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Editorial Address:

Executive Editor,
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Tel: 92-21-4651739-40, 4651741-43

Fax: 92-21-4651738

E-mail: pcsirsys@super.net.pk;

pcsir-sic@cyber.net.pk

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Mass Transfer Rates and Column Heights in Reactive Extraction Processes

Muhammad Tariq Saeed*, Razia Khanam and Muhammad Yar Shaheen
PCSIR Laboratories Complex, Shahrah-e-Jalaluddin Roomi, Lahore-54600, Pakistan

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Abstract. A mathematical model, which is based on the simultaneous interfacial chemical reactions and diffusion processes, is developed for the extraction of zinc ions from sulphate solution by di(2-ethylhexyl) phosphoric acid in *n*-heptane diluent. Actual column heights were compared with the predicted ones, using the design algorithm based on chemical kinetics. The experimental values of mass transfer coefficients could be varied and were in the range of industrial interest. Using the physicochemical data, hydrodynamics, mass transfer coefficient parameters and reaction kinetics pertaining to the system, it was possible to predict the interfacial flux and column height from first principles with a reasonable degree of accuracy.

Keywords: interfacial flux, reactive extraction, chemical kinetics, zinc/DEHPA, spray column

Introduction

Continuous counter current contactors are usually designed assuming interfacial equilibrium. In the case of mass transfer with interfacial chemical reaction, this assumption is only valid if the chemical reaction is very fast with respect to the mass transfer rate (Chapman *et al.,* 1975). In hydrometallurgical processing, the use of liquid-liquid extraction systems are already very popular. The successful application of such related extraction processes has encouraged fundamental research on the extraction equilibria and kinetics leading to development of mathematical models for the solvent extraction of metals. The kinetics of these systems is generally controlled by a combination of diffusion and chemical rate factors and a properly developed model could become a design equation having practical significance. If all the parameters relating to the system, namely physicochemical, hydrodynamics, mass transfer, and the reaction kinetics are known, it would be possible to design extraction column from first principles.

In this paper a mathematical model is developed for interfacial flux, which is based on simultaneous interfacial chemical reactions and diffusion processes. An attempt has also been made to predict the column height from first principles. The system comprises the extraction of zinc ions from acidic aqueous phase by di(2-ethylhexyl) phosphoric acid (DEHPA) in *n*-heptane diluent. In order to check the validity of the model for design purposes, the predicted values are compared with actual heights.

Model formulation. In a column for continuous extraction in aqueous phase, with interfacial chemical reactions, the mass balance of zinc ions over a differential height δh for unsteady state operation may be given as follows (Saeed and Jamil, 1998a):

$$\frac{\partial C_{za}}{\partial t}(1-\phi_D) = \frac{L_c}{S}\frac{\partial C_{za}}{\partial h} + E_{ax,c}(1-\phi_D)\frac{\partial^2 C_{za}}{\partial h^2} - R_z a_c (1-\phi_D) \quad (1)$$

The first term on the right hand side of equation (1) accounts for convection, the second term for axial mixing and the third one represents the overall rate of interfacial transfer. The term on the left hand side represents the variation of zinc concentration with time.

A similar differential mass balance equation can be derived for metals in the dispersed organic phase (Saeed and Jamil, 1998a):

$$\frac{\partial C_{zo}}{\partial t}\phi_D = -\frac{L_d}{S}\frac{\partial C_{zo}}{\partial h} + E_{ax,d}\phi_D\frac{\partial^2 C_{zo}}{\partial h^2} + R_z a_d \phi_D \quad (2)$$

Under steady state conditions, assuming constant flow rates and neglecting backmixing, equations (1) and (2) reduce to:

$$L_c\frac{dC_{za}}{dh} = R_z a_c S (1-\phi_D) \quad (3)$$

and

$$L_d\frac{\partial C_{zo}}{\partial h} = R_z a_d S \phi_D \quad (4)$$

which on integration give the contactor height:

$$H_c = \frac{L_c}{S}\int_1^2 \frac{dC_{za}}{R_z a_c (1-\phi_D)} \quad (5)$$

and

$$H_c = \frac{L_d}{S}\int_1^2 \frac{dC_{zo}}{R_z a_d \phi_D} \quad (6)$$

*Author for correspondence

In the extraction column the local volumetric extraction rate, r_z , is given by:

$$r_z = R_z a_c \quad (7)$$

Since chemical reactions are also involved, the interfacial flux, R_z, would be a function of chemical as well as mass transfer parameters, while the specific interfacial area depends on the operating conditions of the column, i.e., dispersed phase holdup and mean drop diameter.

In order to integrate equations (5) and (6) for calculating contactor height in a given extraction system, knowledge of R_z, a_c, a_d and ϕ_D is essential.

Interfacial area. Interfacial area between droplets and continuous phase is a function of drop size and dispersed phase holdup. The interfacial area per unit volume of the continuous phase is expressed as (Pilhofer and Schroter, 1986):

$$a_c = \frac{\partial \phi_D}{d_{32} (1 - \phi_D)} \quad (8)$$

$$d_{32} = \frac{\Sigma n d^3}{\Sigma n d^2} \quad (9)$$

A correlation has been proposed that predicts the drop size in spray columns which claimed to be valid both for the absence or presence of mass transfer (Kumar and Hartland, 1984):

$$\frac{d_{32}}{d_n} = E\ddot{o}^{-0.38} \left[1.28 \left[\frac{\Delta\rho}{\rho_d}\right]^{0.3} + \exp(-0.16\,Fr)\right] \quad (10)$$

for, $We_n < 8.64$

With a view to obtaining the holdup for the static continuous phase, ϕ, the following equation should be employed for calculating the countercurrent holdup, ϕ_D:

$$u_s = \frac{U_d}{\phi} = \frac{U_d}{\phi_D} + \frac{U_c}{(1 - \phi_D)} \quad (11)$$

Mass transfer coefficients. In liquid-liquid extraction processes, the mass transfer coefficient would be dependent on whether the drops are rising, falling, internally circulating, or in oscillating state through continuous but immiscible liquid phase. However, it was observed both visually and from the analysis of photographic films taken with a cine camera for the dispersion in the column that the rising drops were oscillating (Saeed and Jamil, 1998a). Accordingly, the correlations for continuous phase and dispersed phase, mass transfer coefficients are dewelled upon only for oscillating drops.

The following correlation has been proposed for continuous phase mass transfer coefficient (Yamaguchi *et al.,* 1975):

$$K_c = 1.4 \frac{D_c}{d_{32}} \left[\frac{\rho_c \omega d^2_{32}}{\mu_c}\right]^{0.5} \left[\frac{\mu_c}{\rho_c D_c}\right]^{0.5} \quad (12)$$

The correlation of Rose and Kintner (1966) for dispersed phase mass transfer coefficient is:

$$K_d = 0.95\, D_d^{0.5} \left[\frac{8\sigma (100\, d_{32})^{0.225}}{d^3_{32}(3\rho_d + 2\rho_c)}\right]^{0.25} \quad (13)$$

Diffusion coefficients. The diffusivity of strong electrolytes at infinite dilution on the assumption of complete dissociation can be calculated from the Nernst-Haskell equation (Reid *et al.,* 1977) as given below:

$$D^\circ_{12} = \frac{RT}{F^2} \frac{\frac{1}{n^+} + \frac{1}{n^-}}{\frac{1}{\lambda^\circ_+} + \frac{1}{\lambda^\circ_-}} \quad (14)$$

Harned and Hudson (1951) introduced a correction factor by taking into account the influence of ion-pair formation on the diffusion coefficient given as:

$$D_{12} = D^\circ_{12} \left[1 + 38.16 \times 10^{-2} (1 - \alpha)\right] \quad (15)$$

The most general and widely used correlation for the prediction of diffusion coefficients of non-electrolytes in organic solvents at infinite dilution is that of Wilke and Chang (1955):

$$D^\circ_{AB} = 1.173 \times 10^{-16} \frac{(\Phi M_B)^{0.5} T}{\mu_B V_A^{0.6}} \quad (16)$$

A modified expression was proposed by Leffler and Cullinan (1970) for higher concentrations, taking viscosity into due account:

$$\frac{D_{AB}\mu_{AB}}{f^{th}} = (D^\circ_{AB}\mu_B)^{X_B} (D^\circ_{BA}\mu_A)^{X_A} \quad (17)$$

Extraction equilibria. The extraction chemistry of zinc with di(2-ethylhexyl) phosphoric acid (DEHPA) has been studied by numerous workers. Ajawin *et al.* (1983) reported the overall equilibrium as:

$$Zn^{2+} + 1.5\,\overline{(HL)}_2 \rightleftharpoons \overline{ZnL}_2.\overline{HL} + 2H^+ \quad (18)$$

From the extraction equilibrium studies, Murthy (1987) reported that two complexes of zinc-DEHPA are formed in *n*-heptane according to the stoichiometry of the reactions:

$$Zn^{2+} + 1.5\,\overline{(HL)}_2 \rightleftharpoons \overline{ZnL}_2.\overline{HL} + 2H^+ \quad (19)$$

$$Zn^{2+} + 2\,\overline{(HL)}_2 \rightleftharpoons \overline{ZnL}_2\,\overline{(HL)}_2 + 2H^+ \quad (20)$$

where bars indicate the species in the organic phase and $(HL)_2$ in DEHPA in dimer form.

Extraction kinetics. From the kinetic studies (Murthy, 1987; Ajawin *et al.,* 1983; 1980) conducted in a cell with constant

interfacial area in the chemical control regime, it has been reported that reactions (19) and (20) take place at the interface. The extraction rate of zinc ions is proportional to their concentration in the aqueous phase, dimeric DEHPA activity and inversely proportional to hydrogen ion concentration:

$$r_{ze} = 2\,k\,a_c \frac{C_{za}\,C_{oD}}{C_H} \quad \text{(Ajawin } \textit{et al.,}\text{ 1983; 1980)} \tag{21a}$$

$$r_{ze} = k_I\,a_c \frac{C_{za}\,C_{oD}\,\gamma_o}{C_H} = k_I\,a_c \frac{C_{zo}\,a_o}{C_H} \tag{21b}$$

where:

$$k_I = 2\,k/\gamma_o$$

While stripping rate is first order with respect to zinc-DEHPA complex concentration, it is first order with respect to hydrogen ion concentration and is inversely proportional to the dimeric DEHPA activity factor ($a_o + 0.75\sqrt{a_o}$):

$$r_{zs} = k'_I\,a_c \frac{C_{zo}\,C_H}{a_o + 0.75\sqrt{a_o}} \tag{22}$$

From equations (21b) and (22), the overall extraction rate can be expressed as:

$$r_{zs} = k_I\,a_c \frac{C_{za}\,a_o}{C_H} - k'_I\,a_c \frac{C_{zo}\,C_H}{a_o + 0.75\sqrt{a_o}} \tag{23}$$

or

$$R_z = \frac{r_z}{a_c} = k_I \frac{C_{za}\,a_o}{C_H} - k'_I \frac{C_{zo}\,C_H}{a_o + 0.75\sqrt{a_o}} \tag{24}$$

The above equations indicate that reaction is independent of mass transfer control and, therefore, expected to yield the maximum interfacial flux for a given set of concentrations.

Baes and Baker (1960) have proposed the following relationship for the activity coefficients of the DEHPA dimer in aliphatic diluents:

$$\log \gamma_o = -0.586\,C_{oD}^{1/3} + 0.565\,C_{oD} \tag{25}$$

Activity coefficient correlation for zinc-DEHPA complex proposed by Koncar *et al.* (1988) is:

$$\frac{1}{\gamma_{Zo}} = 1 + 0.616 \left[\frac{1.7\,C_{zo}}{a_o}\right]^{1.387} \tag{26}$$

The mechanism of transfer of the solute from one phase to the other may be very complexed. The trend of mass transfer is assumed to proceed according to the following three steps:

(i) zinc ions and DEHPA diffuse to the interface from the aqueous and the organic phases, respectively,
(ii) zinc ions and DEHPA react according to reactions (19) and (20), and
(iii) the liberated hydrogen ions and zinc-DEHPA complex diffuse from the interface into the aqueous and organic phases, respectively.

The three steps take place simultaneously and thus interfere mutually. A schematic diagram of the concentration profiles for all the species at the interface during zinc extraction is shown in Fig. 1. In the mixed control regime, the interfacial flux depends on the kinetics of both mass transfer and interfacial reaction. In the present case, the interfacial rate of extraction is still given by equation (24), but for the interfacial instead of bulk concentrations. Since the interfacial concentrations are normally not directly measureable quantities, these, it involve some mathematical manipulation as given below:

$$R_z = K_{za}(C_{za} - C_{zai})$$
$$R_z = 1/1.7\,K_o(a_o - a_{oi})$$
$$R_z = -1/2\,K_H(C_H - C_{Hi})$$
$$R_z = -K_{zo}(C_{zo} - C_{zoi}) \tag{27}$$

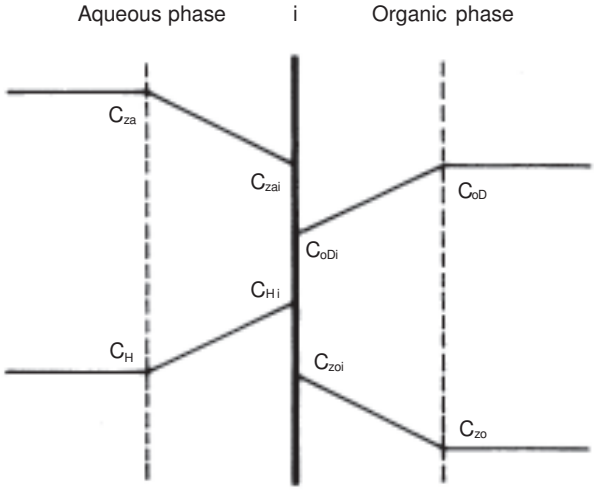

Fig. 1. Concentration profiles at the interface during metal extraction.

