	a	vr	vr	a
63.	*S. quadricauda* (Turp.) Breb.	c	c	c	c
64.	*S. quadricauda* var. *parvus* Smith	a	vr	vr	a
65.	*Tetrallantos lagerheimii* Teiling	a	a	a	a
Order: Tetrasporales					
Family: Palmellaceae					
66.	*Gloeocystis ampla* Kuetz. Legerheim	r	r	r	r
67.	*G. major gerneck* ex Lemm.	r	r	r	r
68.	*G. vesiculosa* Naegeli	r	r	r	r
69.	*Gloiococcus mucosus* West	a	vr	vr	a
70.	*Palmella miniata* Naegeli	vr	vr	r	r
71.	*Sphaerocystis schroeteri* Chodat	vr	r	r	r
Family: Cocomaxaceae					
72.	*Elakatothrix virdis* (Snow) Printz	a	a	a	a
Family: Tetrasporaceae					
73.	*Schizochlamys compacta* Prescott	a	a	vr	a
74.	*S. gelatinosa* A. Braun	a	vr	vr	a
75.	*Tetraspora cylindrical* (Wahl.) Agardh.	a	r	r	r
Order: Ulotrichales					
Family: Ulotrichaceae					
76.	*Geminella crenulatocollis* Prescott	a	a	a	a
77.	*G. ordinata* (W. & West) Heering	a	a	a	a
Order: Volvocales					
Family: Chlamydomonadaceae					
78.	*Chlamydomonas ehrenbergii* Gorosh Pascher	vr	vr	r	r

(Cont'd....)

(Table 3 Cont'd....)

S.no	Species	Summer J-A	Autumn S-N	Winter D-F	Spring M-M
79.	*C. epiphytica* G.M. Smith	r	r	r	r
80.	*C. polyperenoideum* Prescott	a	vr	vr	a
81.	*C. pseudopertyi* Pascher	a	vr	vr	vr
Family: Volvocaceae					
82.	*Eudorina elegans* Ehr.	a	vr	vr	vr
83.	*Pandorina morum* Bory	a	vr	vr	a
84.	*Volvox aureus* Ehr.	a	a	vr	a
Order: Zygnematales					
Family: Desmidiaceae/Closterieae					
85.	*Closterium acerosum* var. *elongatum* Breb.	a	a	a	a
86.	*Cl. dianae* Ehr.	r	r	r	r
87.	*Cl. leibleinii* Kuetz.	a	a	vr	vr
88.	*Cl. praelongum* var. *porosum* Kr.	r	a	vr	a
89.	*Cl. ralfsii* var. *hybridium* Rabenk.	vr	vr	r	r
Sub-family: Cosmarieae					
90.	*Arthrodesmus incus* var. *extensus* Anderson	vr	vr	vr	vr
91.	*Cosmarium contractum* Kirchner	c	c	c	c
92.	*C. contractum* var. *ellipsoideum* W. & West	r	r	r	r
93.	*C. crenatum* Ralfs	a	a	a	a
94.	*C. excavatum* Nordstedt	vr	vr	vr	vr
95.	*C. gibberulum* Lutkem.	r	vr	vr	r
96.	*C. granatum* Breb	r	r	r	r
97.	*C. margaritatum* (Lundell) Roy et Bisset	vr	vr	r	vr
98.	*C. maximum* (Borgesen) W. & West	vr	vr	r	r
99.	*C. moniliforme* f. *pandriforme* Heimerdl	a	vr	a	a
100.	*C. moniliforme* var. *limneticum* W. & West	a	vr	vr	a
101.	*C. pachydermum* Lund	a	vr	vr	a
102.	*C. subtumidum* Nordst.	a	vr	vr	a
103.	*C. sulcatum* Nordstedt.	a	vr	a	a
104.	*C. tinctum* var. *tinctum* Ralfs.	a	vr	vr	a
105.	*C. turpinii* Breb.	a	vr	vr	a
106.	*C. turpinii* Breb var. *intermedium* n.v.	a	vr	vr	a
107.	*Cosmocladium constrictum* Archer	a	vr	vr	a
108.	*Euastrum binale* (Turpin) Her.	a	vr	vr	a
109.	*E. dubium* var. *tropicum* (W. & West) Krieger	a	vr	vr	a
110.	*E. pectinatum* Breb.	a	vr	vr	a
111.	*Micrasterias pinnatifida* Ralfs	a	a	a	vr
112.	*Penium polymorphum* Perly	vr	vr	r	r
113.	*P. simplex Nov.*sp.	r	r	r	r
114.	*Pleurotaenium ehrenbergii* (Breb.) Bory	vr	r	r	vr
115.	*Staurastrum brevispinum* Breb.	a	vr	vr	a
116.	*S. gracile* Ralfs	a	a	a	a
117.	*S. inflexum* Breb.	a	vr	vr	a
118.	*S. hexacerum* (Ehr.) Wittr.	a	a	a	a
119.	*S. hexacerum* (Ehr.) *forma pentagona*	a	vr	vr	a
120.	*S. iotanum* var. *longatum* Hirano.	a	vr	vr	a
121.	*S. orbiculare* var. *depressum* Roy it Bisset	a	a	a	a
122.	*S. orbiculare* var. *ralfsii* West & West	a	vr	vr	a

a, Absent; r, Rare; Vr, Very rare; c, Common; J-A, June-August; S-N, Sep-Nov; D-F, Dec-Feb; M-M, March-May

Table 4

Seasonal variation of unicellular phytoplankton of Lake Phoosna

S.no	Species	SummerJ-A	Autumn S-N	Winter D-F	Spring M-M
Division: Chlorophyta					
Class: Chlorophyceae					
Order: Chlorococcales					
Family: Oocystaceae					
1.	*Ankistrodesmus convolutes* Corda	c	c	c	c
2.	*A. falcatus* (Corda) Ralfs	c	c	c	c
3.	*A. falcatus* var. *stipitatus* (Chod) Lemm	c	c	c	c
4.	*Chlorella vulgaris* Beyerinck	a	a	a	a
5.	*Chodatella baltonica* Scherfell	a	a	a	a
6.	*C. chodatti* (Ber.) Leg. var. *chodatii*	r	r	r	r
7.	*Closteriopsis longissima* var. *tropical* W. & W.	a	a	a	a
8.	*Excentrosphaera virdis* Moore	a	a	a	a
9.	*Gloeotaenium loitelsbergerianum* Hansgirg	a	a	a	a
10.	*Kirchneriella lunaris* (Kirch) Moebius	a	a	a	a
11.	*Nephrocytium obesum* West & West	a	a	a	a
12.	*Oocystis borgei* Snow	a	a	a	a
13.	*O. crassa* (West & West) Nordstedt	a	a	a	a
14.	*O. elliptica* W. & West	a	a	a	a
15.	*O. eremosphaeria* Smith	a	a	a	a
16.	*O. gigas* Archer	a	a	a	a
17.	*O. lacustris* Chodat	a	a	a	a
18.	*O. marsonii* Lemm.	a	a	a	a
19.	*O. parva* West & West	a	a	a	a
20.	*O. pyriformis* Prescott	a	a	a	a
21.	*O. solitaria* Wittrock & Nordstedt	a	a	a	a
22.	*Planktosphaeria gelatinosa* Smith	a	a	a	a
23.	*Scotiella antacotica* Fritsch	r	r	r	r
24.	*Tetraedron asymmetricum* Prescott.	a	a	a	a
25.	*T. caudatum* (Corda) Hansgirg	a	a	a	a
26.	*T. muticum* (A. Broun) Hansgirg	c	c	c	c
27.	*T. miticum* f. *punctulatum* Toni	r	r	r	r
28.	*T. regulare* var. *incus forma* major Pres.	a	a	a	a
29.	*T. regulare* var. *torsum* (Turner) Braun.	a	a	a	a
30.	*T. trigonum* (Naegeli) Hansgirg	a	a	a	a
31.	*T. tumidulum* (Reinsch) Hansgirg	a	a	a	a
32.	*T. victoriae* Woloszynska	a	a	a	a
33.	*Trochiscia hirta* West.	a	a	a	a
34.	*T. granulata* (Reinsch) Hansgirg	c	c	c	c
35.	*T. obtusa* (Reinsch) Hansgirg	a	a	a	a
36.	*T. reticularis* (Reinsch) Hansgirg	a	a	a	a
37.	*Westella botryoides* (West) Wildemann.	a	a	a	a
Family: Dictyosphaeriaceae					
38.	*Dictyosphaerium pulchellum* Wood	a	a	a	a
39.	*Dimorphococcus lunatus* A. Braun	a	a	a	a
Family: Characiaceae					
40.	*Characium obtusum* A. Braun	c	c	c	c
41.	*C. ambiguum* Hermann	r	r	r	r
42.	*C. spharicum* Naeg.	r	r	r	r

(Cont'd....)

(Table 4 Cont'd....)

S.no	Species	Summer J-A	Autumn S-N	Winter D-F	Spring M-M
Family: Micractiniaceae					
43.	*Golenkinia paucispina* West & West.	a	a	a	a
Family: Coelastraceae					
44.	*Coelastrum astrodeum* var. *astrodeum*	r	r	r	r
45.	*C. cambricum* Archer	a	a	a	a
46.	*C. microporum* Naegeli A. Braun	a	a	a	a
47.	*C. scarbrum* Reinsch	a	a	a	a
48.	*C. sphaericum* Naegeli	a	a	a	a
Family: Hydrodictyaceae					
49.	*Pediastrum boryanum* (Turp.) Men.	a	a	a	a
50.	*P. tetras* (Ehr.) Ralfs	a	a	a	a
Family: Scenedesmaceae					
51.	*Actinastrum hantzschii* var. *elongatum* Smith	vr	vr	vr	vr
52.	*Crucigenia apiculata* (Lemm) Schmidle.	a	a	a	a
53.	*C. irregularis* Wille	vr	vr	vr	vr
54.	*C. quadrata* Morren	a	a	a	a
55.	*C. rectangularis* (A. Broun) Gay	c	c	c	c
56.	*C. tetrapedia* (Kirch) W & West	c	c	c	c
57.	*Scenedesmus arcuatus* Lemm	c	c	c	c
58.	*S. arcuatus* var. *arcuatus* K. Oshima	r	r	r	r
59.	*S. arcuatus* var. *platidisca* Smith	a	a	a	a
60.	*S. bijuga* (Turp.) Lagerheim	a	a	a	a
61.	*S.ecornis* var. *deciformis* f. *obiciturus* Uherkovich	a	a	a	a
62.	*S. hystrix* Legerheim	a	a	a	a
63.	*S. quadricauda* (Turp.) Breb.	c	c	c	c
64.	*S. quadricauda* var. *parvus* Smith	a	a	a	a
65.	*Tetrallantos lagerheimii* Teiling	vr	vr	vr	vr
Order: Tetrasporales					
Family: Palmellaceae					
66.	*Gloeocystis ampla* Kuetz. Legerheim	a	a	a	a
67.	*G. major* Gerneck ex Lemm.	a	a	a	a
68.	*G. vesiculosa* Naegeli	a	a	a	a
69.	*Gloiococcus mucosus* West	a	a	a	a
70.	*Palmella miniata* Naegeli	a	a	a	a
71.	*Sphaerocystis schroeteri* Chodat	a	a	a	a
Family: Cocomaxaceae					
72.	*Elakatothrix viridis* (Snow) Printz	r	r	r	r
Family: Tetrasporaceae					
73.	*Schizochlamys compacta* Prescott	a	a	a	a
74.	*S. gelatinosa* A. Braun	a	a	a	a
75.	*Tetraspora cylindrical* (Wahl.) Agardh.	a	a	a	a
Order: Ulotrichales					
Family: Ulotrichaceae					
76.	*Geminella crenulatocollis* Prescott	vr	vr	vr	vr
77.	*G. ordinata* (W & West) Heering	vr	vr	vr	vr
Order: Volvocales					
Family: Chlamydomonadaceae					
78.	*Chlamydomonas ehrenbergii* Gorosh Pascher	a	a	a	a
79.	*C. epiphytica* G.M. Smith	a	a	a	a

(Cont'd....)

(Table 4 Cont'd....)

S.No	Species	Summer J-A	Autumn S-N	Winter D-F	Spring M-M
80.	*C. polyperenoideum* Prescott	a	a	a	a
81.	*C. pseudopertyi* Pascher	a	a	a	a
Family: Volvocaceae					
82.	*Eudorina elegans* Ehr.	a	a	a	a
83.	*Pandorina morum* Bory	a	a	a	a
84.	*Volvox aureus* Ehr.	a	a	a	a
Order: Zygnematales					
Family: Desmidiaceae/Closterieae					
85.	*Closterium acerosum* var. *elongatum* Breb.	r	r	r	r
86.	*Cl. Dianae* Ehr.	a	a	a	a
87.	*Cl. Leibleinii* Kuetz.	a	a	a	a
88.	*Cl. Praelongum* var. *porosum* Kr.	a	a	a	a
89.	*Cl. Ralfsii* var. *hybridium* Rabenk.	a	a	a	a
Sub-Family: Cosmarieae					
90.	*Arthrodesmus incus* var. *extensus* Anderson	a	a	a	a
91.	*Cosmarium contractum* Kirchner	c	c	c	c
92.	*C. contractum* var. *ellipsodieum* W. & West	a	a	a	a
93.	*C. crenatum* Ralfs	r	r	r	r
94.	*C. excavatum* Nordstedt	a	a	a	a
95.	*C. gibberulum* Lutkem.	a	a	a	a
96.	*C. granatum* Breb	a	a	a	a
97.	*C. margaritatum* (Lundell) Roy et Bisset	a	a	a	a
98.	*C. maximum* (Borgesen) W. West	a	a	a	a
99.	*C. moniliforme* f. *pandriforme* Heimerdl	a	a	a	a
100.	*C. moniliforme* var. *limneticum* W. & West	a	a	a	a
101.	*C. pachydermum* Lund	a	a	a	a
102.	*C. subtumidum* Nordst.	a	a	a	a
103.	*C. sulcatum* Nordstedt.	a	a	a	a
104.	*C. tinctum* var. *tinctum* Ralfs.	a	a	a	a
105.	*C. turpinii* Breb.	a	a	a	a
106.	*C. turpinii* Breb var. *intermedium* n.v.	a	a	a	a
107.	*Cosmocladium constrictum* Archer	a	a	a	a
108.	*Euastrum binale* (Turpin) Her.	a	a	a	a
109.	*E. dubium* var. *tropicum* (W. & West) Krieger	a	a	a	a
110.	*E. pectinatum* Breb.	a	a	a	a
111.	*Micrasterias pinnatifida* Ralfs	a	a	a	a
112.	*Penium polymorphum* Perly	a	a	a	a
113.	*P. simplex Nov.* sp.	a	a	a	a
114.	*Pleurotaenium ehrenbergii* (Breb.) Bory	a	a	a	a
115.	*Staurastrum brevispinum* Breb.	a	a	a	a
116.	*S. gracile* Ralfs	vr	vr	vr	vr
117.	*S. inflexum* Breb.	a	a	a	a
118.	*S. hexacerum* (Ehr.) Wittr.	a	a	a	a
119.	*S. hexacerum* (Ehr.) *forma pentagona*	a	a	a	a
120.	*S. iotanum* var. *longatum* Hirano.	a	a	a	a
121.	*S. orbiculare* var. depressum Roy it Bisset	vr	r	r	vr
122.	*S. orbiculare* var. *ralfsii* West & West	a	a	a	a

a, Absent; r, Rare; vr, Very rare; c, Common; vc, Very common.