Eliminating the interfacial values from equations (24) and (27), the final kinetic expression for the interfacial flux comes out to be:

$$R_z = k_I \frac{\left[C_{za} - \frac{R_z}{K_{za}}\right]\left[a_o - 1.7\frac{R_z}{K_o}\right]}{\left[C_H + 2\frac{R_z}{K_H}\right]} - k'_I \frac{\left[a_{zo} + \frac{R_z}{K_{zo}}\right]\left[C_H + 2\frac{R_z}{K_H}\right]}{\left[a_o - 1.7\frac{R_z}{K_o}\right] + 0.75\sqrt{\left[a_o - 1.7\frac{R_z}{K_o}\right]}} \tag{28}$$

This gives R_z in terms of bulk concentrations of the species involved, individual mass transfer coefficients, and specific reaction rate constants for the extraction and stripping reactions.

Aqueous phase ionic equilibria. The aqueous phase under consideration is a solution of zinc sulphate, sulphuric acid and sodium sulphate. It may be assumed that sodium sulphate completely dissociates, whereas dissociation of zinc sulphate and bisulphate ions is incomplete, being according to the following equations:

$$ZnSO_4 \rightleftharpoons Zn^{2+} + SO_4^{2-} \quad (29)$$

$$H\bar{S}O_4 \rightleftharpoons H^+ + SO_4^{2-} \quad (30)$$

In the aqueous phase, the species namely zinc, sulphate, hydrogen, sodium, bisulphate and zinc sulphate ion-pair are being considered to be present in ionic form while higher order associations are ignored.

The ionic strength, I, of any solution is defined by the following equation:

$$I = \tfrac{1}{2} \sum_{i=1}^{n} C_i Z_i^2 \quad (31)$$

where:

C_i and Z_i are the molar concentration and ionic charge of the species i, respectively.

In the light of equations (29) and (30), equilibria constants and mass balances for various ionic species may be represented by the following relationships:

zinc sulphate ion-pair dissociation constant:

$$K_m = \frac{[Zn^{2+}][SO_4^{2-}]}{[Zn\,SO_4]} \quad (32)$$

bisulphate ion dissociation constant:

$$K_b = \frac{[H^+][SO_4^{2-}]}{[H\,SO_4^-]} \quad (33)$$

hydrogen ion balance:

$$[H^+] = 2M\,H_2SO_4 - [HSO_4^-] \quad (34)$$

sulphate ion balance:

$$[SO_4^{2-}] = MSO_4^{2-} - [HSO_4^-] - [ZnSO_4] \quad (35)$$

zinc ion balance:

$$[Zn^{2+}] = C_{zt} - [ZnSO_4] \quad (36)$$

sodium ion balance:

$$[Na^+] = 2\,(MSO_4^{2-} - MH_2SO_4 - C_{zt}) \quad (37)$$

where:

$M_{H_2SO_4}$, $M_{SO_4^{2-}}$ and C_{zt} are the formal concentration of sulphuric acid, the formal total sulphate concentration and formal total concentration of zinc sulphate, respectively.

Nomenclature used in the paper. a_c = interfacial area per unit volume of continuous phase, m^{-1}; a_d = interfacial area per unit volume of dispersed phase, m^{-1}; a_o = activity of DEHPA dimer, $kmol/m^3$; a_{zo} = activity of organic zinc, $kmol/m^3$; C = molar concentration, $kmol/m^3$; D = diffusion coefficient, m^2/s; d_{32} = Sauter mean drop diameter, m; d_n = inside nozzle diameter, m; Eö = nozzle Eotvos number, $\Delta\rho d_n^2\, g/\sigma$; E_{ax} = axial dispersion coefficient, m^2/s; F = Faraday constant, 9.65×10^7 C/kg equiv; Fr = Froude number, $u_n^2/g\,d_n$; f^{th} = thermodynamic factor; H_c = column height, m; K = mass transfer coefficient, m/s; k = concentration-based extraction rate constant, m/s; k_l = extraction rate constant, m/s; k_l' = stripping rate constant, m/s; L = volumetric flow rate, m^3/s; L' = volumetric flow rate m^3/s; M = molecular weight; n_+, n_- = valences of cation and anion respectively; R = gas constant, 8.314×10^3 J/kmol K; R_z = zinc interfacial flux, $kmol/m^2\,s$; r_z = volumetric extraction rate, $kmol/m^3\,s$; S = cross-sectional area of column, m^2; T = absolute temperature, K; t = time, s; U = superficial velocity through the column, m/s; u_s = slip velocity of drops relative to continuous phase, m/s; V_A = molar volume of extractant at its normal boiling point, $m^3/kmol$; We_n = nozzle Weber number, $\rho_d d_n u_n^2/\sigma$; x = mole fraction.

Greek letters used in the paper. α = degree of dissociation of zinc sulphate; γ = activity coefficient; σ = interfacial tension, N/m; ρ = density, kg/m^3; $\Delta\rho$ = desnity difference, kg/m^3; μ = viscosity, kg/m s; ω = frequency of oscillation, $\sqrt{48\,\sigma/\pi^2\,d_{32}^3\,(3\rho_d + 2\rho_c)}$, s^{-1}; λ_+^0, λ_-^0 = limiting (zero concentration) ionic conductances, S m^2/kg equiv; Φ = an association parameter for solvent; ϕ = dispersed phase holdup for $U_c = 0$; ϕ_D = dispersed phase holdup for countercurrent flow.

Subscripts used in the paper. 1 = outlet; 2 = inlet; A = extractant; B = organic solvent; AB = organic solution; c = continuous phase; d = dispersed phase; H = hydrogen ion; i = interface, interfacial values; o,oD = DEHPA dimer; za = aqueous phase zinc ions; zo = organic phase zinc; zt = total aqueous phase zinc.

Materials and Methods

The continuous aqueous phase contains zinc sulphate, sodium sulphate and sulphuric acid, whereas the dispersed phase consists of di(2-ethylhexyl) phosphoric acid (DEHPA) dissolved in *n*-heptane. The *n*-heptane used was of knock-testing grade without purifying anymore. The DEHPA was of technical grade obtained from BDH and was further purified by Partridge and Jensen (1969) method. The use of DEHPA to extract zinc was considered as a recommended system for liquid-liquid extraction studies (Hancil *et al.,*1990). These authors claimed that the use of glycol alone to remove monoester impurities from DEHPA adversely affected the

subsequent zinc extraction kinetics. For impure DEHPA with monoester content ≤ 3 mol%, the kinetics of extraction were the same as for highly purified DEHPA produced using the copper precipitation method. The zinc sulphate, sodium sulphate and sulphuric acid were Analar grade. All the experiments were carried out at 25 °C and an ionic strength of 1 mol/dm^3.

Experiments were performed in a glass spray column of 0.05 m diameter with provision to adjust height. Effective heights of 1.25-2.4 m were used. The column was operated in a semi-batch mode, that is, the continuous aqueous phase was kept stagnant and the dispersed organic phase was not recirculated. The coalesced dispersed organic phase was drawn off from the top of the reservoir by a glass capillary siphon that resulted in a continuous flow of the coalesced phase. Full detail of the apparatus description, procedure and photographic set-up is given elsewhere (Saeed and Jamil, 1994a). The concentrations and operating conditions in the column are given below:

C_{zt}, initial aqueous phase zinc conc = 1.5x 10^{-3}-0.02 kmol/m^3; initial pH = 2.7-3.07; C_{oD}, DEHPA conc (dimer) = 0.025-0.075 kmol/m^3; diluent = *n*-heptane; L_d, dispersed phase flow rate = 3.67 x 10^{-7}-2 x 10^{-6} m^3/s; d_n, nozzle diameter = 0.8 x 10^{-3}-3.0 x 10^{-3} m; H_c, effective column height = 1.25-2.4 m.

Results and Discussion

Equations (32-36) are solved simultaneously for constant values of K_m and K_b to yield a cubic equation in zinc ion concentration. For the calculation of the values of $M_{H_2SO_4}$ and $M_{SO_4^{2-}}$, which would give the required composition of the aqueous phase, that is, $[Zn^{2+}]$, $[H^+]$ and ionic strength, the iteration method was employed by using guessed values of $M_{H_2SO_4}$ and $M_{SO_4^{2-}}$ for a given value of zinc sulphate. Baes (1957) reported the values of dissociation constant of bisulphate ion, K_b, for the system sodium sulphate-sulphuric acid, as a function of total sulphate concentration. His results showed that at constant total sulphate concentration, K_b is nearly constant as the composition is changed, even though the accompanying change in ionic strength is considerable. Baes values were employed for calculating K_b, while K_m value was taken from elsewhere (Smith and Martell, 1976).

Comparison of actual and predicted column heights. Analysis of photographic films gave drop size distribution, dispersed phase holdup and specific interfacial area. The values of hydrodynamic and mass transfer parameters for the system are given in Table 1. Considering R_z and a_c constant along the height and the column was operated while there was no net flow of continuous phase across the section, equation (1) becomes:

$$\frac{dC_{za}}{dt} = -R_z a_c \qquad (38)$$

The physicochemical data for zinc/DEHPA system understudy is given below:

λ^o_+ (½ Zn^{2+}) = 5.3 S m^2/kg equiv; λ^o_- (½ SO_4^{2-}) = 8.0 S m^2/kg equiv; μ_B = 4 x 10^{-4} kg/m s; Φ = 1.0 (for *n*-heptane solvent); M_B = 100.2; V_A = 0.853 m^3/kmol (for DEHPA dimer); D_{za} = 1.08 x 10^{-9} m^2/s; D_o = 7.34 x 10^{-10} m^2/s; D_{zo} = 5.78 x 10^{-10} m^2/s; ρc = 1040 kg/m^3; ρd = 695 kg/m^3; μ_c = 1.023 x 10^{-3} kg/m s; μ_d = 4.733 x 10^{-4} kg/m s; σ = 20.5 x 10^{-3} N/m; k = 4.25 x 10^{-7} m/s (Ajawan *et al.,* 1983) and $k_I = 2k/\gamma_0$; $k_I^{/}$* = 2.96 x 10^{-5} m/s (*modified value based on DEHPA and zinc-DEHPA complex activities).

Table 1. Measured and calculated hydrodynamic and mass transfer parameters in a spray column for Zn/DEHPA system; C_{oD} = 0.075 mol/dm^3

d_n x 10^3 m	$L_d^{/}$ x 10^8 m^3/s	d_e x 10^3 m	d_{32} x 10^3 m	ϕ x 10^3	a_c m^{-1}	K_{za} x 10^4 m/s	K_o x 10^4 m/s
0.8	9.2	3.99	3.99	1.55	2.32	2.43	1.1
	11.2	3.68	3.68	1.88	3.07	2.23	1.12
1.1	10.8	5.22	5.25	1.77	2.08	2.05	0.91
	12.5	5.0	5.04	2.09	2.58	2.12	0.92
	17.5	4.94	5.0	3.0	3.7	2.22	0.92
	23.3	2.81	2.86	4.0	8.5	2.65	1.24
	23.3	2.99*	3.03	4.12	8.27	2.55	1.39
3.0	24.2	6.28	6.31	4.15	4.1	1.98	0.69
	33.3	6.23	6.25	5.73	5.7	2.11	0.8
	50	6.07	6.09	8.54	8.73	1.95	0.79

*C_{oD}: 0.025 mol/dm^3

The values of various physical properties for the system needed in the calculation of Sauter mean drop diameter, diffusion and mass transfer coefficients were determined experimentally. The solution of DEHPA in *n*-heptane behaves like a non-ideal solution. The thermodynamic factor for the system was obtained from the slope of $\ln a_o$ versus $\ln x_A$ graph. The mass transfer coefficient of organic zinc complex was estimated using the penetration theory model which provides that the mass transfer coefficient is directly proportional to the square root of the molecular diffusivity. Although the intrinsic diffusivities of zinc and hydrogen ions are different, in order to maintain electric neutrality, K_{za} is taken equal to K_H. The calculated values of hydrodynamic and mass transfer parameters using different correlations agree well with the experimental values (Saeed and Jamil, 1998b; Saeed *et al.,* 1994; Saeed and Jamil, 1994a). The experimental interfacial flux data fits well in the design equation based on interfacial chemical kinetics (Table 1) (Saeed and Jamil, 1994b).

The values of chemical reaction rate constants at 25 °C and an ionic strength of 1 mol/dm^3 have been reported by Murthy (1987) and Ajawin *et al.* (1983; 1980). The authors have assumed complete dissociation of zinc sulphate in the aqueous phase for the purpose of calculations of reaction rate and equilibrium constants. The modified values of these constants, taking into account the incomplete dissociation of aqueous phase zinc sulphate, were used in the interfacial flux model. Equations (2) and (38) were solved numerically by the method of finite difference using the equation (28) for the interfacial flux. A numerical calculation routine (CO_2AEF), written by Numerical Algorithm Group (NAG), was used to solve the polynomial equation (28). In all cases, which were considered, there was only one real root for positive interfacial concentration.

Samples of the outlet dispersed organic phase were taken directly from the outflow of the siphon to estimate the zinc concentration. A known volume of the organic phase sample was stripped with 2 mol/dm^3 H_2SO_4 and the aqueous phase was analyzed using the atomic absorption spectrophotometer at λ 213.9 nm. In order to predict column heights for the corresponding outlet organic phase zinc concentrations, the required constants and other parameters such as Sauter mean drop diameter, mass transfer coefficients for continuous and dispersed phases, etc., were calculated using respective relationships as detailed in the preceeding paragraphs.

In the calculations, the following assumptions were made:

- DEHPA diffuses in the diluent as a dimer;
- mass transfer rate during drop formation and coalescence was the same as the one rate of rising drops;
- the rising velocity of the drops is the same throughout the column; and
- analysis of the samples of the continuous aqueous phase taken at different points along the column showed no variation of zinc concentration with height, therefore, the dispersive term in equation (2) was assumed to be negligible.

Comparison of actual and predicted heights using the design equation based on the interfacial chemical kinetics is shown in Fig. 2. The predicted values agree well with the actual ones showing that it is possible to design spray columns from first principles provided that the reliable physicochemical data, hydrodynamic and mass transfer parameters are available.

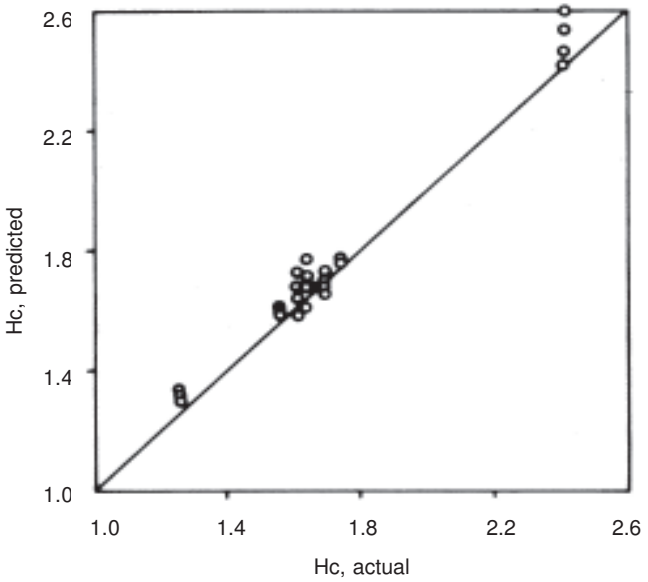

Fig. 2. Comparison of actual and predicted heights using design algorithm based on interfacial chemical kinetics.

Conclusion

The extraction of zinc from an acidic aqueous sulphate solution by di(2- ethylhexyl) phosphoric acid in *n*-heptane diluent has been carried out in a spray column of variable heights operated under semi-batch mode.

A comparison between actual heights and those predicted using the design algorithm based on the interfacial chemical kinetics indicates its applicability, being in good agreement. If all the parameters relating to the system, namely, physicochemical, hydrodynamics, mass transfer and the reaction kinetics are known, it is possible to design extraction column from first principles.

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Humidity Effect on the Disintegrant Property of α-Cellulose and the Implication for Dissolution Rates in Paracetamol Tablets

Michael U. Uhumwangho* and Roland S. Okor
Department of Pharmaceutics and Pharmaceutical Technology, University of Benin, Benin City, Nigeria

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Abstract. A study has been carried out to determine the effect of humidity on the disintegrant property of α-cellulose in tablet formulations. Paracetamol tablets containing α-cellulose (5% w/w) as disintegrant were employed in the study. The tablets were tested for hardness, disintegration time and dissolution rates before and after their exposure to different relative humidities (RH) of 1%, 78% and 100% at 30 °C (room temperature) for various time intervals upto a maximum of 2 weeks. Humidity effect on the particle structure of α-cellulose was determined by photomicroscopy. Tablets exposed to RH of 1% and 78% disintegrated very fast, within a minute, similar to the fresh samples. In contrast, tablets exposed to RH 100% for ≥ 24 h failed to disintegrate within 60 min even though the tablets became softer. Tablet dissolution rate was also markedly impaired in this set of tablets. Exposure of the α-cellulose powder to RH 100% for 24 h caused the particles to gel, which accounted for the impairment of its disintegrant property.

Keywords: α-cellulose, disintegrant property, gel formation, humidity effect

Introduction

The polymer α-cellulose is that part of cellulosic materials which is insoluble in 17.5% w/w solution of sodium hydroxide at 20 °C (Seymour, 1971). This property distinguishes it from β- and γ-celluloses. It is obtained from wood pulp (Nitz, 1994) or more recently from agricultural wastes such as maize cob, rice husk or groundnut shell (Okhamafe *et al.,* 1991). It has potential in tablet formulations as a disintegrant and a direct compression base (Okhamafe *et al.,* 1992). The polymer is readily hydrated being capable of absorbing approximately 4 and a 1/2 times its own weight of water (Okhamafe *et al.,* 1991). This swelling ability is its greatest asset as a disintegrant in tablet formulations. Swelling of the α-cellulose inside the tablet causes localized stress, which leads to tablet rupture. The hydrophilic swelling property of α-cellulose has also been exploited in controlled drug release from matrices which are non-disintegrating tablets (Okor *et al.,* 1992).

The previous studies (Nitz, 1994; Okor *et al.,* 1992; Okhamafe *et al.,* 1992; 1991) on the applicability of α-cellulose in tableting relate to freshly made tablets only, with no consideration for ageing effects. Therefore, information on its long-term performance under different conditions of storage is rare in the literature. In the tropics, high humidities prevail throughout the year. Hence, in the present study, humidity effect on the particle structure and disintegrant property of α-cellulose was investigated.

*Author for correspondence

Materials and Methods

α-Cellulose powder. The polymer α-cellulose was used as the test disintegrant. It was obtained locally as a fine white powder of irregular shaped particles from an agricultural waste, maize cob, by sodium hydroxide and sodium sulphite digestion process already described in detail elsewhere (Okhamafe *et al.,* 1991). It is readily hydrated and swells in water and other aqueous fluids. Maize starch (BP grade) was also used as disintegrant in a comparative study. Magnesium stearate (BDH) was used as lubricant. Paracetamol powder (pharmaceutical grade) was used as the test drug. It was selected for the study because it forms poorly disintegrating tablets on its own (i.e., without a disintegrant).

Granulation and tableting. Paracetamol granules were formed by wet granulation technique using starch mucilage (20% w/w) as the binder fluid and dried on a tray in a hot air oven (Kottermann, Germany) to moisture content, 1.3 ± 0.2% w/w. The lubricant (1% w/w) and the disintegrant (5% w/w) were added to the granules and compressed with a single punch machine (Manesty, Type F_3) to form flat faced tablets of diameter 12.5 mm, thickness 3.38 mm, and weight 550 mg. The compression load was 27.5 (arbitrary unit on the load scale) and held on the tablet for 30 sec for consolidation before releasing the load.

Evaluation of the tablets. ***Storage tests.*** Twenty tablets, freshly made, were stored in each of the three chambers of different relative humidities (RH) of 1%, 78% and 100% for various time

intervals up to a maximum of two weeks to avoid possible microbial degradation. To obtain RH 1%, a desiccator was charged with dried silica gel and to obtain RH 78% or 100% a beaker containing a supersaturated solution of sodium chloride or distilled water was placed, respectively, in a glass chamber. Ambient temperature in the chambers was 30±2 °C.

Moisture uptake experiments. The weight of 10 freshly made tablets was individually determined and the mean weight (M_0) obtained. The tablets (10 each) were stored under the different RH values described above at room temperature for two weeks. At selected time intervals, the samples were removed from the chambers to determine their mean weight, M_t. The percent of moisture uptake (degree of hydration of the tablet) was calculated from the expression as follows:

$$\frac{M_t - M_0}{M_0} \times 100\%$$

The experiment was carried out in triplicate by using different batches of the tablets.

Disintegration test. The method described in the British Pharmacopoeia (BP, 1988) was employed. Six tablets were used in each determination, which was carried out in triplicate.

Dissolution test. The stirred beaker method was employed, details of which have been described previously (Okor *et al.*, 1991). The leaching fluid was 0.1 N hydrochloric acid maintained at 37±2 °C. Samples (5 ml) were withdrawn from the leaching fluid at selected time intervals and analysed for content of paracetamol, spectrophotometrically at λ_{max}, 245 nm. The dissolution rates were obtained by dividing the maximum amount of drug released by the time (M/T). The determination was carried out in triplicate and the mean results reported.

Hardness test. This was carried out using the monsanto hardness tester (Brook and Marshall, 1968). Ten tablets were used in each determination, which was applied to three batches of tablets to obtain mean results.

Test for reversibility of humidity effect on the tablets. Tablets of an initial moisture content 1.3±0.2% (w/w) were placed in a humidity chamber (RH 100%) for 24 h, after which they were dried at 60 °C for 3 h in a hot air oven to a moisture content of about 1.2% (w/w). The dried tablets were re-evaluated for hardness, disintegration times and dissolution rates. The test was carried out in triplicate to obtain mean results. Control tablets were stored in a desiccator (RH 1%) for 24 h and similarly tested.

Test for humidity effect on the particle structure of the disintegrant powders. The disintegrant powder (α-cellulose or maize starch) was dried at 60 °C for 3 h in a hot air oven. A sample of the dried powder was spread thinly on a microscope slide, which was stored in a desiccator (RH 1%) or in a humidity chamber (RH 100 %) for 24 h at 30±2 °C. The slides were examined under a microscope at the magnification of x40. Photomicrographs of representative fields of view were taken.

Results and Discussion

Moisture uptake profiles of the tablets. No measurable moisture uptake was recorded for tablets stored in the desiccator (RH 1%) while those stored under RH (78%) showed no appreciable moisture uptake over the 2-week period. The results for tablets exposed to RH 78% and 100% are given in Table 1 where it can be seen that moisture uptake was about twice greater in tablets containing α-cellulose compared with maize starch. The maximum uptakes were about 4% (tablets with α-cellulose) and 2% (tablets with maize starch).

Humidity effect on tablet disintegration time. The results on the effect of humidity on tablet disintegration time are presented in Table 2. Tablets stored in the desiccator or in the humidity chamber (RH 78%) disintegrated rapidly within

Table 1. Effect of humidity on the moisture uptake (degree of hydration) of tablets containing α-cellulose or maize starch as disintegrant (5% w/w)

Storage time (h)	Moisture uptake (% w/w) in the tablets containing the disintegrant	
	α-Cellulose	Maize starch
RH 78%		
3	0.2	
6	0.2	
9	0.2	0.1
12	0.3	0.1
24	0.7	0.3
48	0.9	0.4
72	1.0	0.4
96	1.2	0.7
RH 100%		
3	0.8	0.3
6	0.9	0.5
9	1.0	0.6
12	1.2	0.8
24	2.1	1.7
48	2.3	1.8
72	3.1	2.0
96	4.3	2.0

Note: there was no measurable moisture uptake at RH 1% (i.e., when tablets were stored in a desiccator)

a minute, similar to the freshly made tablets. Also, tablets with maize starch as the disintegrant, which were exposed to the higher humidity (RH 100%), disintegrated rapidly. This means that humidity had no effect on the disintegration profile of these tablets. However, in the case of tablets with α-cellulose the higher humidity increased the disintegration time remarkably with an increase in the duration of exposure. These tablets failed to disintegrate within 60 min after their exposure to the high humidity for ≥ 24 h. This humidity seriously affected the disintegration time of these tablets.

Humidity effect on tablet hardness. There was no marked change in the hardness of tablets stored at RH 1% and RH 78%, but at the higher relative humidity (100%) tablet hardness decreased appreciably with an increase in the duration of exposure (Table 3). For instance, after 24 h exposure, tablet hardness decreased from an initial value of about 11.5 kg to 5.1 kg (α-cellulose) and from about 10.5 kg to 7.4 kg (maize starch). The decrease was, therefore, more pronounced in tablets containing α-cellulose as the disintegrant.

Humidity effect on tablet dissolution rates. The amounts of drug dissolved were plotted against time (Fig. 1). The disso-

Table 2. Effect of humidity on the disintegration time of tablets containing α-cellulose or maize starch (5% w/w) as disintegrant

Storage time (h)	Disintegration time (min) of tablets with disintegrant	
	α-Cellulose	Maize starch
RH 1%		
0	0.7	0.5
3	0.7	0.5
6	0.7	0.5
9	0.7	0.5
12	0.7	0.5
24	0.7	0.5
48	0.7	0.5
72	0.8	0.5
96	0.7	0.5
RH 78%		
0	0.7	0.5
3	0.7	0.6
6	0.6	0.6
9	0.7	0.7
12	1.2	0.7
24	1.4	0.7
48	2.3	0.7
72	2.3	0.7
96	2.2	0.8
RH 100%		
0	0.70	0.51
3	0.68	0.50
6	0.65	0.88
9	2.05	0.85
12	21.00	1.01
24	> 60.00	1.62
48	> 60.00	2.10
72	> 60.00	2.82
96	> 60.00	3.02

Table 3. Hardness profile of tablets stored under different relative humidities for various time intervals containing α-cellulose or maize starch as the disintegrants

Storage time (h)	Hardness (kg) of tablets with disintegrant	
	α-Cellulose	Maize starch
RH 1%		
0	11.5	10.5
3	11.2	10.5
6	11.0	10.2
9	11.1	10.1
12	11.4	10.1
24	11.5	10.5
48	11.2	10.8
72	11.5	10.2
96	11.1	10.4
RH 78%		
0	11.5	10.5
3	11.0	10.3
6	10.8	10.0
9	10.1	9.3
12	10.0	9.0
24	9.0	8.6
48	8.2	8.0
72	7.6	7.9
96	7.0	7.2
RH 100%		
0	11.5	10.5
3	10.0	10.0
6	9.0	9.6
9	8.8	9.1
12	8.2	8.8
24	5.1	7.4
48	4.0	6.1
72	2.0	4.2
96	2.0	3.5

lution rates were obtained as described above and are presented in Table 4. The results showed that storage of the tablets at RH 78% had no appreciable effect on their dissolution rates. At the higher RH of 100%, tablets with α-cellulose exhibited a retarded dissolution rate. In contrast, the dissolution rates of the tablets with maize starch were not affected by exposure to the higher humidity to any appreciable extent.