J-A, June-August; S-N, Sept.-Nov.; D-F, Dec.-February; M-M, March-May

Generally inlet in Bakar Lake is from June to December and outlet from January to May every year. Water level fluctuation are particularly striking because of semi arid climate where evaporation rates are greater than precipitation and the irregular inflows generally are not balanced by out flow. As a whole, the phytoplankton seems to be strongly related to the water level fluctuation and the climatological features, and it seems reasonable to hypothesize that abiotic (e.g. flooding, dewatering, light, mixing, temprature, turbidity, rain, storm, wind, etc.) factors mainly affected the phytoplanktons in the study period also by inhibiting or delaying the development of that biotic relationship (i.e. fry predation efficiency) which commonly takes place in aquatic environments.

Phytoplankton are cosmopolitan species, found throughout the year. They are abundant in the inlet waters of the Lake, where grazing and other disturbances occur. Some of the phytoplankton species survive in cold waters and in low orthophosphate ratio containing waters. Their abundance can easily be judged by measuring the density of water samples. Normally water density is one (1) and any increase in the density is attributed to the ratio of phytoplanktons in the water samples.

An increase in the temperature of water help to dissolve the minerals resulting in pH increase which results in the increase of phytoplankton production.

Similarly the change in the colour of water from grey to greenish to dark green also results in the high production of phytoplankton. The reason may be that dark colour resists the light and ultimately temperature of water increases. The distribution of phytoplankton is also affected by the direction of the winds. In summers the winds blow from South to North and this drifts the concentration of phytoplankton in the northern banks of the Lakes and reverse happens in winters, when the winds blow from North to South concentrating the phytoplankton flora in the southern banks.

It has also been observed that floods cause increase in turbidity causing changes in the concentration of phytoplankton. They become rare with the storm and disturbed in turbidity. But as soon as the particles of clay, mud, sands etc. in water, settles down there is increased flora of the Lakes. The sampling were done throughout the year and effects of these factors on the distribution and concentration of phytoplankton were observed during the study and sampling period (Table 3 and 4). It is interesting to note that abundant species of the phytoplankton isolated from these studies were recorded from the gut content of the fish caught from these waters.

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ASSESSMENT OF DRINKING WATER QUALITY OF A COASTAL VILLAGE OF KARACHI

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The drinking water quality of a coastal community in Rehri village, Karachi was assessed for human consumption by studying the chemical and microbiological parameters to determine the suitability of domestic drinking water usage. Water samples were collected at household levels from storage tanks and storage containers (earthen jars), main supply line and springs present in the vicinity. Samples collected from different sources indicated that bacterial counts were high for the storage tanks than the earthen jar containers. In storage tanks 71% samples were in high to very high health risk category whereas, in earthen jars 50% samples were in low health risk category. Water samples from two springs showed that samples of Chashma spring had high bacterial count (336 MPN index/100 ml) coupled with high concentration of NO_3 (29.681 mg/l) as compared to Rehri spring (41 MPN index/100 ml, 8.417 mg/l, bacterial count and nitrate concentrations, respectively). All samples collected at household level showed that bacterial contamination exceeded the maximum acceptable concentration. Other parameters (NO_3, NO_2, NH_3 PO_4, free Cl, Ca and Mg hardness) studied including trace elements (Fe, Cu, Cr) in the drinking water were below the WHO drinking water quality guidelines. Fecal coliform, *Escherichia coli* was also detected including important pathogens *Serratia* sp. and *Enterobacter* sp. which were isolated and indicated possible fecal contamination of drinking water at all levels.

Key words**:** Coliforms, Coastal area, Drinking water, *Escherichia coli*, Nutrients, Trace metals.

Introduction

The most and widespread health problems in developing countries are water borne diseases, associated with contamination of water either directly or indirectly by microbial or chemical pollutants. The microbial contamination is by human or animal excreta, particularly feces. An estimated 2.2 million deaths in 1998 have been caused by diarrhoea, almost exclusively in the developing countries where safe drinking water is not readily available (WHO 1999) and an estimated 1.3 billion persons do not have access to safe drinking water (UNEP 1996). In Pakistan the access to safe water is available to only sixty one percent population (85% urban, 47% rural). The proper sanitation facility is only available to thirty percent population of which sixty percent are urban and thirteen and half percent are rural (Aziz 1998). In Karachi, a city of more than 10 million people forty percent of the population is living in slums and has limited water and sanitary infrastructure and poor water quality (Anon 2000).

The presence of any living bacteria in drinking water, even in small numbers, indicates, not necessarily a health hazard, but certainly a failure in the chlorination system or recontamination of the water after disinfections. The presence of 'indicator bacteria' conventionally known as fecal coliform *(Escherichia coli*) indicates fecal contamination but it does not prove that water-borne disease is occurring. *E. coli* are always present in feces; the majorities are not pathogenic, although some strains can cause diarrhoea. In testing untreated water supplies the fecal coliform are most commonly used but other groups of bacteria (such as fecal streptococci) would also indicate contamination. Muneer *et al* (2001) found variety of fecal coliforms contamination by *Streptococcus*, *Enterococcus*, *Staphylococcus*, *Clostridium* and *E. coli* in the drinking water in University of Punjab. Raza *et al* (1998) studied the seasonal variation of drinking water quality of Northern Areas and Chitral. They found low quality of spring water and the storage vessels (containers) were of high health risk category. Kandhar and Ansari (1998) reported that the Hyderabad dwellers were supplied unchlorinated and contaminated drinking water. Zubair and Rippey (2000) in their evaluation of shallow ground water quality in urban areas of Karachi found presence of inorganic nutrients and bacterial contamination.

Rehri village is one of the oldest coastal villages located in the southeast, 9 km from Quaidabad near Malir in Karachi, on the landward coast of the Korangi creek channel of the Indus River Delta system. The predominant occupation of the people living in this area is fishing with mangrove as a source of fuel wood and fodder. The sources of water supply to the community are from main supply line to household; to share com-

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munity taps and tank and water supplied or collected from springs. The assessment of drinking water quality of Rehri, a coastal village was done by investigating the microbiological parameters and other physical and chemical parameters of drinking water at different points, main supply line and at household storage levels and springs.

Materials and Methods

Water distribution system was surveyed to assess the possible sources of contamination in the study area. Drinking water quality samples were collected according to distribution system and population density in different segments (*para* or Mohalla) of Rehri village. There are thirteen different *paras* (mohallas) living in this village (Qureshi and Hasnie 2001). Based on the socio-economic survey (Qureshi and Hasnie 2001) total nine household stations were sampled for assessment of drinking water quality. Additionally, two springs (water collection points for domestic use) were sampled, one from Rehri Goth and other from Chasma Ghot. In each household, samples were obtained from the main supply line (taps), household storage tanks (ST) and drinking water storage containers (EJ earthen jars) except one site where there was only a shared community storage tank (Musarani-II). Drinking water samples were collected in sterile bottles separately for analysis of microbial and chemical parameters. Samples were iced in the field and immediately brought back to the laboratory for further analyses. Water temperature, and pH were noted in field. Total Aerobic Count (TAC) and Total Coliform Count (TCC) were determined by using the most probable number (MPN) technique of serially diluted samples (diluted 1:10, 1:100 and 1:1000 with 0.01 M sterile phosphate-buffered saline) employing US-Standard Methods (APHA 1985). The estimation of bacterial densities was accomplished by comparison with McCardy's (1918). For sub-culture of coliform positive colonies to differentiate the pathogenic microorganisms in water, blue-purple colonies with a greenish metallic sheen were selected as possible *E. coli* and were inoculated in MacConkey broth and incubated at 44°C for 24 h (Cheesbourgh 1984). Those colonies that fermented lactose (as indicated by a change in the color of MacConkey broth) and produced gas and indole at 24 hours were classified as thermo-tolerant coliform *E. coli*. Concentrations were determined by colorimetric analysis following standard methods: for ammonia (Nessler method), nitrate (Cadmium reduction), nitrite (Ferrous Sulphate), phosphate (Ascorbic acid), iron (Phenantroline), chromium (Diphenylcarbohydrazide), copper (Bicinchoninate reagents), and manganese (Periodate oxidation) along with, free chlorine, calcium (Ca) and magnesium (Mg) hardness of drinking water. All parameters were analyzed using Multi-parameter bench spectrophotometer (Hanna C 100 series). All parameters studied are presented as average of three samples.

Results and Discussion

It is alarming that none of the samples collected from water taps, springs, storage tanks and earthen jars (bacterial counts range 40 to 1600 MPN index/100 ml) were free from contamination (Table 1). The main supply line water samples collected from taps at household levels were slightly better, although it also showed significant bacterial count (mean 59 MPN index/100 ml). Spring water samples were found grossly contaminated especially water sample from Chashma village spring (336 MPN index/100 ml) as compared to Rehri village spring water sample (41 MPN index/ml).

Water sample collected from storage tanks and earthen jar containers at household levels in all localities (*Paras* or Mohallas) showed significant bacterial contamination (Fig 1). Bacterial counts for total coliform and fecal coliform were much higher in storage tank samples than in the earthen jars. There was positive and significant correlation in the bacterial

Table 1
Presence of fecal coliform and pathogenic bacteria in drinking and domestic water storage tanks in Rehri village

Sample	Fecal coliform	Medically important pathogens
Dabla 1 S.T	present	*Serratia*
Dabla 1 E.J	present	*E. coli*
Dabla 2 S.T	present	*Enterobacter* sp.
Dabla 2 E.J	present	*E. coli*
Dabla 3 S.T	Absent	
Dabla 3 E.J	Absent	
Khaskheli 1 S.T	present	*E. coli*
Khaskheli 1 E.J	Absent	
Khaskheli 2 S.T	present	*E. coli*
Khaskheli 2 E.J	present	*E. coli*
Khaskheli 3 S.T	Absent	
Khaskheli 3 E.J	present	*E. coli*
Jat S.T*		
Jat E.J*		
Pann E.J*		
Chashma spring*		
Rehri spring*		
Main line	Absent	

S.T: storage tank; E.J: earthen jar container; *Fecal coliform and isolation of pathogenic bacteria was not done.

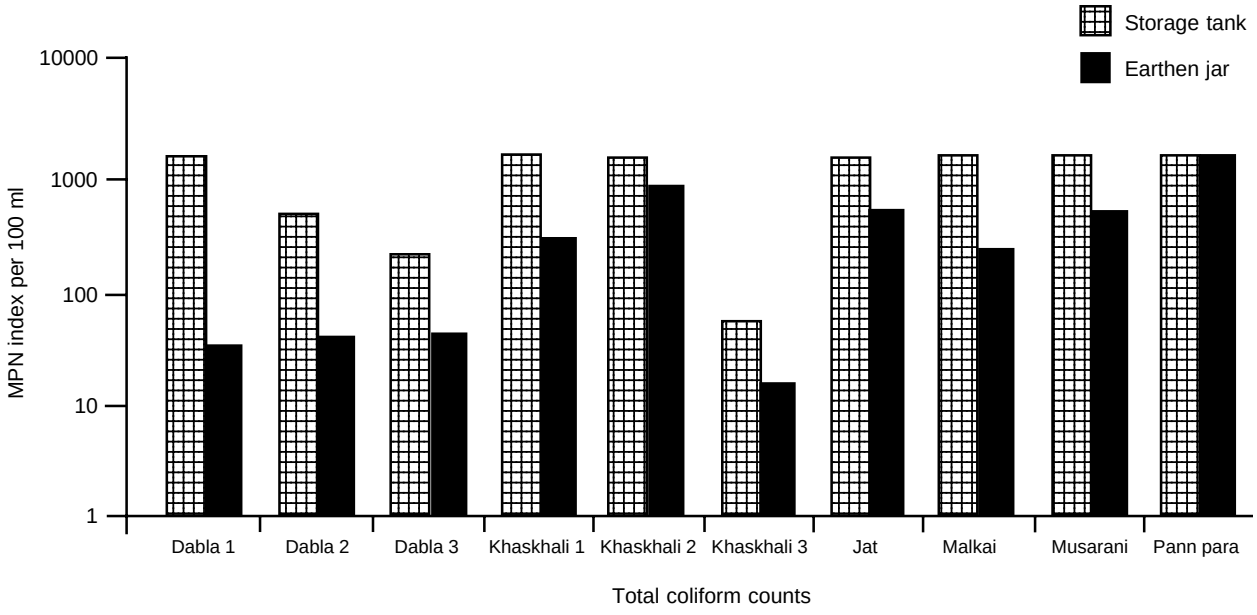

Fig 1. Comparison of drinking water bacterial counts in domestic water of different residential areas (*paras*) in Rehri village.

Table 2
Mean concentrations of nutrients (mg/l) and metals in drinking water samples of Rehri village

	WHO (1984) mg/l	Storage tank	Earthen jar	Chashma spring	Rehri spring
Ammonia		0.306	0.318	0.024	0.340
Nitrate	10.00	2.658	0.310	29.681	8.417
Nitrite		0.022	0.009	0.006	0.006
Phosphate		0.030	0.038	0.453	0.059
Total Chlorine	0.20	0.004	0.003	0.010	0.010
Calcium hardness	500.00	0.107	0.145	1.200	0.530
Magnesium hardness	500.00	7.995	7.947	0.150	0.000
Cromium-hexavalent	0.05	0.007	0.005	0.000	0.000
Copper	1.00	0.368	0.231	0.077	0.542
Iron	0.30	0.066	0.055	0.000	0.050

counts between storage tank and earthen jar (r = 0.96). Fecal coliforms were detected in samples collected from Dabla para and Khaskheli para (Table 1). Medically important pathogens *Serratia sp.* and *Enterobacter* sp. were isolated in samples collected from Dabla para and *E. coli* was also present in the samples obtained from Dabla para and Khaskheli para (Table 1). Low coliform counts were found in the main supply line sample of Dabla para and no fecal coliforms were found.

The mean pH was 7.8 and 7.9. The mean temperature was 25°C and 25.7°C, respectively for ST and EJ. The temperature and pH showed no significant difference between samples collected from storage tanks (ST) and earthen jars (ET).

Table 2 shows the mean concentrations of ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), iron (Fe), copper (Cu), chromium VI (Cr), chlorine (Cl), calcium (Ca) and magnesium (Mg) hardness in drinking water collected from domestic storage tanks (ST), earthen jar (EJ) and springs. The mean concentrations of Cl, Cr, Cu, Fe, NO_3, NO_2 and Mg hardness were found high in storage tanks as compared to the earthen jar containers (Table 2). The higher concentrations in storage tanks in comparison to earthen jars reflect integration and accumulation over time as water in the earthen jars is utilized and replaced daily. The mean concentrations of NH_3, PO_4 and Ca hardness were slightly high in the earthen jars. Water samples collected from two springs, showed NH_3, NO_2, PO_4, Cl, Cr, Cu and Fe were negligible. Concentration of NO_3 was high (29.681 mg/l) for Chashma Ghot spring indicating contamination and exceeded the minimum allowable limits. Nitrate concentration was high (8.417 mg/l) in water sample

Table 3
Pearson correlation matrix for drinking water samples

	Ammonia	Nitrate	Nitrite	Phosphate	Chlorine	Ca hardness	Mg hardness	Cromium-hexavalent	Copper
Nitrate	-0.320								
Nitrite	-0.165	-0.135							
Phosphate	-0.276	0.940	-0.105						
Chlorine	-0.445	0.530	-0.306	0.513*					
Ca hardness	-0.398	0.787	-0.353	0.752*	0.470				
Mg hardness	0.689*	-0.415	0.318	-0.332	0.456	-0.647*			
Cromium-hexavalent	0.212	-0.384	0.612	-0.313	-0.356	-0.617*	0.849*		
Copper	0.465	-0.230	0.213	-0.373	-0.106	-0.445	0.647*	0.563*	
Iron	0.047	-0.376	0.259	-0.516	-0.153	-0.530	0.565*	0.733*	0.733*

*Significant at P > 0.05

collected from Rehri village spring but was within the permitted limits (Table 2). Concentrations of Ca hardness were high in both Chashma and Rehri springs (1.2 and 0.53 mg/l), whereas concentrations of Mg hardness were high in storage tanks and earthen jars (Table 2).