Reversibility of humidity effect on the tablets. Three sets of tablets were involved in this study: (i) tablets stored in a desiccator (RH 1%) for 24 h, (ii) tablets exposed to RH 100%, and (iii) dried tablets previously exposed to RH 100%. Results of their hardness, disintegration time and dissolution rate are presented in Table 5. The results showed that exposure of the tablets to the higher humidity decreased tablet hardness, prolonged the disintegration time (> 60 min), and retarded the dissolution rates of tablets with α-cellulose. Of these three parameters, drying reversed only tablet hardness.

Humidity effect on the particle structure of the disintegrant powders. Photomicrographs of the powder samples stored in a desiccator (RH 1%) and under high humidity (RH 100 %) are presented in Fig. 2. In the control samples,

Table 4. Amount dissolved in 45 min from tablets stored for 24 h in a desicator (RH 1%) and in humidity chambers, RH 78% and 100%

	Dissolution rate (mg/min) of the tablets with the disintegrant	
RH %	α-Cellulose	Maize starch
1 (control)	11.33	11.24
78	10.56	10.78
100	3.29	10.00

Table 5. Comparison of hardness (kg), disintegration time (min) and disolution rate (mg/min) of tablets containing α-cellulose (i) stored for 24 h in a dessicator, (ii) exposed to RH 100% for 24 h, and (iii) dried after previous exposure to RH 100% for 24 h

Parameters evaluated	Set of tablets		
	(i)	(ii)	(iii)
Hardness (kg)	11.5	2.0	9.2
Disintegration time (min)	0.7	60.0	60.0
Dissolution rate (mg/min)	10.6	3.3	4.4

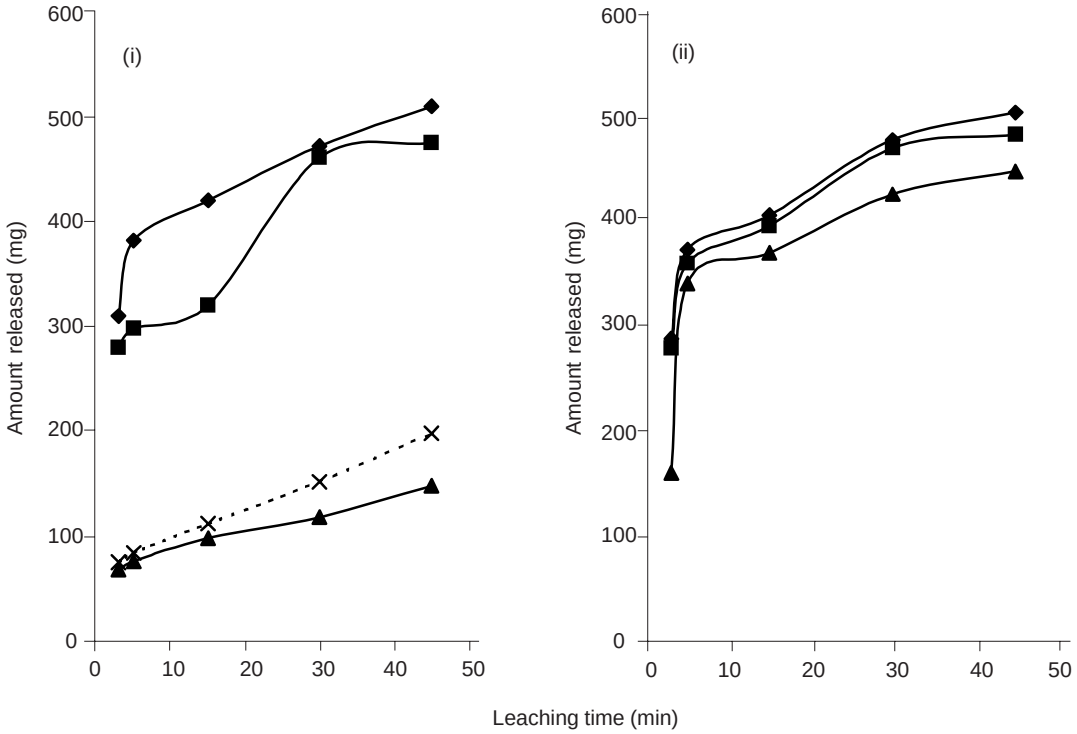

Fig. 1. Dissolution profile of tablets stored under different relative humidities RH 1% (◆), 78% (■) and 100% (▲) for 24 h: disintegrant (i) α-cellulose or (ii) maize starch, (5% w/w); (×) α-cellulose released from redried tablets after previous exposure to RH 100% for 24 h.

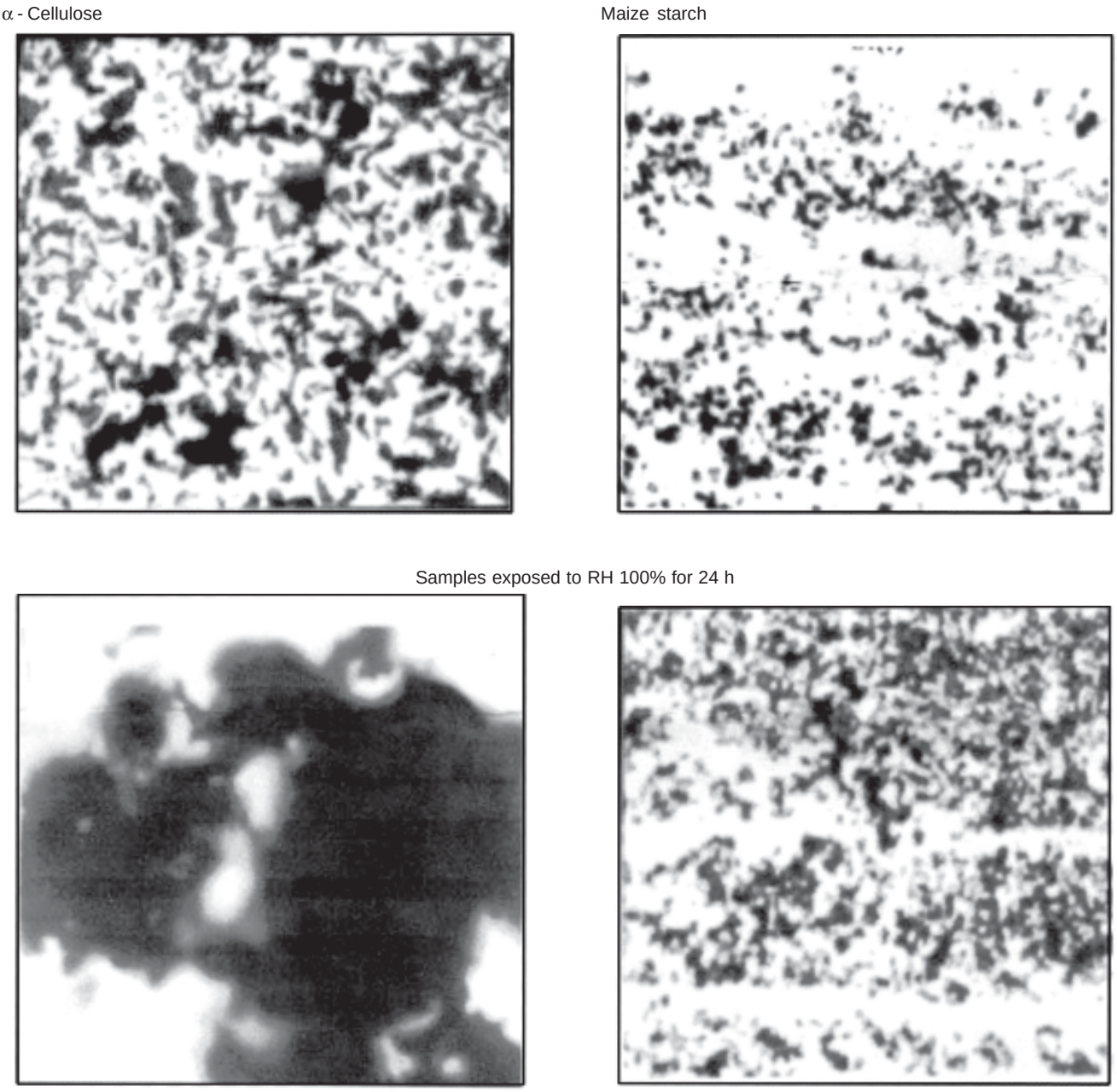

Fig. 2. Photomicrographs showing the gelling effect of humidity on the particle structure of α-cellulose and maize starch powder.

the particles were discrete and appeared in the micrographs as elongated fibres. Exposure of the maize starch powder to high humidity appeared not to have any effect on the structure of the particles, as they remained discrete. In the case of α-cellulose, however, high humidity caused the particles to swell and fuse to a coherent mass, indicating that the particles had gelled.

Exposure of the tablets to the higher RH of 100% impaired the disintegrant property of α-cellulose, whereas maize starch was not susceptible to this humidity effect. This finding relates to the observation that α-cellulose powder gelled at room temperature upon moisture sorption, while maize starch did not display a similar gelling. The results of the moisture uptake experiments under these conditions showed that the tablets containing α-cellulose as the disintegrant were more easily hydrated than similar tablets containing maize starch as the disintegrant. Besides, maize starch would only gel at high temperatures (> 60 °C), which explains why the disintegration time of tablets containing this disintegrant was not suscep-

tible to the humidity effect at room temperature. The disintegrant property of α-cellulose depends on its ability to swell in the tablet to cause its rupture whenever the tablet is placed in an aqueous fluid (Okhamafe *et al.*, 1992, 1991). Having swelled and gelled due to moisture sorption, the capacity of α-cellulose to further swell when the tablet was placed in the disintegration fluid will be compromised. The lower RH (1% and 78%) did not impair its disintegrant property because of the negligible moisture uptake under these conditions.

Drying of the tablets after their initial exposure to higher humidity did not reverse the observed humidity effect on the tablets with α-cellulose. Instead, the tablets became harder. This finding suggests that the α-cellulose gel in the tablets may have formed a xerogel (dried gel) during drying of the tablets. Xerogels are known to function as binders rather than as disintegrants because of their tensile strength and rigidity (Richards, 1972).

The tablets became softer due to hydration when stored under higher humidity. The decrease in hardness was more pronounced in the tablets with α-cellulose compared with maize starch because of the higher potential of the former for moisture uptake (Table 1). This decrease in hardness was expected to lead to a faster disintegration rate since it reflects a weaker interparticulate bonding within the tablets. On the contrary, the disintegration time (in case of the tablets with α-cellulose) actually became prolonged. This apparent abnormality is attributable to the impairment of the disintegrant property of α-cellulose, as already discussed above . Although the tablets became softer, some degree of internal swelling was required before the tablets can disintegrate.

The dissolution rates of the tablets with α-cellulose, that were exposed to higher humidity, were markedly retarded (Table 4) as the tablets failed to disintegrate throughout the time course of the leaching experiment. Tablets with maize starch, which were similarly exposed to the higher humidity, gave fast dissolution rates because they disintegrated readily. The explanation is that disintegration increases the particle surface area for dissolution.

Conclusion

The study has shown that humid conditions can cause gelling of α-cellulose powder at room temperature and by this mechanism impairs its disintegrant property in tablet formulations, with serious implications for dissolution rates. This finding underlines the need to protect such tablets from moisture.

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Proximate, Mineral and Phytate Profiles of Some Selected Spices Found in Nigeria

E. I. Adeyeye*[a] and E. D. Fagbohun[b]

[a]Chemistry Department, University of Ado-Ekiti, PMB 5363, Ado-Ekiti, Nigeria

[b]Microbiology Department, University of Ado-Ekiti, PMB 5363, Ado-Ekiti, Nigeria

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Abstract. The proximate, mineral and phytate (phy) compositions, as well as the calculations for fatty acid, metabolisable energy, phy:Zn, Ca:phy and [Ca] [phy]/[Zn] were determined in 13 spices (S_{11} - S_{23}) used as seasoning agents in Nigeria. The mean values of various parameters for proximate composition (g/100 g) were: moisture (3.61±3.56), dry matter (96.39±3.56), crude fat (5.46±10.02), crude fibre (27.0±17.34), crude protein (13.78±9.84), ash (4.57±2.22) and carbohydrates (45.58±22.25). Fatty acids were noted to be 4.37±8.02 (g/100 g) and energy was 1211.23±317.64 (kJ/100 g). Significant differences ($P < 0.05$) existed in moisture, dry matter, fat, fibre, crude protein and fatty acid levels. Minerals (mg/100 g) included: Na (183.08±144.19), K (1621.54±1703.99), Ca (505.38±463.24), Mg (243.08±235.74), Zn (434.92±945.86), Fe (72.54±92.38) and P (740±624.64), while Pb, Cu and Co, were not detected. The relationships between Na and K as well as between Ca and P were mostly within the desirable range with the respective ratios of Na/K (0.59±0.87) and Ca/P (2.20±3.32). Significant differences existed among the levels of Na, K, Ca, Mg, Zn, Fe, Na/K and Ca/P. The [Ca] [phy]/[Zn] had an overall mean value of 1.45±1.74 showing that the bioavailability of zinc in the spices may be low (except in S_{21}, S_{22} and S_{23}) due to the high phytate content of the spices.