The (Pearson) correlation matrix showed all metals were positively and significantly correlated (Table 3). The concentrations of PO_4, Cl and Ca hardness were positively correlated with NO_3 concentration (r = 0.94, r = 0.53 and r = 0.79, respectively). There was positive correlation between Cl and PO_4 (r = 0.51, Table 3). Ca hardness was negatively correlated with Cr, Cu and Fe, where as Mg hardness was negatively correlated with Ca hardness (r = -0.65) and positively correlated with NH_3, Cr, Cu and Fe (r = 0.69, r = 0.85, r = 0.65 and r = 0.57, respectively).

Water quality analysis of drinking water samples collected from different sources at house hold level showed that 71 percent samples from storage tanks were in high to very high health risk category (>500 MPN index/100 ml) and only 50 percent samples from earthen jars were in low health risk category (Feachem 1980). Three categories related to water quality with respect to fecal coliform counts 100/ml are less than 100 is satisfactory, between 100 to 1000 strong contamination and health risk, and greater than 1000 seriously pathogenic (Zubair and Rippey 2000).

The high bacterial counts and fecal counts indicate fecal contamination of drinking water possibly leading to gastro enteric diseases. High incidence of diarrhoeal diseases has been reported from Karachi with increased death rates (Zubair and Rippey 2000) and during epidemiological and socio-economic surveys of Rehri village (Qureshi and Hasnie 2001). High incidence of diarrhoea diseases in summer were also reported by Aga Khan Health Service, Gilgit, along with peak fecal contamination suggesting possible linkage between drinking water and diarrhoea diseases (Raza *et al* 1998).

The low bacterial counts in the main water supply system and springs indicate that these water sources were less contaminated in comparison to water samples collected at house hold level in the storage tanks and earthen jars, where handling of water may contribute to the source of contamination. In most of the households, the water storage tanks or earthen jars were near the cattle sheds and open toilets (personal observations), which increases the likelihood of fecal contamination. Moreover, the counts were significantly higher in all cases than the recommended WHO guidelines for drinking water. The WHO microbiological criteria for potability is <1 thermo-tolerant coliform 100/ml water. The same guideline also says that the total coliform bacteria must not be detected in any 100 ml sample, and for treated water in a larger distribution system must not be present in 95 % of samples taken throughout the year (WHO 1999). Kandhar and Ansari (1998) reported that the Hyderabad dwellers were supplied un-chlorinated and contaminated drinking water and the total suspended solids (40 to 2250 mg/l) and fecal coliforms (66 to 170 per100 ml) were high in summer in the drinking water in Hyderabad. They also found iron and hexavalent chromium higher than WHO guideline values (Kandhar and Ansari 1998).

The presence of fecal coliform and *E. coli* indicate water contamination with human or animal wastes. Disease causing microbes (pathogens) in these wastes can cause diarrhoea, cramps, nausea, headaches or other symptoms. These pathogens may pose a special health risk for infants, young children and people with severely compromised immune systems.

The spring water at Chashma Goth was highly contaminated with fecal coliform. The unprotected springs are likely contaminated due to human and animal activities in the surroundings. Zubair and Rippey (2000) found high level of contamination of ground water with nitrate and fecal coliform in urban areas of Karachi and suggested that ground water quality was likely affected by wastewater infiltration.

In some cases water quality deteriorates in the distribution system itself due to cross contamination. The communities living in the coastal areas are directly discharging their domestic and sewage wastes through surface drains that are mixed with various other urban sources and run perennially. Many water distribution lines are either a few centimeter distances with sewerage lines or are nearly submerged in the ground saturated with the sewerage discharge (Zubair and Rippey 2000). Some of these lines when subjected to leakages result in cross contamination (personal observation at the site).

The water supplied to the Rehri village appeared to be comparatively clean at the heads from main supply line whereas the water supply or distribution gets contaminated in the project area by multiple sources and at multiple points including cross contamination of water distribution system by the wastewater channel in the vicinity. The high contamination in storage containers is simply due to unhygienic ways and water handling practices. Earthen jars, covered with lids and had daily replacement of water had lower contamination levels than the storage tanks that integrated and accumulated contamination level over time. The quality of drinking water was certainly not fit for drinking without boiling or disinfections with chlorination. Luby *et al* (2000 and 2001) used plastic water storage vessel with home chlorination as a potential inexpensive, sustainable means to achieve cleaner water. An inexpensive, home-based water decontamination and storage system was tested in a pilot project in a low-income neighborhood of Karachi. The baseline drinking water samples among intervention households were found contaminated with a mean 9397 cfu per 100 ml of thermo-tolerant coliform compared to a mean 10,990 cfu per 100 ml from controls (Luby *et al* 2001). It is possible that in Rehri village, a specifically designed water storage container and at home chlorination of water acceptable to the communities is introduced that would markedly improve water quality and ultimately the health.

Conclusion

The study patently highlighted the problems of water quality in a coastal village. Even where water supply from source is relatively pure the point where water is used, the likelihood of contamination is high. It is therefore, imperative that a programme of water and sanitation should encompass not only the supply, but also take into account the knowledge, attitude and practices of the community for relevant interventions as required. A regular sanitary survey will help in identifying the potential source of contamination. However, the preferred method to provide quality drinking water in Karachi would be not only to develop but also maintain effective municipal water purification, delivery and sanitary sewerage systems.

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SOME ECOLOGICAL STUDIES ON *LINUM USITATISSIMUM* LINN

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The application of fertilizers appeared to have no significant effect on the overall growth and productivity. Various salt types affected various parameters with a tendency of better growth in light textured soils. The plant grew better in full light condition with optimal soil moisture. The plants wilted to death under shady condition. Both the water stressed and waterlogged conditions reduced the growth performance but the waterlogged condition adversely affected the plants. Therefore, it is concluded the *L. usitatissimum* could be grown in semi-arid condition on marginal lands.

Key words: *Linum usitatissimum,* Soil moisture, Growth, Productivity.

Introduction

Linum usitatissimum Linn. (Family Linaceae) a medicinal plant has various names such as linseed, flaxseed, common flax, flas, lint (English); alsi, alish, tisi (Punjabi and Urdu); Bazarug, kuman, kuta, tukhme-katan, zaghir, zaghu (Persian). It has many medicinal and other uses (Khan 1969; Khan 1970; Sahid Leitch 1994). Its seeds are used as demulcent in catarrhal complaints and acute or chronic gastritis. Powdered seeds are used as an emollient in poultices for boils, carbuncles, festering sores and other skin affections. The seeds contain galactose, arabinose, rhamnose, xylose, galacturonic and manuronic acids; 30-49% fixed oil, 25% protein, sterols, trilierpenes, 0.1-1.5% cyanogenic glycosides and monoglycosides (Bissent 1994). The cultivation of linseed has several advantages for arable farming interms of profitability, expanding demands and adaptability of the crop to various soil types and seasons. Several workers have made studies on its cultivation (Smid 1998) and under different soil moisture levels (Teo *et al* 1989; Lambert *et al* 1990; Singh and Sharma 1991; Ranney and Bir 1991). The present study was conducted to see the effect of different soil types, light conditions, fertilizers and water levels on the growth performance of this plant.

Materials and Methods

Seedlings of *L. usitatissimum* were raised in nursery beds with loamy soils on October 30, 1998. At an average height of 5 cm, 10 seedlings were transplanted to equal size (18 x 22 cm) pots containing loamy soil. After 7 days, they were thinned to 5 uniform seedlings per pot. There were 5 replicates for every treatment in each of the experiments. In all the experiments loamy soil was used except where the effect of soil type was to be investigated. Weeding was done by hand. The pots were maintained at field capacity (Hussain *et al* 1989) during the experimental period except in experiment where the water and different levels of soil moisture was tested.

To see the effect of soil types plants were grown in similar pots with equal volume of sandy, clayey or loamy soils. The pots were kept in open uniform condition in net house and maintained at field capacity (Hussain *et al* 1989). The effect of fertilizers was determined by applying urea, diammonium phosphate (DAP) and organic matter in each pot following Jalis and Khan (1982). The organic matter was added @ of 0.05% to each pot. The pots were placed in full sunlight, partial light and shade to see the effect of different light conditions (Mubarak *et al* 1983).

To determine the effect of soil moisture on plant growth the pots were maintained at field capacity (control condition), water stressed and waterlogged condition. Initially all the pots were saturated. The water stress was created by withholding the water till the temporary wilting was evident. They were rewatered and again allowed to reach the temporary wilting point. In this way four stress cycles were run. Pots subjected to waterlogging were kept saturated all the time.

In all the experiments, the data on growth behaviour such as number of leaves, branches, height of plants, number of flowers, number of fruits and seeds were taken on April 20, 1999. The amount of total chlorophyll, a and b of shoots was determined following the method of Hussain (1989). The results were subjected to t-test.

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Table 1

Effect of different ecological condition on the vegetative growth performance of *L. usitatissimum*. Each value is an average of 5 replicates, each with 5 plants

Growing condition	Height (cm)	No. of leaves	No. of branches	Fresh weight (g)		Dry weight (g)		Moisture contents (%)	
				Shoots	Roots	Shoots	Roots	Shoots	Roots
a. Effect of soil type									
Sand	39*	48*	21	2.26*	0.33	1.62	0.20	40*	65*
Clay	48*	45*	25	2.44	0.34	1.56	0.20	56*	70*
Loam	48	49	20	1.99	0.38	1.50	0.27	33	41
b. Effect of fertilizer									
Control	44	60	26*	2.00	0.29	1.60	0.16	25	81
Urea	35*	49	11	1.41*	0.22*	1.00	0.15	41*	47*
DAP	49*	69*	27*	1.37*	0.22*	0.99*	0.13	37	69*
OM	40	60	19	1.21*	0.27	0.86*	0.16	41*	69*
c. Effect of different light condition									
Full light	53*	55	27	1.67	0.30	0.99	0.18	69	67
Partial light	41	49	19	0.86*	0.20	0.66*	0.13	30	54
Shade	--	--	--	--	--	--	--	--	--
d. Effect of different levels of soil moisture									
Field capacity	49	69	30	1.46	0.21	1.09	0.11	32	91
Water stress	32*	19*	23	0.45*	0.11	0.35*	0.06	29	83
Water logged	19*	12*	12	0.37*	0.04	0.27*	0.02	37	100

DAP and OM, respectively diammonium phosphate and organic matter. * Significant at P=0.05.

Results and Discussion

Effect of soil types. The plant height was minimum (39 cm) in sandy soil (Table1). The least number of leaves were observed in clay soil while the number of branches were more in clay soil. The fresh and dry weight of shoots and roots was slightly high in sand and clay soil. The moisture content of shoots and roots was slightly high in clayey soil (Table 1). The total chlorophyll and chlorophyll a and b were generally high in plants growing in clayey (Table 2). The number of fruits/plant and seeds/plant were high in loamy soil (Table2). Samui *et al* (1995) also reported better growth of plants in clay, sandy and loamy and the present findings agree with them. Similarly, Guadchau and Marquard (1995) and Khodyankora (1995) reported that flax grew better in sandy and loamy soil. The present findings show that the number of branches and chlorophyll contents were greater in clayey soil suggesting its suitability for the growth of *L. usitatissmum* and this agrees with findings of Connor (1994).

Effect of fertilizer. Although the application of DAP enhanced the height, number of leaves and number of branches but plants had greater fresh and dry weight of shoots and roots in control (Table 1). The plants grown in control and DAP, the shoot moisture contents were least in control and roots moisture contents were least in urea. The chlorophyll contents differed insignificantly in various treatments with a tendency of least amount in organic matter treatment (Table 2). The number of fruits and seeds were maximum in DAP treatment (Table 2). The application of fertilizers appeared to have no profound affect on the overall growth and yield of *L. usitatissimum.* However, Chaubey and Dwived (1995) and Shahidullah *et al* (1996) reported that flax and other plants had improved growth with the application of fertilizers.

Effect of different light conditions. The variation in light conditions severely affects the overall growth performance of plants as observed in this case. All the plants died in shady conditions while the number of leaves and branches, fresh and dry weight and moisture contents (%) of shoots and roots and number of fruits and seeds (Table 2) were comparatively better in plants growing to full sunlight condition (Table 1). However, chlorophyll a and b and total chlorophyll contents were high in plants grown in partial light (Table 2). It appeared that *Linum* showed best growth in full light. Queida and Desbeiz (1992) and Antuono (1994) also observed that various light condition affected the yield of *Linum* and this agrees with our findings. Burel *et al* (1994) stated that bright light conditions induced thickness and hardness of stem and leaves that increased the weight of *L. usitatissimum.*

Table 2
Effect of different ecological conditions on the chlorophyll contents of *L. usitatissimum*

Growing condition	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	No. of fruits plant	No. of seed/plant
a. Effect of soil type					
Sand	0.56	0.79	0.34	420*	422*
Clay	0.61	0.86	1.47	410*	406*
Loam	0.56	0.79	1.35	530	529
b. Effect of fertilizer					
Control	0.56	0.80	1.36	219	317
Urea	0.52	0.80	1.48	182	195*
DAP	0.56	0.82	1.37	343	355
OM	0.49*	0.63	1.12*	100	263*
c. Effect of light condition					
Full light	0.56	0.80	1.36	350	352
Partial light	0.62	0.89	1.05*	300	294*
d. Effect of different levels of soil moisture					
Field capacity	0.56	0.80	1.36	410	417
Water stress	0.56	0.82	1.38	40	143*
Water logged	0.37	0.54	0.91	18*	54*

DAP and OM, respectively diammonium phosphate and organic matter. * Significant at P=0.05.

Effect of different levels of water. The plants showed better height, number of leaves and branches, fresh and dry weight of shoots and roots and number of fruits and seeds in control, followed respectively by plants growing in water stressed and water logged conditions (Table 1). The total chlorophyll, chlorophyll a and b were least in waterlogged condition (Table 2). The water stressed plants were relatively better that those growing in waterlogged condition. Both the water stress (Gust *et al* 1991; Dubey 1994) and waterlogged condition (Dubey 1994; Mahto and Haque 1994; Malik *et al* 1999) reduced the yield attributes of *L. usitatissimum* and our findings agree with them. The present study suggests the possibility of growing *L. usitatissimum* in dry marginal lands as a source of biomass, oil and cover for the soil.