Keywords: spices, chemical composition, metabolisable energy, phytate levels

Introduction

Broadly speaking, spices are aromatic vegetable products of tropical origin that are used, in a pulverised state, primarily for seasoning or garnishing foods and beverages. They are characterised by pungency, strong odour, and sweet or bitter taste. Included in this category are hard or hardened parts of plants such as pepper, cinnamon, cloves, ginger, cardamom, turmeric, nutmeg and mace, all spices, and vanilla. In ancient times, they were valued as basic components of incense, embalming preservatives, ointments, perfumes, antidotes against poison, cosmetics and medicines, and were little used in food. It was only in the first century AD that spices found their way into the kitchen (Kochhar, 1986). Spices cannot be classed as foods since they are used in foods at levels that yield no significant nutritive value, but impart certain aroma and flavour to the food. The importance of spices in our daily diet is as follows (Kochhar, 1986): (1) to give an agreeable flavour and aroma (piquancy or tang) to otherwise monotonous or insipid food, particularly in the tropics where it consists mainly of starchy grains or roots, thereby adding greatly to the pleasure of eating; (2) to stimulate appetite and increase the flow of gastric juices, for which reason they are often termed as food 'accessories' or 'adjuncts'; (3) to camouflage or disguise the slightly unpleasant taste of many dried meats; and (4) to increase the rate of perspiration, thus having a cooling effect on the body.

The spices analysed in this work have been variously described (Akinadewo, 2001; Gill, 1992; McGraw-Hill Encyclopedia of Science and Technology, 1987; Kochhar, 1986; Shaw, 1973). Despite the wide utilization of spices, little work has been reported on their nutritional composition. Most works have been concentrated on tropical chillies (Adeyeye and Otokiti, 1999; Fagbemi and Oshodi, 1993; Bamgbose *et al.*, 1991; Keshinro and Ketiku, 1981). Other works on spices include: isolation of vitamin C in paprika in 1937 (Kochhar, 1986), proximate and mineral composition of black pepper (*Piper guineense*) (Udosen, 1995) and the determination of calcium, zinc, phytate, phy/Zn, Ca/phy and [Ca] [phy]/[Zn] molar ratios in bell and cherry peppers, okro, tomato, onion and sugarnut (Adeyeye *et al.*, 2000).

The importance of a foodstuff as a source of dietary zinc depends upon both the total zinc content and the level of other constituents in the diet that affect zinc bioavailability. Phytic acid (myoinositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate), a compound found only in plant foods, may reduce the bioavailability of dietary zinc by forming insoluble mineral chelates at the physiological pH (Oberleas, 1983). The formation of the chelates depends on relative levels of both zinc and phytic acid (Davies and Olpin, 1979). Conse-

*Author for correspondence

quently, the phytate:Zn molar ratio is considered a better predictor of zinc bioavailability than total phytate level alone. The critical phytate:Zn molar ratio may also depend on dietary calcium level. A kinetic synergism exists between the calcium and zinc ions resulting in a Ca:Zn:phytate complex which is less soluble than phytate complexes formed by either ion alone (Oberleas, 1973). Unfortunately, only limited data are available on the critical phytate:Zn and [Ca] [phy]/[Zn] ratios associated with decreased zinc bioavailability in human diets. Consequently, we have determined proximate and mineral composition, metabolisable energy, fatty acids, phytate, phy:Zn, Ca:phy and [Ca] [phy]/[Zn] in 13 spices available for study.

Materials and Methods

Samples of spices. Samples of the spices were obtained from the Oba Market, Ado-Ekiti, Nigeria. All the samples were obtained in dry form. The names of the samples (in English language, botanical nomenclature and vernacular) are given in Table 1. The identification numbers ranged from S_{11} to S_{23} corresponding to 13 samples. Various parts of the vegetables used as spices are also indicated under the column, 'part used'. Table 2 shows the group arrangement of the samples according to the phylogenetic sequence of orders and families (Hutchinson and Dalziel, 1968; 1963; 1958; 1954). The samples were screened by removing stones and other foreign bodies. Each sample was separately ground in an all glass mortar into fine powder and packed in plastic bottles and kept in the laboratory freezer until used for analysis.

Analysis of the samples. The proximate analyses of the samples for moisture, ash, fibre and ether extract were done by the method of AOAC (1990). Nitrogen was determined by the micro-Kjeldahl method as described by Pearson (1976) and the percentage nitrogen was converted to crude protein by multiplying with 6.25. Carbohydrates were determined by difference. All determinations were performed in duplicate.

The minerals were analysed by dry-ashing the samples at 550 °C to constant weight and dissolving the ash in volumetric flasks using distilled, deionised water with a few drops of concentrated hydrochloric acid. Sodium and potassium were determined by using a flame photometer (Model 405, Corning, UK), using NaCl and KCl to prepare the standards. Phosphorus was determined colourimetrically using Spectronic 20 (Gallenkamp, UK) as described by Pearson (1976) with KH_2PO_4 as the standard. All other metals were determined by atomic absorption spectrophotometer (Perkin-Elmer Model 403, Norwalk CT, USA). All determinations were done in duplicate. All chemicals used were of analytical grade (BDH, London). Earlier, the detection limits of the metals had been determined according to Techtron (1975). The optimum analytical range was 0.1 to 0.5 absorbance units with a coefficient of variation of 0.87-2.20%. All the proximate values were reported as g/100 g, while the minerals were reported as mg/100 g.

Phytate was quantified using the method described by Harland and Oberleas (1986). The blank was also prepared as described by Harland and Oberleas. The colourimeter used was a Spectronic 20 (Gallenkamp, UK). The amount of phytate

Table 1. The part used, scientific and vernacular names of the Nigerian spices analysed

Identification number	Common English name	Vernacular name (Y)[a]	Botanical name	Part[b] used
S_{11}	Ethiopian pepper	eeru	*Xylopia aethiopica*	fruit
S_{12}	black pepper	iyere	*Piper guineense*	fruit
S_{13}	African nutmeg	ariwo	*Monodora myristica*	seed
S_{14}	ginger	aje	*Zingiber officinale*	rhizome
S_{15}	alligator pepper	atare	*Aframomum melegueta*[c]	seed
S_{16}	alligator pepper	atare	*Aframomum melegueta*[d]	seed
S_{17}	garlic	ayuu	*Allium sativum*	bulb
S_{18}	clove	konofuru	*Eugenia caryophyllus*	fruit
S_{19}		aridan	*Tetrapleura tetraptera*	seed
S_{20}		aridan	*Tetrapleura tetraptera*	seedcoat
S_{21}	cinnamon		*Cinnamomum tamala*	leaf
S_{22}	nutmeg		*Monodora fragrans*	seed
S_{23}	rose seed		*Rosa* sp	seed

[a]yoruba; [b]all parts used were dry; [c]bigger variety; [d]smaller variety

in the sample was calculated as hexaphosphate equivalent by using the formula:

phytate, mg/g sample = "mean K" x A x 20/(0.282 x 1000)

where:

A: absorbance
"mean K": std P(μg)A/n (std)
phytate: 28.2% P; the phytate values were reported in mg/100 g

Statistical analysis of the samples. Calcium/phosphorus (Ca/P) and sodium/potassium (Na/K) ratios were calculated for all the samples (Nieman *et al.,* 1992). The fatty acid values were obtained by multiplying crude fat value of each sample with a factor of 0.8 (i.e., crude fat x 0.8 = corresponding fatty acid value) (Paul and Southgate, 1978). The energy values were calculated by adding up the carbohydrates (x17 kJ), crude protein (x17 kJ) and crude fat (x37 kJ) for each of the samples (Kilgour, 1987). The phy:Zn, Ca:phy and Ca x phy:Zn values were calculated according to the method of Wyatt and Triana-Tejas (1994). Mean, standard deviations and coefficients of variation were also calculated. Also, F test calculations were done to find out if significant differences occurred in the various parameters determined among themselves, setting the level of significance at $P < 0.05$ (Christian, 1980).

Results and Discussion

The data on the proximate composition, energy and fatty acid values of the spices are shown in Table 3. The moisture content ranged between 1.10-12.23 g/100 g with a grand mean value of 3.61±3.56 g/100 g. The low values of moisture in most of the samples ensured a long shelf life of the samples without microbial spoilage but the large variation resulted in high value of coefficient of variation (CV) among them, which was 98.61. The dry matter values were generally close with

Table 2. Arrangement of samples in phylogenetic sequence of Orders and Families

Botanical grouping	Identification	Species
A. Angiospermae, Dicotyledons		
Division Archichlamydeae		
Order Annonales		
Family Annonaceae	S_{22}	*Monodora fragrans*
	S_{13}	*M. myristica*
	S_{11}	*Xylopia aethiopica*
Order Laurales		
Family Lauraceae	S_{21}	*Cinnamomum tamala*
Order Piperales		
Family Piperaceae	S_{12}	*Piper guineense*
Order Myrtales		
Family Myrtaceae	S_{18}	*Eugenia caryophyllus*
Order Rosales		
Family Rosaceae	S_{23}	*Rosa* sp
Order Fabales		
Family Fabaceae	S_{19}	*Tetrapleura tetraptera* (seed)
	S_{20}	*Tetrapleura tetraptera* (seedcoat)
B. Angiospermae, Monocotyledons		
Division Calyciferae		
Order Zingiberales		
Family Zingiberaceae	S_{15}	*Aframomum melegueta*[c]
	S_{16}	*Aframomum melegueta*[d]
	S_{14}	*Zingiber officinale*
Division Corolliferae		
Order Liliales		
Family Allaceae	S_{17}	*Allium sativum*

a mean value of 96.39±3.56 g/100 g and low value of CV (3.69). The crude fat values varied highly with values ranging between 1.03-38.46 g/100 g with a high CV of 183.50, hence this sample fits into the group of oil seeds (Adeyeye *et al.,* 2000) as reported for sugarnut (*Irvingia gabonensis*) (Oshodi and Ipinmoroti, 1990). Fat is important in diets because it promotes fat soluble vitamin absorption (Bogert *et al.,* 1994). It is a high energy nutrient and does not add to the bulk of the diet. The crude fibre values were high (except in sample S_{20}, *Tetrapleura tetraptera*) having a mean of 27.0±17.34 g/100 g. Dietary fibre has beneficial effects on the muscles of the large and small intestines (Fisher and Bender, 1995) and prevents diseases such as colon diverticula (Eastwood, 1974). The crude protein was low to high in value (5.73-38.92 g/100 g). Hot spots for the protein values were observed in S_{22} (*Monodora fragrans*, 38.92 g/100 g) and S_{23} (*Rosa* sp., 30.12 g/100 g). These values were better than the results in dry bell pepper (18.28 g/100 g) and cherry pepper (18.67 g/100 g) (Adeyeye and Otokiti, 1999). An adult man of 70 kg body weight requires 0.57 g/kg of protein (FAO/WHO, 1973), i.e., 39.9 g of protein daily. This meant that samples S_{22} and S_{23} would almost supply the required protein, assuming complete protein absorption. The available carbohydrates were high for most of the samples with the exception of S_{13} (*Monodora myristica*, 5.49 g/100 g) and S_{22} (*Monodora fragrans*, 17.94 g/100 g). The ash levels ranged between 1.72-8.48 g/100 g. The ash content is a reflection of the mineral content obtained in this study.

The calculated fatty acid values showed that many of the samples have very low values, < 0.1 g/100 g. However, S_{13} (*Monodora myristica*) had a value of 30.77 g/100 g fatty acids. This sample needs a further study to evaluate the nutritional quality of the fatty acid composition. The calculated metabolisable energy values showed that most of the samples were concentrated sources of energy. The energy from cereals ranged from 1.3-1.6 MJ/100 g (Paul and Southgate, 1978) indicating that most of the samples have energy concentrations favourably comparable to cereals.

All the parameters were subjected to F test analysis and the following parameters were significantly different ($P < 0.05$) among themselves, moisture, dry matter, crude fat, crude fibre, crude protein and fatty acids. The statistical comparison was based on between the groups' variations. This is so, as comparison within the group variation would not make any sense because of the small number of samples within the groups (Table 3).