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SERA FROM NIGERIAN CHILDREN WITH GENITOURINARY SCHISTOSOMIASIS HAVING IMMUNE COMPLEXES AND HEAT LABILE LEUCOCYTE MIGRATION INHIBITORY FACTORS WITHOUT IMPAIRED CELLULAR IMMUNITY

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There was a significant increase in the rate of synthesis of both albumin and globulin as a result of good adaptive mechanism which prevent hypoproteinemia in children with genitourinary schistosomiasis from endemic areas in Edo and Delta States, South Eastern Nigeria. Single radial immunodiffusion method and the Mantoux test were used to evaluate serum acute phase proteins and delayed hypersensitivity skin assay. While the Nytrel filter method of World Health Organization was employed in the counting of *Schistosoma haematobium* ova and the serum inhibitory factor to leucocyte migration was determined in accordance with WHO specifications with modifications in the preparation of the two antigens and a mitogen - BCG, IMV and PPD. Results were interpreted statistically using spearman's coefficient of correlation and regression analysis. Complement factors present in circulating soluble immune complex and complement dependant cell mediated and killed schistosomula. C_4 decreased with increase in number of *S.haematobium* eggs while C_3 (C_3C) products increased with severity of infection. There was an acute phase response to tissue damage by all stages of schistosomes and inflammatory response of immune competent cells against schistosome antigens from eggs and worm organs, which resulted to increase in transferrin. It was suggested that the heat labile leucocyte migration inhibitory factors were present in the sera of *S.haematobium* infected children, and there was a reduced negative skin response to tuberculin antigen in the infected children. These facts establish the possession of adequate functioning of the cytotoxic $CD8^+$ T lymplocytes in the infected children. The heat labile migration inhibitory factors are products of immune complexes which when activated by complements result to patechial haemorrhagic manifestations. Leucopenia, atypical lymphocytes and plasma cells were observed in the blood which are characteristics of children who have experienced previous attack of *S. haematobium* infection.

Key words: Genitourinary schistosomiasis, Immune-complex, Leucocyte-migration, Cellular immunity.

Introduction

Most studies on *Schistosoma haematobium* are on the level of epidemiology, pathology, biology, control and prevention. However, immunology of genitourinary schistosomiasis in relation to urinary egg count among Nigeria school children has not been fully studied. Available information on the immunological studies of urinary schistosomiasis by Lucas and Boros (1992) and Newar *et al* (1992), on experimental animals are inconsistent and focused attention on a few indices.

The belief that immunity is a major factor controlling the prevalence and intensity of schistosomiasis in men is a deep seated one. It seems impossible to most observer that subjects in endemic areas who are exposed to infected waters are not constantly re-infected. Furthermore, there is a continuous trickle of anecdotal evidence of the infectivity of the parasite supplied by case reports of individual from non-endemic areas who became infected after only a short term exposure (Warren 1973).

During infection patients undergo immunologic modulation, which results to decrease inflammation around the eggs, and this allows patients to survive up to chronic stage of infection (Boros *et al* 1975). The evasion of penetrating schistosomula from the destruction of eosinophils and non-specific antibody of the host activates the macrophage, as further defensive machanism (Olds and Ellner 1984). The importance of macrophage in schistosome infection derides from increased secretion of granule-poietic colony stimulation factor (CSF) from monocyte-macrophage and lymphocytes (Bolin and Robinson 1977; Verman *et al* 1979). Schistosomiasis is associated with a significant decrease in the bacteria-phagocytic

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Table 1
Mean serum levels of total proteins albumin and globulin in schistosomiasis infected children and control

Subjects	No. examined	Total proteins (g/100ml)	Albumin (g/100ml)	Globulin (g/100ml)
Control	50	7.33 + 0.62	2.96 + 0.38	2.60 + 0.45
Lightly infected children (<50 eggs/10ml urine)	75	7.02 + 0.41	2.54 + 0.15	2.48 + 0.42
Heavily infected children (>50 eggs/10ml urine)	75	5.49 + 0.61	2.15 + 0.32	1.96 + 0.43
Control and heavily infected compared		2.40 < 0.02	1.50 < 0.01	0.40 < 0.30
Control and heavily infected compared		3.10 < 0.01	9.60 < 0.02	1.40 > 0.02
Lightly infected and heavily infected compared		0.60 < 0.02	1.00 < 0.03	2.30 < 0.02

index of macrophages due to reduced proportion of cells engaged in phagocytosis (Wellhansen and Boros 1981). The objective of this study was to investigate the immunobiological effects of *S. haematobium* on children with chronic genitourinary schistosomiasis.

Materials and Methods

Two hundred primary school children from *S. haematobium* endemic areas were enrolled in the study. These children were 5-15 years old, comprising of 75 lightly and heavily infected children. Their sexes were in equal ratio in each category.

Controls. There were 50 primary school children whose ages were within 5-15 years old and were sex matched with the infected children. These were all apparently healthy and immune from *S. haematobium* or any other blood helminth infection.

Blood collection. Consent were sought from both parents and teachers before blood was drawn from each of their children. About 10ml of venepuncture blood was drawn from each child and 5ml placed on clean plane glass container till refraction for serum operation.

Exclusion of other parasites. Plasmodia, trypanosomes and blood micro filarial agents were all included by thick smear stained with Geimsa of pH 7.2. This was examined under x40 and later confirmed under x100 objective lens using light microscope.

Serum acute phase proteins. Serum acute phase protein like the C_4 ,C_3C, transferrin, C-reactive proteins, α-2-macroglobulin, haptoglobulin and caeruloplasmin were determined and evaluated using single radial immunodiffusion method of Mancini *et al* (1965).

*Counting of S.haematobium eggs.*Highly turbid or haematuric urine sample were diluted appropriately with normal saline or phosphate buffered saline of pH 7.2 before counting of *S. haematobium* eggs. Nytrel filter was used to count the eggs microscopically in accordance with World Health Organization (WHO 1983).

Serum inhibitory factor to leucocyte migration. The serum inhibitory factor was based on the Hudson and Hay (1976), with slight modifications by including both the sera of test and control children in the solution used in the preparation of the antigens and mitogen (BCG, IMV and PPD).

The walls in migration chamber contain cut capillary tubes topped with medium or antigen - sera medium solutions. These were done in duplicates. About one volume of 1 in 50 of each antigen prepared was mixed with equal volume of 1 in 50 of both test and control sera each. Then the percentage migration due to the presence of antigens and sera were calculated by using:

$$MIS = \frac{Ms}{Mc} \times \frac{100}{1}$$

Where MIS is the migration index in the presence of antigen and serum, Ms is the sera of the migration in the presence of antigen and serum solution and Mc is the sera of migration in medium (15% foetal calf serum).

Delayed hypersensitivity skin assay using Mantoux test. The administration of Mantoux test was through injection of 0.1ml (5 tuberculin units-TU) of phenol-preserved and Tween-80 stabilized purified protein derivative (PPD) using a plastic insulin syringe and gauge 27 steel needle. The injection site was the middle third of the anteromedial aspects of the left forearm. Each vial of 10 dose of PPD was used up

Table 2
Distribution of acute phase proteins amongst children with urinary schistosomiasis

Subjects	No. examined	C_3 (mg/100ml)	C_4 (mg/100ml)	c- reactive proteins (mg/100ml)	Caeruloplasm in (mg/100ml)	α-2-macro globulin (mg/100ml)	Haptoglobul in (mg/100ml)	Transferrin (mg/100ml)
Control	50	98.1±6.5	19.6±6.2	1.2±0.1	30.6±5.2	23.7±0.1	70.1±42.1	197.2±31.0
Lightly infected children (1-50 eggs/10ml urine)	75	105±11.2	25±12.1	3.11±1.1	33.4±6.2	25.2±1.2	88.6±58.2	182±9.6
Heavily infected children (>50 eggs/10ml urine)	75	110±7.5	12.6± 6.3	3.15±0.2	32.5±5.4	26.5±4.3	94.5±60.2	172±6.5
Control and lightly infected compared		3.6<0.02	0.30>0.02	6.0 <0.01	1.4 >0.02	2.1 <0.02	0.7 >0.1	1.2 >0.03
Control and heavily infected compared		6.2<0.02	4.2<0.01	7.3 > 0.01	0.9 >0.02	2.0 <0.01	1.2 >0.1	1.9 <0.01
Lightly infected and heavily infected compared		1.0>0.01	2.4<0.02	2.4 <0.02	0.1 >0.01	1.4 >0.1	1.2 >0.01	1.6 >0.2

before the next was opened. The result was read at the third day after administering the injection and children were instructed not to scratch the site/ point of the injection. The results were read as follows: an induration measuring zero to 4mm was interpreted as negative, 5mm to 9mm as intermediate and above 10mm as positive.

Statistical methods. Results were interpreted using Student's t-test, Chi-square (X^2) test, Spearman's Coefficient of Correlation and Regression analysis.

Results and Discussion

Table 1 shows the mean serum levels of total proteins, albumin and globulin in genitourinary schistosomiasis infected children and control. The mean value of total proteins reduced significantly among the heavily infected children and lightly infected children when compared with the control. This was not the case when heavily and lightly infected were compared. There was a marked reduction of mean albumin level comparing lightly infected with control, lightly infected with heavily infected and control with heavily infected. The mean serum globulin level was not significantly reduced, comparing control with lightly infected but there was a significant reduction when globulin level in heavily infected was compared with control and lightly infected. There was no significant correlation between total protein, albumin and globulin with egg count ($r = 2.1$, $p > 0.01$; $r = 0.4$, $p > 0.02$ and $r = 2.0$, $p > 0.20$) in that order.

Table 2 shows the distribution of acute phase proteins amongst children with genitourinary schistosomiasis. Significant difference existed between the infected children and the control when the levels of C_3C, C-reactive protein and α-2-macroglobulin were compared. Significant difference also existed between the lightly infected when the mean values of C_4 and C- reaction protein were compared. C- reactive protein and α-2-macroglobulin showed a marked correlation with eggs counted ($r = -0.16$, $p<0.005$, $r = -0.15$, $p < 0.005$), respectively. Both C-reactive protein and haptoglobulin were detected in 26 (52.0%) and 12 (24.0%), respectively from the control.

Table 3 shows the leucocyte migration index from control, lightly and heavily infected children, vis-à-vis the serum inhibitory substances, using tuberculin purified protein derivative antigen, with either pooled sera from control, lightly and heavily infected, or heat inactivated pooled sera from heavily infected. Migration index of leucocyte from control was significantly decreased by the addition of sera of children with either lightly or heavily infected. The addition of sera from heavily infected children showed highest migration index. The migration index from control was highest in the presence of inactivated pooled sera. Only the sera from heavily infected children imparted a marked reduction in the mean migration index of leucocytes from lightly infected, and mean migration index of leucocytes from heavily infected.

Table 4 shows the result of purified protein derivative (PPD-Mantoux skin test) on both the control and the infected children- both treated and untreated. The result revealed marked increase in mean diameter of induration - Mantoux skin test reaction, in the heavily infected when compared with the control and in the lightly infected, when compared with the heavily infected before and after treatment. Out of 55 untreated and 70 treated infected children, 11 and 13 chil-

Table 3
The leucocyte migration index from control, lightly and heavily infected children compared to the serum inhibitory substances

Sources of sera	Control		Lightly infected (1-50 eggs/10ml urine)		Heavily infected (>50 eggs /10ml urine)	
	No. examined (n=50)	Mean % age migration index of leucocytes from control	No. examined (n=75)	Mean % age migration index of leucocytes from lightly infected	No. examined (n=75)	Mean % age migration index of leucocytes from heavily infected
i) Foetal calf serum	10	65.7 ± 1.20	15	61.5 ± 4.30	15	57.9 ± 6.10
ii) Sera from control children	10	60.5 ± 10.1	15	58.3 ± 8.40	15	52.3 ± 7.20
iii) Sera from lightly infected	10	54.1 ± 9.40	15	56.2 ± 9.20	15	48.2 ± 5.30
iv) Sera from heavily infected	10	42.2 ± 8.30	15	48.9 ± 10.4	15	33.1 ± 10.2
v) Heat inactivated sera from heavily infected	10	72.8 ± 5.20	15	60.1 ± 8.30	15	55.5 ± 3.40
Comparison						
i and ii		1.3 > 0.01		0.2 > 0.01		1.2 < 0.20
i and iii		2.4 > 0.02		0.4 > 0.02		1.4 < 0.01
i and iv		7.3 < 0.01		1.2 < 0.04		7.2 > 0.10
i and v		0.4 > 0.10		0.4 > 0.10		0.5 < 0.02
Ii and iii		1.2 > 0.01		1.6 > 0.01		0.2 > 0.01
Ii and iv		3.4 < 0.01		0.1 < 0.02		0.4 < 0.02
Ii and v		5.1 > 0.01		2.1 > 0.01		0.3 > 0.02
iii and iv		1.0 < 0.01		1.3 < 0.02		0.1 < 0.03
iii and v		1.2 > 0.01		1.0 > 0.01		1.1 > 0.01
iv and v		3.2 < 0.02		3.2 < 0.01		2.3 > 0.01

dren, respectively had their Mantoux skin test negative by having the diameter of their skin reaction less than 4mm (X^2 = 4.15, p> 0.01 and X^2 =2.21, P > 0.01, respectively). While 17 out of the 50 uninfected control children showed negative skin reaction. The correlation coefficient of the skin reaction diameter of Mantoux test and eggs counted using tuberculin PPD antigen showed a significant positive result (r = 0.26, P< 0.01). While the correlation coefficient of skin reaction diameter of Mantoux test and leucocyte migration index using tuberculin PPD antigen showed a negative result (r = 0.41, P> 0.02).

There was loss of protein from the urine of schistosome infected children as reported by Ukwandu *et al* (2001) yet, the mean values of the total protein in both the lightly and heavily infected children still falls within the normal reference range of (6.7-8.2)g / 100ml. This could be as a result of good adaptive mechanisms, which prevent hypoproteinemia in genitourinary schistosomiasis patients in the form of increase in the synthesis of albumin and globulin. The mean synthesis of these protein have been observed in this study to be significantly higher in the heavily infected children when compared with the lightly infected children and control. It has been established by Rasheed *et al* (1990) and Hussein *et al* (1993) that the presence of complement factors (C1q C_4 and C_3) in circulating soluble immune complexes and in complement dependent cell mediated kill schistosomula.

However, none of the studies did relate complement factors and other acute phase reactants to number of eggs of schistosoma. This study has established that C_4 decreases with increase in the number of *Schistosoma* eggs; while C_3 (C_3C) products increase with severity of genitourinary schistosomiasis. This has tallied with the works of Santoro *et al* (1980) who detected an increase in C_3d amongst heavily infected children with *S. mansoni*. A factor of complement activation by *Schistosoma* antigens was believed to have been responsible for the decreased C_4 and increased C_3C levels vis-à-vis number of eggs excreted and the release of proteinase enzymes by schistosomes. This reacted directly with complements' factor, which resulted in hypocomplementemia.

Irrespective of the severity of the disease, yet remarkable decrease in transferrin was observed when heavily infected children were compared with the control.

This observation could be due to acute phase response to tissue damage by all stages of schistosomes that affected

human. The penetration of the skin by cercariae damaged the skin, while schistosome adults adhered to red blood cells (RBC) and subsequently lysed the RBC. The lysed RBC, thus released iron which then bound with transferrin. This was then passed directly into the developing erythrocyte to form haemoglobin.

In the alternative, the observed low level tranferrin could be due to schistosome eggs which destroyed the tissues during circulation or inflammatory response of immune competent cells against schistosome antigens from eggs and worm origins. The bacterial infection was a result of low transferrin level added to reduce efficiency of phagocytic cells at the late acute and early chronic stages of schistosomiasis. This is supported by the fact that transferrin is an iron-binding protein in serum. Low level means that free iron is abundant for use by bacteria i.e. iron for their multiplication.

Haptogblobulin was observed to increase with increase in number of eggs. Haptoglobulin is known to bind free haemoglobulin which is then degraded for iron to be saved in the liver. Stites (1980) showed that a high plasma haptoglobulin concentration occurred non-specifically as a part of acute phase reaction during acute inflammation. This work confirmed inflammation superceding haemolysis in genitourinary schistosomiasis.

C - reactive protein (CRP) was observed to increase in the infected children when compared with control. This was as a result of inflammatory response to schistosome antigens. Although, it was observed that, CRP reduced insignificantly in the lightly *S. haematobium* infected children as compared with the heavily infected. The explanation for this could be as a result of more complement activation by CRP in heavily infected.

This CRP, is known to initiate alternate pathway of complement activation (Stites 1980) and expected to be significantly higher in heavily infected than lightly infected as an inflammatory response to higher number of eggs laid by schistosome adults. Nonetheless most of the CRP produced were observed to have been consumed at a faster rate in heavily infected by alternate pathway of complement activation.

Both α-2-macroglobulin and haptoglobulin were observed to increase significantly amongst the infected children when compared with schistosomiasis free controls. This can be explained in line with inflammatory response to schistosoma antigens.

Stites (1980) opined that caeruloplasmin could function as an oxygen-radical scavenger in inflammatory circumstances and therefore caeruloplasmin increase, during genitourinary schistosomiasis was obvious, given the possible increase in immunological reactions by immune competent cells against schistosome antigens.

Table 4
Purified protein derivation (PPD - Mantoux skin Test) on control, and *S. haematobium* infected children (treated and untreated)

Subjects	No. examined	Mean diameter (mm) of Mantoux skin test in treated children	Mean diameter (mm) of Mantoux skin test in untreated children
Control	50	4.8 ± 2.5	4.0 ± 2.0
Lightly infected (1-50 eggs/10ml urine)	75	5.2 ± 3.4	4.4 ± 3.3
Heavily infected (>50 eggs/10ml urine)	75	8.7 ± 5.6	7.6 ± 5.2
Control and lightly infected compared		> 0.02	> 0.01
Control and heavily infected compared		> 0.01	> 0.01
Lightly infected and heavily infected compared		< 0.02	< 0.04

Immune modulation of host immune systems against schistosome antigens, to permit adult schistosome worms existed and has been established by high serum concentration lymphokines being associated with acute intestinal schistosomiasis (Robert *et al* 1993) and a low level of lymphokines associated with chronic stage of intestinal schistosomiasis (El Missiry *et al* 1994).

To date, inhibitory substances to leucocyte migration in the serum of *S. haematobuim* infected patients have not been determined. This work has established that leucocyte migration inhibitory factors are present in the sera of *S.haematobium* children. This was because the sera from both lightly and heavily infected children significantly reduced the migration index of leucocyte when compared with the sera from the non-infected control. The result showed the presence of serum factor(s) particularly in heavily infected children prevent phagocytes from moving off the sites of infections. This augmented inflammation, phagocytosis and granuloma formation. It increased leucopenia from the peripheral blood counts. Acute phase proteins and other serum factors had contributed to this inhibition of leucocyte migration, since high concentration of C-reactive protein was suppressive in *in vitro* lymphocyte responses (Stites 1980). Incubation of sera from

S.haematobium infected pupils at temperature of 56˚C for 30 min did not significantly inhibit migration of leucocytes in the control and infected children. This showed that leucocytes migration substance is likely to be a heat labile protein. Inhibitory tendency was high amongst the heavily infected, since this category was exposed to constant re-infection and therefore immune complexes were excessively formed due to production of excess antibodies. The immune complexes with inherent activation were responsible for haemorrhage.

Negative skin response of genitourinary schistosomiasis in children to tuberculin antigen was markedly low. This was contrary to the opinions of Wilkin and Brown (1977) who showed a reduced positive response to tuberculin streptococcal antigen among *S. haematobium* infected Gambians. This study affirmed a reduced negative skin response in the infected children and was a factor of adequate functioning of T- lymphocyctes in these infected children. Skin reaction was observed to increase in largeness of diameter of induration amongst the heavily infected when compared with lightly infected children. This was as a result of high amount of lymphokines and immune complexes (leucocyte migration inhibitor) in the serum of the heavily infected.

The high amount of serum migration inhibition lymphokines and immune complexes retained more leucocytes (phagocytes) at the site of tuberculin purified protein derivative injection with the resultant increase in skin reaction diameter. Post treated schistosomiasis children showed more positive and increased diameter of induration to Mantoux skin test when compared to the untreated children. This was as a result of the second injection of tuberculin (PPD) acting as boost.

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PRELIMINARY INVESTIGATION ON THE HERBICIDAL POTENTIALS OF THE EXTRACTS FROM MAIZE INFLORESCENCE ON SEEDS OF THREE TROPICAL COMPOSITAE WEEDS

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The herbicidal potentials of extracts derived from maize inflorescence were collected at 48, 96, 144, 196 and 240 h after formation were examined on the weeds, Siam-weed (*Chromolaena odorata*), Node-weed (*Synedrella nodiflora*) and Tridax (*Tridax procumbens*). Two sets of aqueous extracts (A and B) were prepared. Treatment A involved the dispersion of the powdered extracts from the inflorescence in distilled water, while treatment B involved the addition of sodium chloride to the dispersed powder in distilled water. The extracts inhibited growth of the seeds of the weeds used when compared to the control. In both treatments 48 h extract tends to be the most effective. The effectiveness of the extracts decreases with an increase in the age of the inflorescence. Hence, more extract-treated seeds germinate with an increase in the age of the inflorescence. The addition of sodium chloride to the extracts tends to increase the potency of the extracts in delaying germination of weed seeds.

Key words: Herbicide, Inflorescence, Weeds.

Introduction

The loss incurred as a result of weeds infestation on farms has made the invention of herbicides the miracle of the last millennium. Unfortunately, most of the formulations have low biodegradability rates (Adesina *et al* 1998) hence were detrimental to the flora and fauna species of the environment (Taylor 1998). Also, herbicides are expensive and often beyond the reach of resource of poor farmers who invariably constitute the major stakeholders (Arendsen *et al* 1996).

The search for a sustainable approach to weed control technique that would be environmentally friendly and readily accessible to the resource-poor farmers has now been the focus of ecologists and environmental scientists (Akoroda 2000; Chikoye 2000). Recently, Trebuil (2002) reported the development of a household weed killer by the paddy farmers in northern Thailand. The present study is a part of the ongoing attempt to develop a sustainable weed control method from materials that would be accessible to the stakeholders for growing crops in the field.

Materials and Methods

Maize *(Zea mays)* inflorescences were collected at 48, 96, 144, 196 and 240 h after formation. They were ground in a mortar and later blended with powders in an electric blender.

Portions of 6g each of the 48, 96, 144, 196 and 240h inflorescence powders were measured out. Each of the portions were dispersed in 100 ml of distilled water. The mixtures were then filtered using Whatman No.1 filter paper and the filtrates used for the experiment named as treatment A.

Similary, Portions of 5 g each of the 48, 96, 144, 196 and 240 h inflorescence powders were measured out. Each portion was mixed with 1 g sodium chloride (NaC1, common salt) and dispersed in 100 ml distilled water. The mixtures were then filtered and the filtrates used for the experiment named as treatment B.

Matured weed seeds of *C.odorata, S. nodiflora and T. procumbens* were collected from the campus of the University of Ado-Ekiti. Two sets of Petri dishes were double layered with Whatman No. 1 filter papers. A set of the Petri dishes that consists of 75 Petri dishes were used for treatment A and the other set of 75 Petri dishes for treatment B.

Each set of the Petri dish was divided into 3 sub-sets of 25 Petri dishes each. A sub-set was used for each weed. Five seeds of each of the weeds were placed in each Petri dish and moistened daily with filtrates of the 48, 96, 144, 195 and 240 h of treatments A and B.

Control experiments were set up for the two treatments without adding the extracts. Control experiments for treatment A were moistened with distilled water. In treatment B, 1g of NaC1 was dissolved in 100 ml of distilled water and the resulting solution was used for the control experiments. Thus, each of the extract treatments and the control were replicated five times.

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Table 1
Herbicidal effects of the extracts from maize inflorescence on the germination of *Chromolaena odorata*

Extracts (hs)	Treatment	Herbicidal Effects*					
		C. odorata		*S.nodiflora*		*T.procumbens*	
		1	2	1	2	1	2
48	A	NG	-	NG	-	12	15
	B	NG	-	NG	-	23	11
96	A	10	6	12	38	8	23
	B	NG	-	NG	-	22	13
144	A	9	18	10	54	7	25
	B	NG	-	NG	-	20	15
196	A	9	32	9	63	5	38
	B	NG	-	NG	-	16	28
240	A	8	36	6	71	2	49
	B	NG	-	NG	-	12	36
Control	A	2	93	2	76	2	80
	B	3	50	2	62	2	73

*NG; No Growth, 1; Number of days germination was first observed, 2; Proportion (%) of seed that germinated.

All the petri dishes were kept at room temperatures (28˚C ± 2˚C) and the proportion of germination in them were recorded at an interval of 24h.

Results and Discussion

The effects of the extracts from the maize tassel on the germination of the weed seeds of *C. odorata* is shown in Table 1. The extracts inhibited germination of the seeds when compared to the growth recorded in the control. In both treatments, no growths were recorded in the seeds treated with extracts from the 48h old inflorescence.

The extracts containing NaC1, i.e. treatment B, tends to be more effective than those without NaC1, i.e. treatment A. While there was no germination recorded in all the extracts containing NaC1, germination were recorded in 96, 144, 196 and 240 h extracts that lacked NaC1 where 6, 18, 32 and 36% of the seeds germinated respectively. Table 1 also revealed that the potency of the extracts tend to decrease with an increase in the age of the inflorescence. While germination was first observed on the the 10th day in the 96h extract, it was 9 days in both 144 and 196h extracts and 8 days in the 240h extract. Thus, as the age of the inflorescence increases, there were decreases in the number of days. The germination was first observed in the extract-treated seeds while the proportion of germinated extract-treated seeds increased.

The effects of the extracts on the germination of the weed seeds of *S. nodiflora* are also shown in Table 1. While germination was not recorded in all the seeds treated with salt containing extracts. Also, growths were not observed in the seeds treated with the salt free extract derived from the 48h old inflorescence, but in older inflorescences, growths were observed and the total proportions of germination were directly proportional to the age of the inflorescence.

Similarly, the effects of the extracts on the germination of the weed seeds of *T. procumbens* have been shown in Table1. Though, germination was recorded in all the extracts of both treatments and the trend of the effects were still similar to those obtained in *C.odorata and S. nodiflora*. Extracts containing salts (i.e treatment B) appeared to delay germination considerably than the extracts without salts (i.e treatment A). This might be responsible for the considerable reduction in the proportion of germination of the extract B-treated seeds.

Results from this study suggest that aqueous extracts derived from maize inflorescence possess the potentials to prolong seed dormancy in these weeds. A number of studies had been carried out on the seed physiology of tropical weeds. The range of phenomena discovered amongst them indicated that they possessed short dormancy periods that are easily broken by light which appeared to be the most important germimination trigger in them (Longman 1969; Auld and Martins 1975; Kayode 1999). The depressions in growths might be due to possible release of allelochemicals. The addition of salts to the extracts tends to enhance its potency. Salts were the major components of the household weed killer recently developed in Thailand (Trebuil 2002). The effectiveness of the extract from the 48 h old inflorescence suggests that the active ingredients present in the inflorescence are more likely to be concentrated in the pollen grains of young tassels. Though this study does not consider the effects of the extracts on the already germinated seedlings of these weeds yet field observations had revealed that whenever the maize inflorescence releases the pollen grains, other plants around it do not thrive well. Thus the consideration of the extracts on growing weed seedlings and the determination of the active ingredients in the inflorescence could constitute subjects of further research activities.

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EFFECT OF AQUEOUS EXTRACT OF *AVICINNIA MARINA* ON MYOCARDIAL CONTRACTION OF ISOLATED MAMMALIAN HEART

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Aqueous extract of *Avicinnia marina* commonly known as mangrove showed a marked inhibitory action on isolated mammalian heart *in-vitro*. The degree of inhibition was found to be highly dose dependent. A dose of 100 mg/kg caused cardiac arrest for few seconds. The force and magnitude of cardiac contractions were regained to pre-injection level after 1.6-2 min. Pretreatment with atropine was found to have no effect on myocardial contractions. The amplitude of cardiac contractions was reduced slightly after the administration of adrenaline.

Key words : *Avicinnia marina*, Mangrove, Mammalian heart.

Introduction

Avicinnia marina Linn. (Verbenaceae) commonly known as mangrove is a coastal vegetation. It fringes shores and estuaries and also proliferate luxuriantly in swamp and marshy places. It is found abundantly all along 150 miles coast line of Sindh (Jafri 1966, 1973; Saifullah 1982).

Mangrove plant is not only a land builder and retainer but shows an interesting array of diverse uses i.e commercial and industrial uses in paper, plywood, adhesive, leather, dying and textile industries (Combs and Anderson 1949; Koeppen and Cahen 1956; Julia 1965; Vetter *et al* 1995; Sowunni *et al* 1996) and as a food and feed for livestock and marine organisms.(Borris *et al* 1949; Taedo 1962; Julia 1965). Also used in medicines locally as well as internally (Julia 1965; Shahnaz *et al* 1995; Itoigawa *et al* 2001).

An ample data available on the chemical composition of mangrove reveals the presence of a number of chemical constituents and bioactive compounds (Gosh and Patra 1979; Kokpol Udom *et al* 1990; Adrian *et al* 1998; Sharaf *et al* 2000; Ito *et al* 2000; Bandara-nayake 2002) beside minerals, amino acids, vitamins, fatty acids, lipids and waxes (Drude *et al* 1986; Rashid *et al* 1986; Misra *et al* 1987; Joshi and Kumar 1989; Mohan *et al* 1998; Datta *et al* 2003). While on the other hand very little data is available on its biological and pharmacotoxicological properties. Therefore, it was decided to study the pharmacotoxicological properties of *A. marina* with profound basis regarding the search for a new useful pharmaceutical product capable of benefitting both applied and basic research sides.

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Materials and Methods

Collection and identification. Mangrove plant was collected from the coastal areas of Karachi, identified by a taxonomist and a voucher specimen was kept in our laboratory under PCSIR Herbarium No. 585 for future reference.

Preparation of extract. The collected plant material was washed thoroughly first with tap water and then with distilled water. Dried in air at room temperature and then chopped into small bits. 1.0 kg of chopped material was soaked in 95% ethanol for a period of 5 days with continuous stirring for 6 h per day. Solvent was then decanted and concentrated under reduced pressure at room temperature. The resultant gel like alcoholic extract was partitioned with (V/V 2:1 ratio) water and pet ether with vigorous shaking in separating funnel. The resultant aqueous layer thus formed was separated and concentrated to maximum dryness in rotary evaporator. The mass thus obtained as aqueous extract was used for the study.