Table 3. Fatty acid, energy and proximate composition (g/100 g) of spices analysed (dry weight basis) with respect to the groups

Sample identification/ statistical test	Moisture	Dry matter	Crude fat	Crude fibre	Crude protein	Fatty acids[a]	Carbo-hydrates	Total ash	Energy[b]
S_{11}	7.36	92.64	5.21	36.65	10.85	4.17	34.63	5.30	965.93
S_{12}	1.34	98.66	2.54	18.45	12.33	2.03	57.98	7.36	1289.25
S_{13}	8.69	91.31	38.46	32.25	13.20	30.77	5.49	1.91	1740.75
S_{14}	1.62	98.38	2.60	19.73	5.53	2.08	62.88	7.64	1259.17
S_{15}	1.32	98.68	1.20	62.02	8.45	0.96	23.94	3.07	595.03
S_{16}	1.21	98.79	1.34	57.71	7.00	1.07	29.29	3.45	666.51
S_{17}	2.32	97.68	2.10	18.50	14.66	1.68	57.75	4.67	1308.67
S_{18}	1.12	98.88	3.45	19.53	5.73	2.76	65.12	5.05	1332.10
S_{19}	1.10	98.90	1.03	13.23	9.67	0.82	70.09	4.88	1394.03
S_{20}	12.23	87.70	1.34	1.34	8.56	1.07	74.45	2.01	1460.75
S_{21}	2.14	97.86	5.53	15.68	14.08	4.42	58.72	3.85	1442.21
S_{22}	3.12	96.88	3.68	34.62	38.92	2.94	17.94	1.72	1102.78
S_{23}	3.25	96.75	2.55	21.34	30.12	2.04	34.26	8.48	1188.81
$\bar{X}$[c]	3.61	96.39	5.46	27.00	13.78	4.37	45.58	4.57	1211.23
SD[d]	3.56	3.56	10.02	17.34	9.84	8.02	22.25	2.22	317.64
CV[e]	98.61	3.69	183.50	64.22	71.41	183.52	48.82	48.58	26.22
F test	14.1	352.9	1073.2	7.6	36.2	742.5	3.5	2.4	3.4
Difference	*	*	*	*	*	*	ns	ns	ns

[a]calculated fatty acids (0.8 x crude fat); [b]energy, calculated metabolisable energy (kJ/100g) (protein x17 + fat x37 + carbohydrates x17); [c]$\bar{X}$, mean; [d]SD, standard deviation; [e]CV, coefficient of variation; [*]significant value; ns, non-significant value

The results of the mineral analysis are shown in Table 4. Lead, copper and cobalt were not detected in any of the samples. The samples may generally be regarded as good sources of sodium, potassium, calcium, magnesium, zinc, iron and phosphorus. Calcium in conjunction with phosphorus, magnesium, manganese, vitamins A, C and D, chlorine and protein, are all involved in bone formation (Fleck, 1976). Calcium is also important in blood clotting, muscle contraction and in certain enzymes in metabolic processes. Magnesium is an activator of many enzyme systems and maintains the electrical potential in nerves (Shils, 1973). Phosphorus assists calcium in many body reactions although it also has independent functions (Fleck, 1976). Sodium and potassium are required to maintain osmotic balance of the body fluids, pH of the body, regulate muscle and nerve irritability and control of glucose absorption (Fleck, 1976; Pike and Brown, 1967). Iron is reported to be very important for normal functioning of the central nervous system (Vyas and Chandra, 1984). Iron also facilitates the oxidation of carbohydrates, proteins and fats. Zinc is present in all tissues of the body and it is a component of more than fifty enzymes (Bender, 1992). Consumption of meat (or other animal products) with vegetables enhances the absorption of both iron and zinc (Bender, 1992; National Academy of Sciences, 1971). The values for most of the minerals have positive correlation with the corresponding mineral values in bell and cherry peppers (Adeyeye and Otokiti, 1999). Table 4 also depicts the Na/K and Ca/P ratios. Modern diets, which are rich in animal proteins and phosphorus may promote the loss of calcium in the urine (Shils and Young, 1988). This has led to the concept of the Ca/P ratio. If the Ca/P ratio is low (low calcium, high phosphorus intake), more than the normal amount of calcium may be lost in the urine, decreasing the calcium level in bones. In animals, a Ca/P ratio above two (twice as much calcium as phosphorus) helps to increase the absorption of calcium in the small intestine. Such samples in the present results included S_{11}, S_{14}, S_{21}, S_{22} and S_{23}, which may help in increasing the calcium content of bones. Food is considered "good" if the ratio is above one and "poor" if the ratio is less than 0.5 (Nieman *et al.,* 1992). This means that 38.46% of the studied samples were poor in Ca/P ratio. Sodium to potassium ratio (Na/K) is also of significance, but the Na/K ratio of 0.6 is recommended (Nieman *et al.,* 1992). About 23.08% of the samples had Na/K values greater than 0.6, while about 76.92% had lower than 0.6. This result showed that most of the spices would not promote high blood pressure. The F test values at $P < 0.05$ showed that Na, K, Ca, Mg, Zn, Fe, Na/K and Ca/P were significantly

Table 4. Mineral composition (mg/100 g) of spices analysed (dry weight basis) with Na/K and Ca/P ratios with respect to between the groups' variations

Sample identification/ statistical test	Na	K	Pb	Ca	Mg	Cu	Zn	Fe	Co	P	Na/K ratio	Ca/P ratio
S_{11}	180	90	nd[a]	270	130	nd[a]	10	360	nd[a]	70	2.0	3.86
S_{12}	120	50	nd[a]	170	270	nd[a]	10	100	nd[a]	1550	2.4	0.11
S_{13}	180	1090	nd[a]	200	110	nd[a]	10	50	nd[a]	2040	0.17	0.10
S_{14}	130	980	nd[a]	340	150	nd[a]	10	2	nd[a]	80	0.13	4.25
S_{15}	110	4120	nd[a]	170	90	nd[a]	10	60	nd[a]	1040	0.03	0.16
S_{16}	240	5540	nd[a]	290	20	nd[a]	10	30	nd[a]	980	0.04	0.30
S_{17}	190	2750	nd[a]	1330	240	nd[a]	40	80	nd[a]	1120	0.07	1.19
S_{18}	60	150	nd[a]	400	100	nd[a]	10	100	nd[a]	540	0.40	0.74
S_{19}	630	340	nd[a]	680	240	nd[a]	20	70	nd[a]	1090	1.85	0.62
S_{20}	60	2440	nd[a]	130	100	nd[a]	4.0	1.0	nd[a]	590	0.02	0.22
S_{21}	180	700	nd[a]	1580	330	nd[a]	3110	40.00	nd[a]	130	0.26	12.15
S_{22}	180	690	nd[a]	270	460	nd[a]	650	20.00	nd[a]	130	0.26	2.08
S_{23}	120	2140	nd[a]	740	920	nd[a]	1760	30.00	nd[a]	260	0.06	2.85
$\bar{X}$	183.08	1621.54		505.38	243.08		434.92	72.54		740	0.59	2.20
SD	144.19	1703.99		463.24	235.74		945.86	92.38		624.64	0.87	3.32
CV	78.76	105.08		91.66	96.98		217.48	127.35		84.41	147.46	150.91
F test value	23.90	25.80		76.6	30.6		61873.18	69.9		2.7	13.5	262.0
Difference	*	*		*	*		*	*		ns	*	*

nd[a], not detected; [*]significant value; ns, non-significant value

different among themselves. The statistical comparison was done between the variation in groups.

The phytate, phy/Zn, Ca/phy and [Ca] [phy]/[Zn] levels of the spices are shown in Table 5. All the phytate values in this report were higher than those reported for *Capsicum annuum*, *Piper nigrum*, *Hibiscus esculentus*, *Lycopersicon lycopersicum*, *Allium cepa* and *Irvingia gabonensis* (Adeyeye *et al.,* 2000). The above trend was not consistent for phy/Zn and Ca/phy values in the current report and literature values enumerated above. A high incidence of suboptimal zinc status may exist among rural populations of low income countries consuming cereal-based diets, low in animal products (Prasad, 1983). Indeed, the first case of severe zinc deficiency in humans was reported among rural populations in Egypt and Iran (Halsted *et al.,* 1972; Sandstead *et al.,* 1967; Prasad *et al.,* 1963), where 50-75% of the dietary energy was provided by cereals (Reinhold *et al.,* 1973). The high phytic acid level of cereals in these diets was probably a significant etiological factor in the development of zinc deficiency (Davies, 1982).

Oberleas and Harland (1981) reported that foods with a molar ratio of phy:Zn less than 10 showed adequate availability of Zn, while problems were encountered when the value was greater than 15. In Table 5, samples S_{11}, S_{12}, S_{17}, S_{18}, S_{19}, S_{21}, S_{22} and S_{23}, i.e., in 61.54% of the samples, had phy:Zn ratio less than 15. This means Zn in 61.54% of the samples would be bioavailable. Franz *et al.* (1980) demonstrated a lower availability of Zn in rats when fed with foods of high molar ratios of phy:Zn. In human studies, phy:Zn molar ratios of 15:1 have also been associated with reduced zinc bioavailability (Turnlund *et al.,* 1984). The high phy:Zn molar ratio in most of the Nigerian diets may have serious implications, furthermore, because animal products, which are the alternative sources of zinc, are sold at unaffordable prices, particularly to the rural Nigerians (Adeyeye, 1996).

The solubility of the phytates and the proportion of zinc bound in a mineral complex in the intestines depends on the levels of calcium (Wise, 1983). In this model, phytate precipitation is not complete until dietary Ca:phy molar ratios attain a value of approximately 6:1. At Ca:phy molar ratios lower than 6:1, phytate precipitation is incomplete, so that some of the dietary zinc remains in solution. The proportion remaining in solution increases with decreasing Ca:phy molar ratios (Wise, 1983). In the present studies, only samples S_{17}, S_{18}, S_{19}, S_{21}, S_{22} and S_{23} were above the critical molar ratio of 6:1. These accounted for 46.15% of the samples studied. In the typical rural Nigerian diet, however, a leaf, leg-

Table 5. Phytate and calculated phy:Zn, Ca:phy and [Ca] [phy]/[Zn] molar ratios of the spices analysed (dry weight basis)[a] with respect to between the groups' variations

Sample identification/ statistical test	Phytate[b] (phy) (mg/100g)	phy/Zn[c]	Ca/phy[d]	[Ca] [phy][e] / [Zn]
S_{11}	845	8.42	5.26	0.57
S_{12}	1141	11.37	2.45	4.88
S_{13}	1648	16.42	0.20	0.83
S_{14}	6210	61.90	0.90	5.31
S_{15}	2154	21.47	1.30	0.92
S_{16}	2070	20.63	2.31	1.52
S_{17}	972	2.41	22.53	0.80
S_{18}	929	9.26	7.09	0.94
S_{19}	887	4.39	12.62	0.73
S_{20}	2873	71.36	0.74	2.34
S_{21}	820	0.03	31.79	0.01
S_{22}	390	0.06	11.42	0.004
S_{23}	540	0.03	22.51	0.01
$\bar{X}$	1652.08	17.52	9.32	1.45
SD	1546.42	13.10	10.32	1.74
CV	93.60	131.84	110.73	120.00
F test value	70.9	48.9	19.15	45.79
Difference	*	*	*	*

[a]mean of duplicate determinations; [b]phytate content calculated by assuming that it contains 28.2% phosphorus; [c]mg of phy/MW (molecular weight) of phy: mg of Zn/MW of Zn; [d]mg of Ca/MW of Ca: mg of phy/MW of phy; [e][mol/kg Ca] x [mol/kg phy] / [mol/kg Zn]; [*]significant value

ume, or fish relish is always consumed with spices as seasoning agents. Such relishes, with the exception of legumes, are high in calcium (Adeyeye *et al.,* 2000). Hence, the calcium content of the relishes in these diets may be sufficient to promote phytate-induced decrease in zinc bioavailability (Ferguson *et al.,* 1988). Ferguson *et al.* (1989) showed that the molar ratio varies with different foods and recommended that this value be used in conjunction with other data to explain the availability of Zn using the Ca:phy ratio.

The results for [Ca] [phy]/[Zn] are shown in Table 5. Ellis *et al.* (1987), and Davies and Warrington (1986) indicated that the ratio of Ca x phy:Zn is a better predictor of Zn availability and noted that if the value was greater than 0.5 mol/kg, then there would be interference with the availability of Zn. In the present results, Ca x phy:Zn values were greater than 0.5 mol/kg in S_{11}, S_{12}, S_{13}, S_{14}, S_{15}, S_{16}, S_{17}, S_{18}, S_{19} and S_{20} samples, in other words 76.92% of the samples would interfere with the Zn bioavailability. However, 23.08% of the samples would promote Zn bioavailability among the spices, which had the following corresponding mol/kg molar ratios: S_{21} (0.01), S_{22} (0.004) and S_{23} (0.01). This means only samples S_{21}, S_{22} and S_{23} could satisfy the critical values of phy:Zn (< 10-15), Ca:phy ($\leq$ 6.0) and Ca x phy:Zn ($\leq$ 0.50 mol/kg). Statistical values of F test ($P < 0.05$) showed that phytate, phy:Zn, Ca:phy and Ca x phy:Zn were all significantly different among themselves based on between the groups' variations.

There is a special delicacy in Nigeria called "pepper soup" prepared mainly from fish or meat, water, salt and pepper. The pungent taste of red pepper is due to capsaicin ($C_{18}H_{27}NO_3$) while the pungent taste of black and white peppers is due to the alkaloid piperine ($C_{17}H_{19}NO_3$). The piperine content of pepper is as high as 5%. Formation of N-nitrosopiperidine, a mutagen, by the reaction of nitrite with piperine in an acid solution (human stomach is acidic) has already been reported (Rao *et al.,* 1981). Also, cooked meat is often laced with spices around its whole body surface (suya) before being consumed. Nigerian peasants normally consume large quantities of fruits and vegetables in their diet and these food materials usually contain ascorbic acid in appreciable amounts. This habitual ingestion of vitamin C in the diet is bound to ameliorate the toxic effect of N-nitrosopiperidine as reported by Greenblatt (1973).