Standard reference solutions. Adrenaline, acetylcholine and atropine were used as standard references. These solutions were made in sterile distilled water.

Test solution. Mac. Ewen's solution (Robert 1971) having the following formula was used to perfuse the isolated heart.

NaCl	7.60 g	$NaHCO_3$	2.10 g
KCl	0.24 g	Dextrose	2.00 g
$CaCl_2$ (anhydrous)	0.19 g	Sucrose	4.50 g

Double glass distilled water ———Vol. made to 1 liter.

Animal used. Healthy adult rabbits (2.5 - 3.0 kg) of either sex were used and kept under observation for a period of 7 days before using for the experiment.

Experimental procedure (Lagendorff 1895). Rabbit was killed by dislocating the neck and the beating heart was immediately taken out of the body with at least 1.0 cm aorta attached by cutting the chest wall with sharp scissors. The heart was immediately immersed in a dish containing 200 ml of Heparinized (100 i.u. of Heparin) Mac Ewen solution maintained at 37°C. The heart was gently squeezed to remove blood or any blood clot present in it. All the rudiment and tissues attached to the aorta were removed with care and precaution. The aorta was then cut from the point where it divides and the heart was tied to the perfusion apparatus on glass cannula. Perfusion of the isolated mammalian heart averages 3.24cc per gram of the organ, in conditions similar to those taking place in the body at rest (Aldo 1954). Care was taken to avoid air bubbles to enter the aorta. The oxygenated perfusion solution maintained at a constant temperature of 37°C. The perfusion pressure was also kept constant. One end of the thread was attached to the apex of the heart by a hook and other end to a spring lever through a system of pullies to record the amplitude of cardiac contraction on the smoked kymograph.

The drug was inserted in the heart through a cannula attached to the perfusion apparatus near the aorta. The height of tracings were taken as a force of cardiac contractions. The only modification made in this method was that instead of using ringer solution, McEwen's solution was used, which also resembles much to, plasma minus protein like Ringer's solution (Aldo 1954).

Mangrove extract in a dose of 25 mg/kg, 50 mg/kg and 100 mg/kg was used to assess the affect on myocardial contractions. Before the administration of extract normal responses of an isolated heart against the standard known drugs i.e. adrenaline and acetylcholine were checked.

Results and Discussion

Isolated perfused rabbit heart showed a normal behaviour against the standard known drugs i.e. adrenaline and acetylcholine. Adrenaline showed a positive inotropic effect as shown in Fig 1.

Aqueous mangrove extract showed a significant action on an isolated heart. It significantly reduced the force of cardiac contraction. Decrease in cardiac contractions were found to be highly dose dependent (Fig 2). This effect becomes progressively more intense and marked as the concentration of drug is increased. A dose of 100 mg/kg stopped the heart in partial diastolic condition for few seconds (4 -7 sec) which gradually regained the pre-injection level after 1.6-2 min (Fig 2).

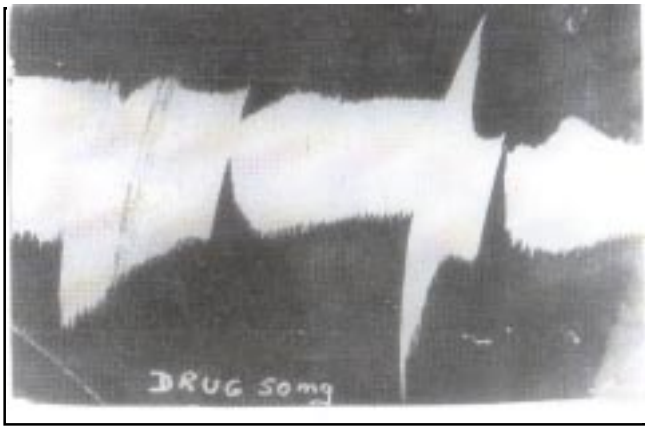

Fig 1. Contractions of isolated rabbit heart on a smoked drum. Note: Positive ionotropic action of adrenaline & a transient reduction in the force of cardiac contraction at a dose of 50 mg of aqueous mangrove extract

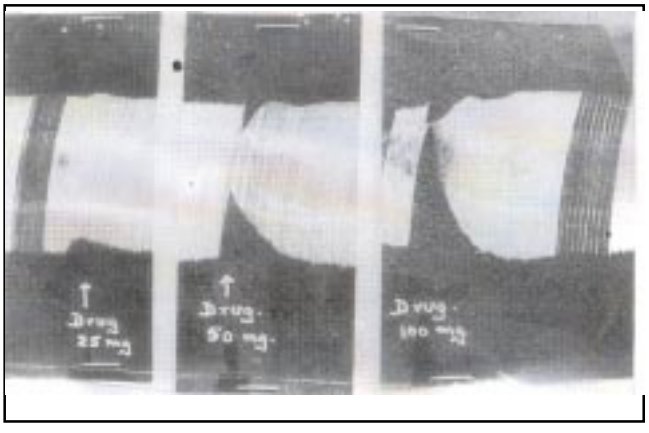

Fig 2. Dose proportionate cardiac inhibition. Extract causes the transient cardiac arrest in partial diastolic condition

Effect of atropinisation. Atropine which is standard known anticholinergic drug when injected in a dose of 2.0 mg/kg abolished bradycardia induced by cholinergic agent (i.e acetylcholine) and resulted in partial heart block. The aqueous mangrove extract in a dose of 100 mg/kg when administered after atropinization produced same inhibitory effects on myocardial contractions as before the administration of atropine i.e heart stopped in partial diastole for 4 - 7 sec and then gradually regained the pre-injection level, (Fig 3). It was further noticed that the amplitude of cardiac contractions were reduced when extract was administered after standard drug i.e adrenaline.

From the study it was evident that the aqueous mangrove extract decreases the contractility of myocardium in isolated

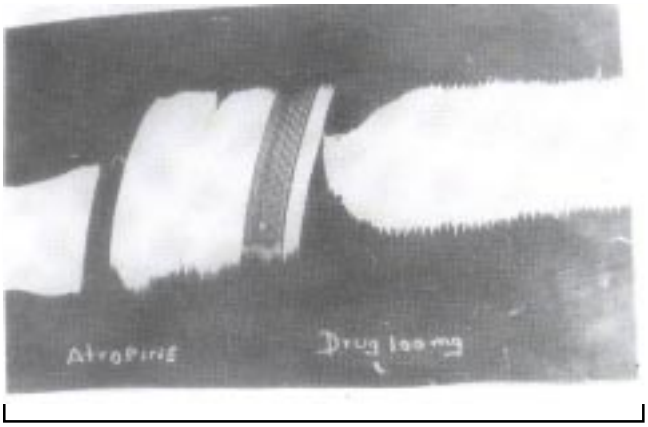

Fig 3. Effect of atropinization and 100mg/kg dose of aqueous mangrove extract on cardiac contraction

rabbit heart preparation in a dose dependent manner (Fig 2). It resulted in cardiac arrest / inhibition for few seconds in higher doses. As soon as the action of the extract was washed, the heart again regained the force and magnitude of contractions to pre injection level.

The amplitude of cardiac contraction was reduced slightly after the administration of adrenaline. Atropinization fails to inhibit the action of extract. This indicates that the extract does not act either through the cholinomimetic or adrenergic mechanism. It can be assumed probably that the mechanism of action of the extract seems to be through sodium channel blockers such as in Quinidine. Literature citation depicts that Quinidine in higher concentration decreases myocardial contraction and produces transient asystole (Andres 1976; Laurence and Bennet 1992) a negative ionotropic effect, as produced by aqueous mangrove extract. Furthermore, the chemical composition of mangrove reveals the presence of polyphenols, tannic acid and napthaquinones (Gosh *et al* 1979; Kokpoludom *et al* 1990; Ito *et al* 2000; Itoigawa *et al* 2001) which also strengthens its mode of action like Quinidine. However, crude extract needs to be further processed to have pure compound(s) which can then be tested for the claimed pharmacological activity.

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TOXICITY OF *CLERODENDRUM INERME* EXTRACT AND CYHALOTHRIN AGAINST *RHIZOPERTHA DOMINICA* PARC STRAIN AND THEIR EFFECT ON ACID PHOSPHATASE AND CHOLINESTERASE ACTIVITY

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Toxicity of *Clerodendrum inerme* (dumdum leaves extract) and cyhalothrin (Pyrethroid) was tested against adult beetles of *Rhizopertha dominica*. Filter paper impregnation method was adopted for the determination of LC_{50} dose. LC_{50} dose of *C. inerme* and cyhalothrin was found to be 1460 µg/cm^2 and 0.00063 µg/cm^2, respectively. Biochemical estimation revealed that both the products inhibited the acid phosphatase and cholinesterase activity to some extent.

Key words**:** *Rhizopertha domimica*, Cyhalothrin, Acid phosphatase, Cholinesterase, *Clerodendrum inerme.*

Introduction

Lesser grain borer, *Rhizopertha dominica* is a cosmopolitan insect and a serious pest of stored grains especially wheat and flour. According to our food requirements wheat has a prominent place in our daily diet. In Pakistan farmers store about 60% of wheat for food and for sowing requirements. Unfortunately about 5-7% of wheat is destroyed by insect pests.

The insect also attacks the germ part of the grain, feeds upon the endosperm and fills the burrows with excrement which leads to poor quality of seed. In Karachi, the lesser grain borer damages more wheat in Dockyard areas probably due to damp climate which softens the hard pericarp of wheat grain. Conventional (synthetic) pesticides are used for the control of this insect pest. But their indiscriminate use causes problems like pollution, residual toxicity and insect resistance (Zettler 1982; Holiday *et al* 1988; Saleem and Shakoori 1993). Synthetic pyrethroids are rapid in action, have low mammalian toxicity and wide controlling range (Bagherwal *et al* 1994). Several workers have reported effect of biopesticides on certain enzyme levels of insects (Bandyopadhyay 1982; Hoyaoka and Dauterman 1982; Shakoori and Saleem 1989; Shakoori *et al* 1994, Ahmad *et al* 2001).

Plant products kill insects, however, they have low mammalian toxicity, leave no toxic residues and do not pollute the environment.

Extracts from dumdum leaves (*Clerodendrum inerme*) can be used effectively to protect stored grains from insect infestations. The extract possesses anti-feedent, repellent, insecticidal and growth disrupter effects on insects. There are no estimates of dumdum leaves production in Pakistan. However, it is widely grown in Sindh and Punjab and some farmers are aware of its pest control properties.

As the synthetic insecticides are toxic and hazardous, so phytopesticides which are much less toxic and biodegradable, the present extract was used against *R. dominica*.

Materials and Methods

Rhizopertha dominica beetles were obtained from PARC, TARC, University Campus, Karachi, reared under controlled conditions i.e., 28± 3˚C and 65 ± 5% R.H. Sterilized wheat was used as rearing medium. All experiments were carried out with 7 days old adults of uniform size. Cyhalothrin was purchased from market and extraction of *Clerodendrum inerme* leaves was prepared in the laboratory.

Extraction of sample. 250g *C. inerme* leaves, collected from Karachi University Campus, were washed for the preparation of extract. Leaves were macerated in 50% methanol (1:1 H_2O: CH_3OH). The macerated leaves were left for 24 h in 250 ml of 50% methanol. Maceration of leaves was done in Ultra Turax grinder and homogenized for 30 min . Finally, it was filtered twice and stored in a refrigerator at 10˚C.

For the treatment of insects filter paper impregnation method was employed. After preliminary test 236, 475, 950, 1900 and 3800 µg/cm^2 doses of *C. inerme* and 0.000316, 0.000633, 0.00126, 0.00253 and 0.00507 µg/cm^2 doses of cyhalothrin were selected. These doses were applied on the filter paper of 2.5 cm diameter with the help of pipette. Twenty *R. dominica* adults of

same age were released in each petri dish separately. For the estimation of acid phosphatase and cholinesterase 300 adults were treated with LC_{50} dose, a day prior to enzyme assay. Thereafter, 150 surviving beetles were crushed in 2 ml double distilled water with the help of mortar and pestle and then homogenized for 10 min at 1,000 rpm at 4°C. The homogenate were centrifuged in Labofuge 15,000 at 3,500 rpm for 20 min , placed in cold chamber. Supernatants were taken in separate tubes and used for biochemical estimation of acid phosphatase activity by thymolphthalein monophosphate using QCA kit and cholinesterase activity was estimated by Clonital's Kit No. KC 060.

Results and Discussion

Toxicity of *C. inerme* extract and cyhalothrin was determined by using five different doses of each compound against *R. dominica*. It was observed that the rate of mortality gradually increased with the increase in dose of each pesticide (Table 1-2). LC_{50} was calculated by using log-log graph paper. By plotting the mean mortality values against the dose of the compound. LC_{50} of *C. inerme* was found to be 1460 µg/cm^2.

R. dominica treated with cyhalothrin showed mean mortality of 32, 48, 60, 72 and 92%, respectively after 24 h . By plotting the mean mortality value against the dose, the LC_{50} of cyhalothrin was found to be 0.00063 µg/cm^2.

Activity of acid phosphatase was found to be inhibited by 20.9% (when treated with *C. inerme* at dose of LC_{50} i.e 1460 µg/cm^2) and 41% (when treated with cyhalothrin at dose of LC_{50} i.e. 0.00063 µg/cm^2, Table 3).

Cholinesterase activity was reduced upto 23.61% with *C. inerme* at a dose of 1460 µg/cm^2 and upto 30.53% with cyhalothrin treatment at a dose of 0.00063 µg/cm^2 (Table 4).

Table 1
Toxicity of *C. inerme* against *R. dominica* beetles

Dose in µg/cm^2	Mean mortality %	S.D. (±)	S.E. (±)	Range at 95% confidence limit
Control	--	--	--	–
0237	12	4.47	2.00	08.08 - 15.92
0475	26	5.47	2.45	21.20 - 30.80
0950	40	7.07	3.17	33.80 - 46.20
1900	62	4.47	2.00	58.08 -65.92
3800	82	4.47	2.00	78.85- 92.41

Table 2
Toxicity of cyhalothrin against *R. dominica* beetles

Dose in µg/cm^2	Mean mortality %	S.D (±)	S.E. (±)	Range at 95% confidence limit
Control	-	-	-	-
0.000316	32	10.90	04.88	22.44 - 41.56
0.000633	48	10.90	04.88	38.44 - 57.56
0.00126	60	14.14	06.34	47.58 - 72.42
0.00253	72	22.80	10.22	51.21 - 92.31
0.00507	95	17.88	08.02	76.28 - 107.72

Table 3
Acid phosphatase inhibition in *R. dominica* treated with *C. inerme* and cyhalothrin

Compound	Mean of unit (µ/l)	S.D. (±)	S.E. (±)	Range at 95% confidence limit	Inhibition %
Control	502.53	565.80	327.0	138.3 - 114.3	00.0
C. inerme	397.30	403.40	233.2	59.7 - 854.3	20.9
Cyhalothrin	296.33	247.20	143.1	16.3 - 576.3	41.0

In present study LC_{50} of cyhalothrin and *C. inerme* was found to be 0.00063 µg/cm^2 and 1460 µg/cm^2 for *R. dominica* beetles. These results are in close conformity with those of Bengeston *et al* (1981) and Ahmad *et al* (2001) for pyrethroids and Islam (1987), Naqvi (1987), Vollinger (1987), Azmi *et al* (1998) and Ahmad *et al* (2000) who used plant products (Safer pesticides).