Conclusion

Looking at the spice samples across board (Table 3-5) it is observed that samples S_{21} (*C. tamala*), S_{22} (*M. fragrans*) and S_{23} (*Rosa* sp.) have low moisture content, average crude fat, high crude fibre, high crude protein, moderate fatty acids and high metabolisable energy. These three are very good sources of Na, K, Ca, Mg, Zn and non-hazardous Na/K and Ca/P ratios. They have the lowest values for phytate and phy:Zn, good values for Ca:phy and lowest values for Ca x phy:Zn, thereby making them the best among the samples. It is, however, advocated that the level of research devoted to the investigation of the nutritional qualities of spices be increased to cover all the spices available in Nigeria. This is because they are less expensive and replacing synthetic seasoning agents which have been blamed for promoting high blood pressure. Where their consumption may interfere with zinc bioavailability, meat or fish or leaves could be eaten with them to get enough supply of calcium in the diet.

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Effects of Exposures to Cement Dust and Powder on Workers in Cement Distribution/Retail Outlets in Benin City, Nigeria

Ifeyinwa Flossy Obuekwe [ab]* and Lovette Isioma Okoh [a]

[a]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

[b]207-10807-47 Avenue, Edmonton, Alberta T6H 5J1, Canada

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Abstract. This study investigated the effects of exposures to cement dust and powder on workers in fifteen cement distribution/retail outlets in Benin City, Edo State, South-West Nigeria. Forty workers from these retail outlets were initially surveyed by using detailed and open-ended questionnaires as well as oral interviews. Twenty of them were finally subjected to microbiological tests and medical examinations after series of oral interviews and depending on the physical effects of the cement dusts on their skins. Skin, nose and eye swabs, as well as sputum samples of the subjects were collected and cultured using various growth media. Organisms isolated included *Staphylococcus aureus, Branhamella catarrhalis, Bacillus* spp., *Klebsiella pneumoniae, Streptococcus* and *Proteus* species, and some fungi, including *Penicillium, Aspergillus, Trichophyton, Mucor* and *Epidermophyton* species. Chest radiographs were also done to detect the occurrence of silicosis (occupational asthma). The results of this study have shown that depending on the length and level of exposure to cement dust and powder, effects may range from contact dermatitis, skin rash, immediate or delayed irritation of the eyes, as well as chest infections.

Keywords: health hazard, cement dust, cement exposure, dermatitis, silicosis

Introduction

Cement can cause ill health on inhalation, and skin and eye contacts. Risk and extent, or severity of injury obtained, depends on the duration and level of exposure, as well as individual sensitivity. Thousands of labourers working at cement distribution outlets are exposed to the product in various forms, ranging from dry cement powder, cement dust, wet cement, and concrete everyday, being absolutely ignorant of the underlying health hazards and consequences of their occupation. Cement dust, released and inhaled during bag handling and bag 'dumping', can irritate the skin causing xerosis, which may result in scaling, itchiness, burning and redness (Yang *et al.,* 1996). Irritant contact dermatitis, as well as allergic dermatitis may develop. When cement is trapped, against the skin, it may take several months to heal and may involve hospitalization and skin grafts. The most hazardous effects of cement dust are on the lungs. In the short term, such exposures irritate the mucous membrane of the nose and throat causing choking, as well as difficulty in breathing (Al-Neami *et al.,* 2001; Yang *et al*.,1996). Cement has also been classified as a carcinogen due to its silica content.

Incidences of occupational health hazards amongst workers have been reported (Mwaiselage *et al.,* 2004; Alvear-Galindo and Mendez-Ramirez, 1999; Ng *et al.,* 1992). Workers in small-scale enterprises make up the second largest employment sector in developing countries. They confront very high social and health risks with poor working conditions, employment insecurity and minimum health care. Most of the workers even do not know that they are being exposed to numerous health risks. Reports show that more than 70% of workers at small-scale enterprises hardly know and believe that they are exposed to certain occupational health hazards (Fell *et al.,* 2003). The minimal occupational exposure standard for cement dust has been suggested to be 10 mg/m^3 total inhalable dust and 4 mg/m^3 total respirable dust. However, in developing countries, these standards are hardly maintained, particularly in small-scale enterprises. Therefore, workers at these sites are exposed to greater risks of developing job-related diseases (Al Neami *et al.,* 2001; Leffler and Milton, 1999; Yang *et al.,* 1993).

It has been reported that cement-related pneumocosis, e.g., silicosis, is attributed to the presence of silica in inhaled cement dust (Mengesha and Bekele, 1998; Ng and Lee, 1995). This is usually due to occupational exposure and inhalation of airborne crystalline silica. Silicosis is a disabling dust-related disease of the lungs. Even materials containing small amounts of crystalline silica may be hazardous if exposed to, in ways that produce high dust concentration, such as 'bag

*Author for correspondence; [b]present address;
E-mail: ifyobuekwe@yahoo.com

dumping' at cement depots during loading and unloading. Inhaling silica dust has also been seen to aggravate lung diseases, such as tuberculosis and lung cancer. It may be noted that pre-existing upper respiratory tract and lung diseases may be aggravated on inhalation of cement dust. Irritation of the moist mucous membranes of the nose, throat and upper respiratory systems also occur leaving unpleasant deposits in the nose. The risk of asthma attributable to occupational exposures is probably under-appreciated due to under-reporting and inappropriate use of narrow definition of exposure (Leffler and Milton, 1999).

This study investigates the health hazards associated with the exposure of workers at various cement distribution outlets to cement dust and powder in Benin City, Nigeria and also provides information to workers and employers on how to maintain a healthy work force and suggests effective measures to protect those at risk.

Materials and Methods

The study site. This study was carried out in Oredo and Egor Local Government Areas of Benin City, Edo State, South-West Nigeria. Fifteen major cement depots used as distribution and retail outlets located in the city were used for the study.

Subjects. The focus of this investigation was the effect of the length of exposure of each study-subject to cement dust. Forty subjects were initially used for the questionnaire-based study and twenty subjects were finally used for the experimental procedures. They were grouped into five, based on the results of an initial questionnaire-based study (Table 1). These workers were not temporary workers, for some had worked with the same company for more than five years.

Group A: those exposed to cement dust for less than 1 year.
Group B: those exposed for 2 years.
Group C: those exposed for between 3 to 4 years.
Group D: those exposed for more than 5 years.
Group E (the control group): those who had not worked or received exposure to cement dust previously.

It was ensured that the subjects used for this study had not been exposed to any other type of dust, like wood dust, grain dust, which may have caused occupational health hazards to the subjects previously. This precaution was taken to forestall any previous exposure to other forms of dust, which may have affected the subjects in a similar manner, in the past.

Sample collection. Sterile swabs were used to collect samples from the skin, nostrils and eyes of the twenty subjects investigated. The swab sticks were appropriately labelled and the samples were immediately shaken in normal saline, within 3-5 min of the collection. Samples were dispensed aseptically into other tubes and serial dilutions were made until a final dilution of 10^{-6} was obtained. The pour plate technique was used for the enumeration of microorganisms.

Plates were incubated at 37 °C for 24-48 h for the growth of bacteria and 5-7 days at room temperature (28±2 °C) for fungal growth. Swab samples of twenty subjects were further studied on sterile blood agar plates, which were incubated at 37 °C for 24 h for the detection of pathogens. Radiological examinations were done to detect the presence of fibrotic nodules or silica deposits on their lungs. Appropriate biochemical tests were used for the identification of all isolated microorganisms.

Results and Discussion

The initial questionnaire-based study showed a response rate of 90%. All the workers had no previous knowledge of the hazards related with exposure to cement dust and powder. 70% of the subjects reported one or more skin problems including rashes, blisters, fissures, burning, dryness, scaling and itchiness (Table 1). None of the subjects with skin problems reported lost work time, or physician visits for their problems; thus, they continued to work without seeking medical treatment, setting themselves up for life-long health problems. 65% reported one eye problem or the other, including redness, pain, burning and itchiness. These occurred only on contact with cement dust and powder. 30% of them reported shortness of breath on exertion, and this was experienced by those who had worked in the cement depots for 4 years and above. 90 % of these subjects were chronic cigarette smokers.

Table1. Results of the questionnaire-based study of the 40 subjects working at different cement distribution outlets in Benin City, Nigeria

No of subjects	Observations	(%)
30	Skin problems, including skin rashes, blisters, abrasions, fissures, burning, scaling, itchiness	75
Nil	Lost work time report or physician visits due to physical problems	Nil
26	Eye problems, including redness, pain, itchiness, burning	65
12	Shortness of breath and physical exertion	30
36	Chronic cigarette smokers	90

Table 2 shows the period of exposure of 20 workers investigated for microorganism studies. The total count of microorganisms isolated from the skin, eye and nose swabs of the subjects are shown in Table 3. The total bacterial count ranged from 2.5 x 10^5 to 5.0 x 10^6 colony forming units per millilitre (cfu/ml) zero to 8.9 x 10^7 cfu/ml, zero to 2.3 x 10^6 cfu/ml from the skin, nose and eye swabs, respectively. The isolated bacteria included *Staphylococcus aureus* and *Bacillu*s species, while the fungi isolated from skin swabs included *Penicillium, Mucor, Aspergillus, Trichophyton* and *Microsporium* species. Results from blood agar medium revealed the presence of pathogenic organisms, namely, *Staphylococcus aureus, Branhamella catarrhalis, Klebsiella pneumoniae* and *Streptococcus* species. (Table 4). Chest radiographs showed clear lung fields and normal heart sizes.

Table 2. Periods of exposure to cement dust and powder of the 20 workers investigated for microbiological examination

Periods of exposure	No of subjects	(%)
< 1 year	4	20
2 years	7	35
3-4 years	3	15
>5 years	3	15
Not exposed	3	15

Table 3. Total count of microorganisms isolated from nose, skin and eye swabs of the subjects

Subjects	Total count of microorganisms (cfu/ml)			
	Nose swab	Eye swab	Skin swab	
	Total bacteria	Total bacteria	Total bacteria	Total fungi
A1	8.9 x 10^7	2.3 x 10^6	2.35 x 10^6	1.75 x 10^6
A2	2.8 x 10^6	Nil	1.75 x 10^6	3.5 x 10^6
A3	3.5 x 10^5	1.05 x 10^6	4.5 x 10^6	1.8 x 10^6
A4	2.45 x 10^6	Nil	2.5 x 10^5	1.7 x 10^6
B1	4.5 x 10^6	6.0 x 10^5	1.65 x 10^6	2.3 x 10^6
B2	4.5 x 10^6	5.0 x 10^5	3.0 x 10^6	1.5 x 10^6
B3	4.6 x 10^6	1.7x 10^6	2.0 x 10^6	1.85 x 10^6
B4	3.35 x 10^6	5.0 x 10^5	8.5 x 10^5	2.25 x10^6
B5	1.27 x 10^7	1.0 x 10^5	3.38 x 10^6	3.4 x 10^6
B6	4.5 x 10^6	5.0 x 10^4	3.38 x 10^6	1.7 x 10^6
B7	2.8 x 10^6	1.5 x 10^5	4.1 x 10^6	1.1 x 10^6
C1	Nil	6.0 x 10^4	1.9 x 10^6	5.5 x 10^6
C2	3.9 x 10^6	2.2 x 10^6	5.0 x 10^6	3.4 x 10^6
C3	1.1 x 10^7	Nil	2.5 x 10^5	1.5 x 10^6
D1	2.35 x 10^6	7.5 x 10^5	2.5 x 10^5	7.0 x 10^5
D2	2.4 x 10^6	1.0 x 10^5	3.35 x 10^6	1.0 x 10^6
D3	3.3 x 10^6	5.0 x 10^4	2.75 x 10^6	1.5 x 10^6
E1	3.4 x 10^6	1.1 x 10^6	2.5 x 10^6	2. 15 x 10^6
E2	4.5 x 10^6	2.0 x 10^5	1.35 x 10^6	7.0 x 10^5

Table 4. Results from sputum cultures on blood agar plates

Subjects	Organisms isolated
A_1/A_2	*Staphylococcus albus, Branhamella catarrhalis*
A_3	*Staphylococcus aureus, Klebsiella* sp.
A_4	*Klebsiella* sp., *Branhamella catarrhalis*
B_1	*Streptococcus* sp.
B_2	*Staphylococcus aureus*
B_3	*Branhamella catarrhalis*
B_4	*Streptococcus* sp.
B_5	*Staphylococci, Streptococci*
B_6	*Staphylococcus aureus, Branhamella catarrhalis*
B_7	*Staphylococcus albus*
C_1	*Proteus* sp., *Staphylooccus albus*
C_2	*Streptococci, Branhamella catarrhalis*
C_3	*Branhamella catarrhalis*
D_1	*Staphylococcus aureus*
D_2	*Streptococcus* sp.
D_3	*Staphylococcus albus*
E_1	*Bacillus* sp., *Branhamella catarrhalis*
E_2	
Control	*Staphylococcus albus, Branhamella catarrhalis*

Results from this study have shown that workers in cement distribution/retail outlets in Benin City, Nigeria are exposed to very serious occupational health hazards. The most prevalent work-related health problem observed among the workers was contact dermatitis. Organisms such as dermatophytes and various bacterial species were isolated from skin samples, though some of the organisms were normal microbial flora of the skin. However, organisms such as *Candida albicans* can cause infections when the host defence mechanism is compromised and other opportunistic microorganisms whose rate and extent of proliferation and pathogenicity could be enhanced by the damaged skin barrier due to maceration of tissues, wounds and abrasions, chemical burns, and intravascular catheter. In this study, some of the workers were found to have experienced skin abrasions as well as chemical burns and were thus predisposed to infections such as dermatomycosis. Furthermore, skin injuries such as burns could predispose an individual to serious staphylococcal infections (Koneman, 1987). *Staphylococcus aureus* was iso-

lated from the skin swabs of all the subjects examined in this study. Cement is abrasive and contains some harmful chemicals that may burn and damage the skin, thus altering its integrity and predisposing workers to various microbial infections associated with contact dermatitis.