In the present investigations cyhalothrin was found to be more effective than *C. inerme* against *R. dominica* adults. Bengeston (1981) also found that fenvalerate, permethrin and diphenothrin were not effective against malathion resistant *T. castaneum* and *S. oryzae*, whereas, cypermethrin and decamethrin showed satisfactory effectiveness.

Treatment of *C. inerme* and cyhalothrin inhibited acid phosphatase and cholinesterase enzymes in *R. dominica* adults.

Many workers reported inhibition of acid phosphatase and cholinesterase enzymes in storage insects after pesticide treatments (Ahmad *et al* 2000; Rizwan *et al* 2000; Azmi *et al* 2001 and Rizvi *et al* 2001).

The enzyme cholinesterase is involved in detoxification of insecticide (Saleem and Shakoori 1993; and Shakoori *et al*

Table 4
Cholinesterase inhibition in *R. dominica* treated with *C. inerme* and cyhalothrin

Compound	Mean of enzyme unit (μ/l)	S.D (±)	S.E (±)	Range at 95% confidence limit	Inhibition %
Control	58.44	45.62	26.36	6.78-110.21	00.0
C. inerme	53.57	12.61	7.31	14.92 - 43.54	23.61
Cyhalothrin	36.52	12.60	7.28	22.3 - 50.72	30.53

1998). However, enzyme inhibition in any insect by pesticide use exhibits potency of the poison.

Conclusion

It could be concluded that *C. inerme* extract prove better alternative pesticide for controlling insect pests. They have low mammalian toxicity, less persistent and do not pollute the environment.

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INFLUENCE OF DIFFERENT TYPES OF MILK AND STABILIZERS ON SENSORY EVALUATION AND WHEY SEPARATION OF YOGHURT

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The influence of seven different stabilizers i.e. pectin, guargum, carboxymethylcellulose (CMC), carrageenan, sodium alginate, corn starch and gelatin was studied at 0.4% levels in buffalo milk with 16.6% total solids, cow milk with13.5% total solids and mixture (1:1) of both having 15.0% on syneresis, body/texture, flavor, acidity and color in yoghurt. Results showed that corn starch gave best results in controlling synerises in yoghurt followed by gelatin, pectin, sodium alginate, carageenan, guargum and CMC in buffalo milk as compare to mixture and cow milk. Treatment (T_{19}) having 0.4% corn starch and 16.6% total solids got maximum scores in flavor, body/texture, acidity and appearance than all other six stabilizers. This sample had firm coagulum, less separating whey, good aroma, pleasant taste and rheologically superior to all other samples. Statistical analysis showed that the treatments, storage intervals and total solids had a significant effect ($P<0.05$) on syneresis, body/texture, flavor, acidity and color of the yoghurt samples.

Key words**:** Yoghurt, Milk stabilizer, Syneresis.

Introduction

Yoghurt is defined as the product of fermented milk made from whole, low fat or skim milk. Yoghurt is so popular that it contains all the food value of the milk from which it was made (Krause and Mehan 1984). It has different forms e.g. stirred, set and frozen liquid yoghurt. Among all varieties, set yoghurt with a rather firm body is most common (Potter and Joseph 1995).

In Pakistan, yoghurt is prepared in two ways i.e. by traditional and by commercial methods. In traditional method "Dahi" is prepared at home and by shopkeepers. It is comparatively cheaper but has short shelf life with poor body characteristic and problem of syneresis. These defects yield a product of variable nature and of low quality. The commercial yoghurt manufacturing in this country is in growing stage, which mainly depends upon a high degree of mechanization and sanitation. With the development of science and technology, chemical additives are used in foods and dairy industries on commercial scales.

In the last few years, attempts have been made to improve the quality of yoghurt but further research is required in this field, particularly on milk composition and whey separation. Milk solid not fat (MSNF) play an important role in preventing whey separation in yoghurt. Richter *et al* (1979) found that MSNF was the most important component affecting the flavor, rheological properties and overall acceptability of the yoghurt.

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Whey separation or syneresis is a major problem of yoghurt which occurs when the body of yoghurt is cut and undesirable watery (whey) comes on the surface of yoghurt. Different stabilizers are used to over come this problem during processing and storage of yoghurt. Stabilizers (sometimes referred as hydrocolloids) have two types of action i.e. the binding of water and increase in the viscosity in yoghurt (Boylw 1972). The stabilizers permitted by FAO/WHO in 1976 are natural gums including plant extracts (pectin), seed flour (guar gum), cellulose derivatives (CMC), seaweed extracts (carrageenan and sodium alginates) and cereal starches (corn starch). From animal source includes gelatin (Glicksman 1979).

The most common inoculating material used by the modern dairy plants is the culture comprising of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in the ratio of 1:1, available either in powder or in tablet form. These grow together symbiotically and are responsible for the production of good taste and aroma in yoghurt. This fermentation process also causes pre-digestion of protein, carbohydrates, fats, increase in B–vitamins, enzymes and enhance the calcium bio-availability (Shahani 1983; Kaup *et al* 1987). So far little research work has been conducted on the effect of stabilizers on the physico-chemical characteristics, particularly on syneresis of yoghurt. It is the continuation of our previous study (Ayub and Siddiq 2003), which has been undertaken to improve the quality of yoghurt by controlling whey separation with added stabilizers in fresh dairy farm milk of buffalo and cow (1:1 and individual), available to common consumer in any season.

Materials and Methods

Preparation and formulation of raw material. Buffalo milk (S_1), cow milk (S_2) and mixture of both (1:1) S_3, with total solids 16.6, 13.5 and 15.0% were used for all trails. Hydrocolloid @ 0.4% was added as a stabilizer in formulation of yoghurt mix. Samples were pasteurized at a temperature of 85°C for 15 min in water bath, and cooled to a temperature 42°C. The mixture was inoculated with already prepared starter culture (@ 2%) of *S. thermophilus* and *L. bulgaricus* having pH 4.2. After stirring for 1 min the mix was filled in polystyrene cups (125 ml) and sealed. The culture mix was incubated at a temperature of 42°C till setting of the body to pH (4.2 ± 0.05) and acidity (0.85 ± 0.05%). The yoghurt was cooled to 4°C in order to stop further fermentation. The product was stored for 15 days at 10°C for further studies. Samples prepared for this study with different milk composition and stabilizers are presented in Table 1.

Table 1
Plan of study for yoghurt samples

Code	Stabilizers	Concentration	Milk	% TS
T_1	Control	Nil	Buffalo	16.6
T_2	Control	Nil	Cow	13.5
T_3	Control	Nil	Mixture	15.0
T_4	Pectin	0.4%	Buffalo	16.6
T_5	Pectin	0.4%	Cow	13.5
T_6	Pectin	0.4%	Mixture	15.0
T_7	Guargum	0.4%	Buffalo	16.6
T_8	Guargum	0.4%	Cow	13.5
T_9	Guargum	0.4%	Mixture	15.0
T_{10}	CMC	0.4%	Buffalo	16.6
T_{11}	CMC	0.4%	Cow	13.5
T_{12}	CMC	0.4%	Mixture	15.0
T_{13}	Carrageenan	0.4%	Buffalo	16.6
T_{14}	Carrageenan	0.4%	Cow	13.5
T_{15}	Carrageenan	0.4%	Mixture	15.0
T_{16}	Sodium alginate	0.4%	Buffalo	16.6
T_{17}	Sodium alginate	0.4%	Cow	13.5
T_{18}	Sodium alginate	0.4%	Mixture	15.0
T_{19}	Corn starch	0.4%	Buffalo	16.6
T_{20}	Corn starch	0.4%	Cow	13.5
T_{21}	Corn starch	0.4%	Mixture	15.0
T_{22}	Gelatin	0.4%	Buffalo	16.6
T_{23}	Gelatin	0.4%	Cow	13.5
T_{24}	Gelatin	0.4%	Mixture	15.0

Syneresis or whey separation. Susceptibility to syneresis was determined by using the drainage test described by Molder *et al* (1983), using 120 ml container of yoghurt.

Organoleptic evaluation. Samples were evaluated for flavor, body texture, physical appearance and taste or sensory acidity by a panel of three judges using the score card as approved by American Dairy Science Association (Nelson and Trout 1964).

Statistical analysis. The data obtained was statistically analysed using three factors factorial design according to Steel and Torrie (1980).

Results and Discussion

Syneresis/whey separation. Table 2 shows the mean values of syneresis of yoghurt samples. The mean values for pectin (T_2), guargum (T_3) and CMC (T_4) treated yoghurt samples (0.9, 0.933, 1, 1.433, 1.333, 1.433, 1.933, 1.967 and 1.9 ml) in S_1, S_2 and S_3 gradually increased (8.533, 8.467, 8.767, 11.367, 11.4, 11.433, 15, 14.833 and 14.7 ml) during 15 days storage. Samples with carrageenan (T_5) and sodium alginate (T_6) showed mean values of 2.333, 2.3, 2.367, 3.2, 3.267 and 3.267 ml which was gradually increased to 18.233, 18.4, 18.567, 16.2, 16.233 and16.233 ml, respectively. The mean values of samples with added corn starch (T_7) and gelatin (T_8) (0.533, 0.467, 0.5, 0.8, 0.733 and 0.733) gradually increased (6.8, 6.867, 6.9, 8, 8 and 7.967 ml) during storage. Control samples (T_1) showed a higher increase (from 1.9, 1.867 and 2.033 to 21.333, 20.833 and 21.1 ml) in syneresis during storage (Foley and Mulchahy 1989; Rouse and Moore 1973), as compared to those samples having stabilizers. Our results showed that syneresis decreased with the increase in total solids in yoghurt samples. Among all added stabilizers corn starch gave best results in controlling syneresis in yoghurt samples.

Statistical analysis showed that storage intervals and stabilizers had a significant effect ($P<0.05$) on syneresis of the product; the interaction between storage intervals and stabilizers was also highly significant. These results are in agreement with the findings of ealiers (Nielson 1974; Christensen and Trudsoe 1980; Anderson 1981; Porsdal and Jakobsen 1983; Anon 1993), who determined that yoghurt with increased total solids, had a resistance in developing syneresis.

Body/texture. The body/texture of the product is the next important factor in organoleptic evaluation. For body/texture excellent score was 30 and 24 for acceptable. The mean score for body/texture of samples from T_2 to T_6 (30, 24, 24, 22, 16, 10, 22, 16, 12, 16, 12, 8, 24, 20 and 12 in S_1, S_2 and S_3) decreased (28, 24, 22, 18, 12, 8, 14, 8, 8, 12, 6, 6, 22, 16 and 12)

Table 2
Effect of storage time on syneresis of different yoghurt samples

Milk	Treatments	Storage time (days)				Average (ml)
		0	5	10	15	
Buffalo (16.6% T.S)	T_1	1.900	7.967	14.100	21.333	11.325
	T_2	0.900	2.500	6.133	8.583	4.517
	T_3	1.433	2.733	6.633	11.367	5.542
	T_4	1.933	2.900	7.167	15.000	6.750
	T_5	2.333	6.067	12.433	18.233	9.767
	T_6	3.200	5.500	11.433	16.200	9.083
	T_7	0.533	1.433	5.200	6.800	3.492
	T_8	0.800	2.067	5.900	8.000	4.192
Cow (13.5% T.S)	T_1	1.867	7.733	13.500	20.833	10.933
	T_2	0.933	2.500	6.233	8.467	4.533
	T_3	1.333	2.700	6.733	11.400	5.542
	T_4	1.967	3.400	7.167	14.833	6.842
	T_5	2.300	6.100	12.467	18.00	9.817
	T_6	3.267	5.667	11.567	16.233	9.183
	T_7	0.467	1.533	5.300	6.867	3.542
	T_8	0.733	2.167	5.967	8.000	4.225
Mixture (15.0% T.S)	T_1	2.033	8.067	14.00	21.100	11.300
	T_2	1.000	2.467	6.333	8.767	4.642
	T_3	1.433	2.667	6.800	11.433	5.583
	T_4	1.900	3.167	7.233	14.700	6.750
	T_5	2.367	6.133	12.500	18.567	9.892
	T_6	3.267	4.800	11.667	16.233	8.992
	T_7	0.500	1.667	5.367	6.900	3.608
	T_8	0.733	2.267	6.033	7.907	4.250

T_1, Control; T_2, Pectin; T_3, Guargum; T_4, CMC; T_5, Carrageenan; T_6, Sodium alginate; T_7, Corn starch; T_8, Gelatin.

during storage. The mean score of samples T_7 and T_8 (30, 30, 24, 30, 24 and 24) remain same during storage, while in control samples it significantly ($P<0.01$) decreased during storage, due to weak body development and lumps production (Table 3).

Comparative study of the samples showed a significant ($P<0.01$) increase in the score of body texture of yoghurt sample, with added corn starch as compared to other stabilizers and control. Our overall results showed that body/texture consistently and gradually decreased during storage in all samples.

Statistical analysis showed that storage intervals, stabilizers and total solids had highly significant ($P<0.01$) effect on body texture of treated yoghurt samples. Interaction between storage intervals and stabilizers was also significant. These results are in accordance with the findings of (Mehanna and Gonc 1988; Rohm and Kneifel 1993), who reported a decrease in the score of texture in yoghurt samples during storage.

Color and flavor. For color maximum score (excellent) was 10 and 7, for minimum. The mean values for color of samples T_2 to T_6 (8.66, 32.66, 6, 5.33, 2.66, 2, 6, 4.66, 2.66, 5.33, 4, 2, 6.66, 5.33 and 3.33) decreased (8, 6.66, 4, 3.33, 2.66, 2.66, 4, 2.66, 2, 4, 2.66, 2, 6, 4, and 2) during storage. The initial mean scores of samples T_7 and T_8 were 10, 9.33, 7.33, 10, 8 and 6.66. In control samples the mean score (7.33, 5.33 and 3.33) decreased (7.33, 4.66 and 2.66) during storage. Among all yoghurt samples treatment (T_{19}) with added corn starch obtained maximum score, while samples with added guargum got minimum score, which developed a velvety and gummy appearance. Sample T_1 also showed same results. The results showed that samples with added corn starch gave best results for color than other six stabilizers and control samples.

Statistical analysis showed that storage intervals, stabilizers and total solids had highly significant ($P<0.01$) effect on appearance of treated yoghurt samples. These results are in agreement with the results of ealiers (Radha Krishna 1972;

Table 3
Mean score of judges for the body/texture of different yoghurt samples

Milk	Treatments	Storage time (days)			Average
		5	10	15	
Buffalo					
(16.6% T.S)	T_1	28.0	22.0	26.0	25.33
	T_2	30.0	28.0	28.0	28.66
	T_3	22.0	14.0	18.0	18.00
	T_4	22.0	10.0	14.0	15.33
	T_5	16.0	8.0	12.0	12.00
	T_6	24.0	18.0	22.0	21.33
	T_7	30.0	28.0	30.0	29.33
	T_8	30.0	28.0	30.0	29.33
Cow					
(13.5% T.S)	T_1	22.0	18.0	20.0	20.00
	T_2	24.0	24.0	24.0	20.00
	T_3	16.0	8.0	12.0	12.00
	T_4	16.0	6.0	8.0	10.00
	T_5	12.0	6.0	6.0	8.00
	T_6	20.0	14.0	16.0	16.66
	T_7	30.0	24.0	28.0	27.33
	T_8	24.0	24.0	28.0	24.00
Mixture					
(15.0% T.S)	T_1	18.0	14.0	14.0	15.33
	T_2	24.0	18.0	22.0	21.33
	T_3	10.0	6.0	8.0	8.00
	T_4	12.0	6.0	8.0	8.00
	T_5	8.0	6.0	6.0	6.66
	T_6	12.0	6.0	12.0	10.00
	T_7	24.0	22.0	24.0	23.33
	T_8	24.0	18.0	20.0	20.66

T_1, Control; T_2, Pectin; T_3, Guargum; T_4, CMC; T_5, Carrageenan; T_6, Sodium alginate; T_7, Corn starch; T_8, Gelatin.

Table 4
Mean score of judges for the acidity of different yoghurt samples

Milk	Treatments	Storage time (days)			Average
		5	10	15	
Buffalo					
(16.6% T.S)	T_1	14.67	14.67	13.33	14.22
	T_2	13.33	14.67	16.00	14.67
	T_3	14.67	18.67	13.33	15.57
	T_4	12.00	13.33	17.33	14.22
	T_5	16.00	16.00	12.00	14.67
	T_6	13.33	12.00	16.00	13.78
	T_7	20.00	17.33	20.00	19.11
	T_8	16.00	20.00	13.33	16.44
Cow					
(13.5% T.S)	T_1	10.67	12.00	12.00	11.67
	T_2	9.33	12.00	12.00	11.57
	T_3	10.67	13.33	10.67	11.57
	T_4	8.00	8.00	10.67	8.89
	T_5	12.00	12.00	9.33	11.11
	T_6	8.00	8.00	12.00	9.33
	T_7	16.00	16.00	16.00	16.00
	T_8	12.00	17.33	8.00	12.44
Mixture					
(15.0% T.S)	T_1	6.67	8.00	8.00	7.56
	T_2	5.33	9.33	6.67	7.11
	T_3	6.67	8.00	6.67	7.11
	T_4	4.00	4.00	4.00	4.00
	T_5	5.33	10.67	5.33	7.11
	T_6	4.00	5.33	8.00	5.78
	T_7	14.67	12.00	12.00	12.89
	T_8	8.00	12.00	4.00	8.00

T_1, Control; T_2, Pectin; T_3, Guargum; T_4, CMC; T_5, Carrageenan; T_6, Sodium alginate; T_7, Corn starch; T_8, Gelatin.

Mehanna and Gonc 1988). In a similar study, Varbioff (1979) determined that yeast and molds mainly affect the appearance of yogurts during storage.

Flavor of the product is one of the most important factor for determining the consumer's response. The flavor score for excellent was 45 and for acceptable 36. The mean values for flavor of samples T_2 - T_6 (42, 36, 36, 24, 24, 15, 21, 15, 12, 24, 18,12, 33, 24 and 21 in S_1, S_2 and S_3) decreased (39, 36, 33, 21, 21, 15, 18, 12, 9,15, 9, 9, 30, 27 and 21) during storage. The mean score of samples T_7 and T_8 (45, 45, 42, 45, 39 and 36) showed no changes in flavor during storage. Our results showed that samples with added corn starch (T_7) obtained maximum scores; compare to other samples (T_2 - T_8). The data showed that fresh samples got an acceptable range for flavour both for treated and untreated samples, which was significantly ($P<0.01$) decreased during storage. The flavor retention in corn starch samples (T_{19}) was comparatively higher than other samples during storage.

Statistical analysis showed that storage intervals, stabilizers and total solids had highly significant ($P<0.01$) effect on the flavor of treated yoghurt samples. Interaction between storage intervals and stabilizers was also significant. Our results are in agreement with the findings of (Abrahamsen 1978; Resubal *et al* 1987; Rehman 1987).

Acidity. The mean score for acidity (excellent) was 20 and for acceptance was 14. The mean score for acidity of samples T_2 to T_6 (13.33, 9.33, 5.33, 14.67, 10.67, 6.67, 12, 8, 4, 16, 12, 5.33, 13.33, 8 and 4) increased (16, 12, 6.66, 13.33, 10.67, 6.67, 17.33, 10.67, 4, 12, 9.33, 5.33, 16, 12 and 8) during storage. The mean values of samples T_7 and T_8 (20, 16, 14.67, 16, 12 and 8) slightly decreased (20, 16, 12, 13.33, 8 and 4) during storage. The mean values of sample T_1 also (14.67, 10.67 and 6.67) decreased (13.33, 12 and 8) during storage (Table 4).

Our results showed that the mean score for acidity decreased (due to increase in acidity) during storage. Among all the yoghurt samples, treatment with added corn starch obtained maximum mean score for acidity followed by samples with added gelaton.

Statistical analysis showed that storage time, stabilizer and total solids had a highly significant ($P< 0.01$) effect on acidity of treated yoghurt samples. Interaction between storage intervals and stabilizers is also highly significant. The results are in agreement with the findings of previous (Salji and Ismail 1983; Rehman 1987; Mehanna and Gone 1988; Shin *et al* 1991).

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Short Communication

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COMPARISON OF HYPER PRODUCER *ASPERGILLUS NIGER* CULTURES (IFS-5, IFS-6 AND IFS-17) FOR CITRIC ACID FERMENTATION IN SURFACE CULTURE

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Citric acid fermentation by *Aspergillus niger* is an aerobic process and the organism needs a fairly high and constant oxygen supply for its growth (Hang 1988; Haq *et al* 2000). Surface culture technique (SCT) is a conventional method of citric acid production. Most of the pilot plants are using this technique due to low energy consumption and manpower involved (Singh *et al* 1998). In SCT, the substrate remains stationary and organism form mycelial mat on the surface of medium. The relation between constitution of the fermentation medium and rate of citric acid production has been investigated (Elimer and Ewaryst 1995). Sucrose salt medium as synthetic fermentation medium while cane or beet molasses as natural fermentation medium have long been employed as usual routine basal media (Ali *et al* 2001). Clark *et al* (1965) obtained 80% conversion of available sugar, 8 days after incubation. Farouk *et al* (1977) pointed out that the age of culture also affect the yield of citric acid. Both of these authors used synthetic medium in their study on citric acid fermentation. The mutant strains have greater ability to produce citric acid. The present investigation deals with the time course study during citric acid production by surface culture technique using three different mutant cultures of *A. niger* (IFS-5, IFS-6 and IFS-17) and their comparison on kinetic basis.

Organism. In the present study, 3 different mutant strains of *A. niger* (hyper producers of citric acid) were used. These strains (IFS-5, IFS-6 and IFS-17), have already been developed by mutation (Ali *et al* 2001) in Biotechnology Laboratories, Government College University, Lahore and maintained on potato dextrose agar medium. The cultures were stored at 4°C in a refrigerator.

Culture medium. Sucrose salt medium containing (g/l); sucrose 150.0, $MgSO_4.7H_2O$ 0.25, KH_2PO_4 2.5, NH_4NO_3 2.5 at pH 3.5 was used as the basal fermentation medium.

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Fermentation technique. Citric acid was produced by surface culture technique, following the method of Singh *et al* (1998). Conidial inoculum ($1x10^7$ conidia/ml), prepared in sterilized distilled water was used. The optimum conditions for citric acid production were investigated in 250 ml Erlenmeyer cotton wool plugged conical flasks, containing 25 ml fermentation medium. The flasks were incubated at 30°C for 7 days. The results are sum mean of three parallel replicates.

Analysis. Dry cell mass was determined according to Kirimura *et al* (1992). Residual sugar was estimated by DNS method (Ghose and Ghen 1970) while pyridine acetic anhydride method was employed for the determination of citric acid as reported by Marrier and Boulet (1958). The kinetics of time course was also undertaken (Pirt 1975).

Time course study is one of the most critical factors, which determines the efficacy of the process along with product formation (Elimer and Ewaryst 1995). The data of Table 1 shows the biosynthesis of citric acid at different intervals of time. Three different mutant strains of *A. niger* (IFS-5, IFS-6 and IFS-17) were used for their time course comparison. These cultures were incubated at 30°C for 1-11 days. The maximum production of citric acid (48.14 g/l) with mutant IFS-5 was obtained 10 days after incubation, which seems to be uneconomical due to longer fermentation period. When IFS-6 was used for inoculating the culture medium, a maximum citric acid production of 31.52 g/l was obtained with a high degree of consumable sugars (96.0 g/l). Although the fermentation period became very short (5 days) as compared to mutant IFS-5 but the yield of product was too low for an economical process.

The maximum production of citric acid (46.22 g/l) by mutant strain of *A. niger* IFS-17, was achieved 7 days after the inoculation. The dry cell mass and sugar consumed were 23.20 and 94.0 g/l, respectively. Further, increase in the incubation period did not enhance the production of citric acid, rather it was decreased. It might be due to the reduction of sugar contents in the fermentation medium and accumulation of other by-products. Thus incubation period of 7 days was found to be optimum for maximal citric acid biosynthesis. Our results are in agreement with the findings of many workers (Jaszwry *et al* 1971; Singh *et al* 1998). For maximum citric acid production, the optimum time of incubation varies from organism to organism depending on fermentation medium provided (Elimer and Ewaryst 1995).

The kinetic study of time course during citric acid fermentation by mutant strains of *A. niger* was also worked out. There was a marked difference of product yield coefficients (Yp/s and Yp/x) among different mutant strains i.e., maximum Yp/s value in case of IFS-17 (0.492 g/g) was much higher as compared to mutants IFS-5 and IFS-17 (0.384 and 0.328 g/g)

Table 1
Time course study during citric acid fermentation by *Aspergillus niger*

Fermentation period (h)	Dry cell mass (g/l)			Sugar consumption (g/l)			Citric acid (g/l)		
	IFS-5	IFS-6	IFS-17	IFS-5	IFS-6	IFS-17	IFS-5	IFS-6	IFS-17
24	2.50	3.72	4.00	46.5	24.5	35.0	1.56	2.13	0.67
48	5.05	9.10	7.50	54.2	56.6	49.5	2.10	6.25	1.26
72	6.55	16.25	10.61	69.5	79.1	56.2	6.26	9.55	4.50
96	9.00	24.80	12.31	79.5	88.5	73.0	9.55	16.12	16.80
120	13.25	26.45	17.22	87.4	96.0	86.0	13.18	31.52	20.04
144	16.40	29.05	20.11	96.0	100.8	89.5	16.52	26.27	38.55
168	21.15	32.46	23.20	98.5	114.5	94.0	23.55	21.05	46.22
192	24.52	36.62	26.19	104.6	121.0	99.5	32.16	18.66	37.50
216	28.90	37.15	29.27	121.0	126.2	100.6	39.25	18.50	29.53
240	34.62	40.65	31.12	126.5	132.5	106.5	48.14	17.16	27.62
264	39.46	41.20	31.50	128.5	135.6	118.0	42.66	17.05	19.40

Sugar added 150 g/l, Initial pH 3.5, Temperature 30°C

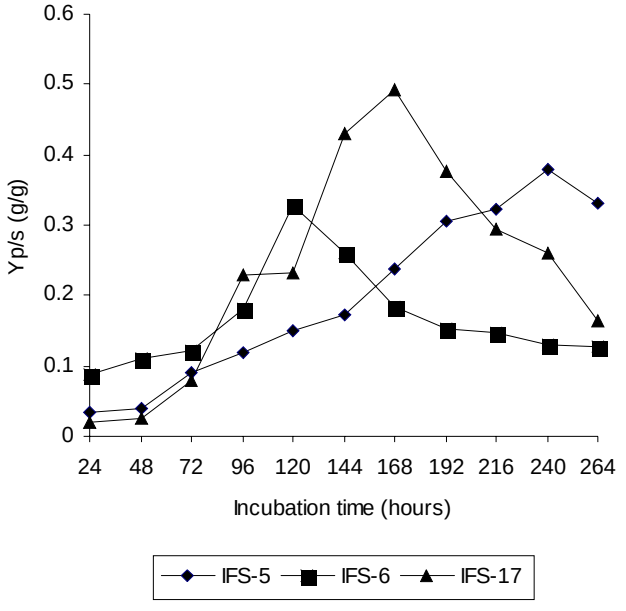

Fig 1. Comparative study of product yield coefficients (Yp/s, g/g) during citric acid fermentation by *A. niger* strains (IFS-5, IFS-6 and IFS-17). The value of Yp/s was determined by product (g/l)/substrate utilized (g/l).

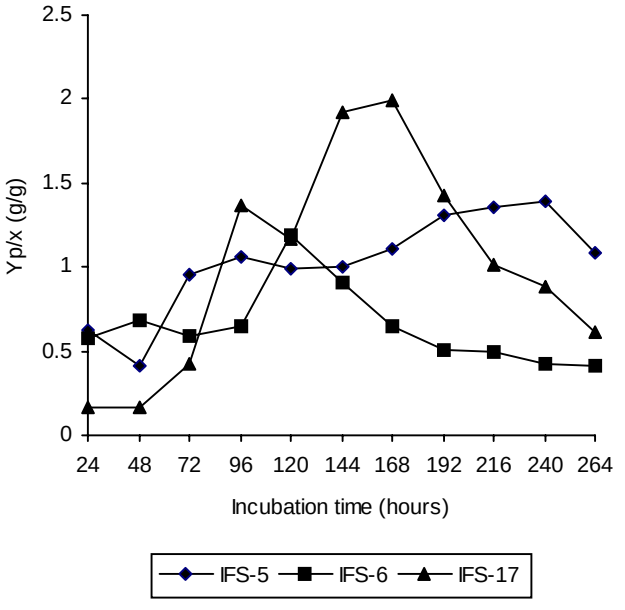

Fig 2. Comparative study of product yield coefficients (Yp/x, g/g) during citric acid fermentation by *A. niger* strains (IFS-5, IFS-6 and IFS-17). The value of Yp/x was determined by product (g/l)/cell mass (g/l).

and the maximum Yp/x value in case of mutants IFS-5 and IFS-6 (1.402 and 1.192 g/g) was lower in comparison with IFS-17. So, the mutant *A. niger* IFS-17 is better producer of citric acid as compared to others. Meyrath and Ahmed (1989) have attempted to reduce the fermentation time by vermiculite addition and found that time is reduced from 0-9 days. Lakshminarayan *et al* (1975) incubated cultures of *A. niger* for 6 days and achieved good results. Shamrai and Orlow (1986) described that the optimum period of fermentation was dependent on the intensity of fermentation and got better citric acid production (28.65 g/l), 8 days after incubation. So, our finding (46.22 g/l) citric acid, 7 days after inoculation by mutant IFS-17 is more encouraging and significant as compared to Shamrai and Orlow (1986).

The mutant strain of *A. niger* IFS-17 is a better citric acid producer (i.e. Yp/s=0.492 g/g) due to faster growth rate. Time

required for maximal citric acid production depends mainly on the fermentation design, type of the strain and composition of basal medium. Mutation can raise the status of fermentation process. The mutant IFS-17 is D-glc-resistant and has been preserved in paraffin oil as a master culture. Proper aeration, suitable depth and Cu^{++} ions addition in the fermentation medium may increase the mycelial branching level and subsequently citric acid productivity. Better performances can be obtained by more quantitative analysis and mathematical modelling, which are left for further study.

***Key words*:** Citric acid, Biosynthesis, Fermentation, *Aspergillus niger*.

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Reid R W, Watson J A 1995 Reaction of lodgepole pine to attack by blue stain fungi. *Can J Bot* **45** (2 Part 1) 45-50.

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