Chest radiographs of these subjects showed clear lung fields as well as normal heart sizes, thus the occurrence of silicosis may not have commenced. However, many of the subjects (30%) reported cases of shortness of breath on exertion (Table 1). Though lung function measurements were not carried out in this study, this observation could be interpreted as a pointer to the development of job-related lung dysfunction. *Branhamella catarrhalis* and *Staphylococcus aureus* were isolated from sputum samples in this study. Though these microorganisms are a part of the normal microbial flora of the respiratory tract, the presence of *Klebsiella pneumoniae* and *Proteus* species could be an indication of chest infections.

Smoking habit is one of the predisposing factors to the occurrence of job-related silicosis and 98% of the subjects in this study were found to be chronic cigarette smokers. Occupational asthma (silicosis) is diagnosed by a history of work-related symptoms and exposure to known causative agents. The diagnosis is usually confirmed by serial pulmonary function testing or inhalational challenge testing. The risk of silicosis attributable to occupational exposure is probably under-appreciated, due to under-reporting and to inappropriate use of narrow definition of exposure in epidemiological studies of attributable risk (Leffler and Milton, 1999; Ng *et al.,* 1992).

Skin problems from cement are widespread. Unfortunately, problems are often tolerated as part of the price of the work in this trade. Definitely, there will always be the occurrence of occupational diseases in different sectors of employment. However, there must be substantial variations in their incidences in various occupations. Work-related factors no doubt play a contributory role in these diseases and their prevalence is bound to vary with different occupations and types of employment. Workers hardly visit physicians for these diseases for fear of losing their jobs. The general level of tolerance permits the high rates of occupational skin problems to continue. There should be a change in this attitude, if these conditions must reduce to a manageable minimum.

Conclusion

This study observed that the workers were ignorant of the health hazards involved in their jobs, and therefore recommends that those working in cement distribution/retail outlets must have clear-cut information about the nature of their jobs, the possible hazards to health and ways of protecting themselves. Health education must be a priority with employers in all cement distribution outlets throughout the country.

One of the challenges is to convince cement product workers that it is possible for them to prevent occupational skin problems as well as occupational respiratory problems. They must, however, first realize how and when the skin and respiratory problems occur. Often, symptoms of lung dysfunction and rashes are downplayed or overlooked as a prelude to chronic disabling diseases. Actual challenge is the motivation, as the health impact of these occupations on the lives of the workers may play a key role. The prevention and monitoring of these work-related health problems present new challenges for the occupational health authorities. Employers also have a major role to play by providing facemasks to minimize exposure of their workers to cement dust and powder. Health education of workers should be a priority in all cement distribution/retail outlets in Nigeria. Research to provide protection and treatment for workers who are susceptible or who have already developed chronic occupational skin and respiratory diseases will be very timely.

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Determination and Seasonal Variation of Heavy Metals in Algae and Sediments in Sewers from Industrial Areas in Lagos State, Nigeria

O. R. Awofolu
Department of Chemistry, Faculty of Science, Lagos State University, Ojo, Lagos State,
PMB 1087 Apapa, Lagos, Nigeria

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Abstract. The level of heavy metals (Cd, Pb, Cu and Zn) in algae and sediments in sewers from industrial areas was determined by atomic absorption spectrophotometry (AAS). In order to evaluate the metal load of the sewers, as a result of discharged effluents, algae and sediments were collected from three major industrial areas in Lagos, Nigeria during the two main seasons (rainy and dry). Using total digestion, the mean concentration of Cd, Pb, Cu and Zn in algae at Oshodi/Isolo industrial area for the two seasons respectively ranged from 0.04-0.15 μg/g, 0.32-1.86 μg/g, 0.42-1.52 μg/g and 0.10-1.80 μg/g, while those in sediments ranged from not detectable (ND)-0.10 μg/g, 0.12-1.32 μg/g, 0.21-2.65 μg/g and 0.18-1.74 μg/g, respectively. At Iganmu industrial area, the range in algae was ND-0.21 μg/g, 0.20-1.84 μg/g, 0.17-1.90 μg/g and 0.05-1.87 μg/g, while those in sediments was 0.04-0.50 μg/g, 0.22-1.85 μg/g, 0.15-1.78 μg/g and 0.48-2.86 μg/g in the same metal order. The range in algae at Ikeja industrial area was 0.05-0.18 μg/g, 0.08-0.82 μg/g, 0.51-1.40 μg/g and 0.24-2.80 μg/g, while the sediments recorded a range of 0.04-0.60 μg/g, 0.16-0.90 μg/g, 0.24-2.35 μg/g and 0.23-2.84 μg/g, in the respective metal order. Levels of the metals were higher in most samples during the dry season and there were significant differences in the metal concentrations from industrial and residential areas.

Keywords: heavy metals, sewer, algae, sediment, atomic absorption spectrophotometry (AAS)

Introduction

The concept of 'save environment' is rapidly gaining vast attention in many developing countries of the world, especially in Africa and South East Asia. However, one of the major impediments to the realization of this concept is due, partly, to inadequate and ineffective monitoring of proper waste treatment and disposal by industries and to non-compliance of waste treatment legislations by the industries where such exist. Most of these industries still consider waste treatment as a profit reduction venture. These scenarios exist in Lagos State, the industrial nerve center and former capital of Nigeria, where over 60% of the industries are located. A sizeable percentage of these industries are the chemical and allied industries. Trace metals, such as Cd, Pb, Cu and Zn, are the common pollutants. These are widely distributed in the environment with sources mainly from the weathering of minerals and soils (O'Neil, 1993; Merian, 1991). However, there has been a greater input to the amount of these metals in the environment as a result of human activities. These inputs are mostly from industrial discharges, domestic effluents, urban runoffs and atmospheric deposition.

Some of these metals, especially Cd, Pb, Zn and Hg, have been found to be harmful even in small quantities (Borgmann, 1983; Tyler, 1981), hence usually monitored for health purposes. Cadmium is one of the most toxic elements with reported carcinogenic effects in humans (Goering *et al.,* 1994). It accumulates mainly in the kidney and liver, and its high concentration has been found to cause chronic kidney dysfunction. It induces cell injury and death by interfering with calcium regulation in biological systems. Copper is among some of the heavy metals that are essential to life but could be toxic at elevated levels. It is toxic at low concentration in water and is known to cause brain damage in mammals (DWAF, 1996). Provisional health-based guideline value of 2 mg/l for copper was proposed and concentration above 5 mg/l can give rise to problems of taste (IPCS, 1998). United States Environmental Protection Agency classified lead as potentially hazardous and toxic to most forms of life (USEPA, 1986). It has been found to be responsible for quite a number of ailments in humans, e.g., chronic neurological disorder, especially in foetus and children. Although Zn has been found to have low toxicity to man, yet prolonged consumption of large doses can result in some health complications such as fatigue, dizziness, and neutropenia (Hess and Schmid, 2002). Health-based guideline value was not derived for zinc, however, drinking water containing zinc at levels above 3 mg/l may not be acceptable to consumers (WHO, 1996).

The determination and evaluation of environmental metal burden have been carried out by using potable water (Garcia *et al.,* 1999; Gulson *et al.,* 1997; Holynska *et al.,* 1996), fresh

E-mail: orawofolu3@yahoo.com

and marine waters (Fiaccabrino *et al.,* 1998; Batterham *et al.,* 1997; Fatoki, 1993), air (Cerda *et al.,* 1999), soil and sediments (Borgmann *et al.,* 2001; van Staden and van der Merwe, 2000; Mellor and Bevan, 1999), algal samples (Kut *et al.,* 2000; Carrilho and Glibert, 2000), and plants (Murphy *et al.,* 2000) by using various methods. However, there has been greater interest in the use of algal species as pollution indicators, mostly present in aquatic environments (Kaewsarn and Qiming, 2001; Tam *et al.,* 1997; Chan *et al.,* 1991). Unicellular green algae have been reported as potential biological materials for removing heavy metals from industrial effluents (Wong *et al.,* 2000; Wong and Pak, 1992; Akzu and Kutsal, 1990). This is due to their hyper-accumulating capacity of metals. They are, therefore, being studied as potential phyto-remediating agents of trace metals in water and sediments. They are able to accumulate metals in thousand-folds higher than the concentration in surrounding water (Bryan and Langston, 1992). The accumulated metals were found to only affect the reproductive activities (stasis) of these plants but are not fatal to them. They also satisfy all the basic requirements of organisms used as bioindicators. These include their sedentary nature, sensitivity to environmental variations, rapid response to pollutants, easy identification and collection, and wide distribution (Lovett-Doust *et al.,* 1994).

The present study was aimed at the determination and evaluation of trace metals (Cd, Pb, Cu and Zn) in common algal species and in sediments from sewers in industrial areas of Lagos State, Nigeria. This is with a view of assessing the environmental metal burden as a result of the perceived inadequate waste treatment and management of the industries and to stimulate the necessary governmental authorities of the need to intensify their monitoring and compliance activities.

Materials and Methods

Sampling area and sampling protocol. Algal samples were collected randomly from sewers using pre-washed stainless steel spoon, rinsed with 1% dilute HNO_3 and then with double distilled water. The samples were placed in clean polyethylene containers, labelled and taken to the laboratory where they were kept refrigerated at 4 °C before analysis so as to preserve them. Sediment samples were also collected randomly from sewers at each site by using a separate and clean stainless steel spoon. The spoon was properly washed, rinsed first with 1% dilute HNO_3 and then with double distilled water. They were placed in clean polyethylene containers, labelled and taken to the laboratory where they were kept frozen at -18 °C in order to preserve the integrity of sediment samples and to avoid possible biological activities before analysis. Freezing has long been an acceptable preservation method for sediments collected for the determination of organic and inorganic constituents. It has been shown that rapid deep-freezing can best maintain sample integrity and thus enable investigation for concentrations of the contaminants (Forstner, 2004).

There are two major dry and rainy seasons in Nigeria. The dry period is characterized by hot climatic/atmospheric temperature, which varies relatively between 30-40 °C and spans between November and April, climaxing in February/March. The rainy season spans between May and October, climaxing in June/July. Consequently, both the algae and the sediment samples were collected in February and June 2000 representing the dry and rainy periods, respectively.

Algae and sediment samples were collected from sewers of three different major roads on which a variety of chemical industries are located within each of the selected industrial areas. Samples were also collected from sewers in residential areas within the respective Local Government Authority of the industrial areas. A total of three major industrial areas were sampled. The different sampling sites and their respective industrial areas are presented in Table 1.

Reagents. All chemicals, purchased from Aldrich Chemical Company, were of analytical grade. Metal standard solutions were prepared from 1000 mg/l stock solutions of the respective metals. All the glassware was properly washed with detergent, rinsed with water, soaked overnight in dilute nitric acid and then rinsed with distilled water before use.

Instrumentation. The analysis was performed with Pye-Unicam Philips (PU900X) atomic absorption spectrophotometer with a digital readout unit and a standard single slot burner head. The operational conditions specified in the instrumentation manual were followed.

Chemical analysis. The algal samples were carefully rinsed with double distilled water to remove any attached particles.

Table 1. List of industrial areas and designation of sampling sites

Industrial areas	Sampling sites	Designation
Oshodi/Isolo	Aswani Road	I
	Ademola Road	II
	Abimbola Road	III
Iganmu	Abebe Road	IV
	Industrial close	V
	Moshood Abiola Way	VI
Ikeja	Acme Road	VII
	Adeniyi Jones Avenue	VIII
	Oba Akran Road	IX

These were then air dried in the oven at 25 °C for about 48 h. Low temperature at 25 °C was used because low temperature drying prevents loss of volatile constituents, avoids chemical changes in labile components, particles of the dried sediments remain dispersed, and aggregation of the particles is minimized. Also, it avoids charring of the filamentous lower plants. The dried plant sample was gently pulverised and 0.5 g weighed into a 100 ml beaker. This was digested using 5 ml of nitric acid and then about 2 ml of perchloric acid in a fume-hood to a final volume of about 2 ml (adapted method, van Loon, 1982). Double distilled 10 ml water was used to rinse the sides of the beaker and the solution was filtered into 50 ml standard flask using 0.45 μm Millipore filter kit (Millipore Corp., Bedford, MA, USA). Triplicate analysis of each sample was carried out and the results were expressed as the mean of the triplicate digestion.

Sediment samples were also air-dried in a circulating oven at 25 °C for 48 h. 0.5 g of the sediment was weighed into a 100 ml beaker and 5 ml concentrated HNO_3 was added. This was digested at low heat on a hot plate for about 20 min in the fume-cupboard until the brown fumes subsided. The digest was allowed to cool and 2, 3 and 5 ml $HClO_4$, HNO_3 and HF, respectively, were added. This was then digested until the content was about 2 ml. The digest was allowed to cool and 5 ml of double distilled water was used to rinse the sides of the beaker. The content was filtered using 0.45 μm Millipore filter kit into a 50 ml standard flask and made up to the mark. The metal concentration was expressed as the mean of the triplicate digestion.

Quality assurance. This was carried out by using the spiking procedure in the absence of standard reference materials. The weighed samples were spiked with 0.02 ppm of Cd and Pb and 0.5 ppm of Cu and Zn. They were taken through the same digestion protocol for the lower plant and sediment samples as described above. Triplicate analysis of this process was carried out and the recoveries of the spiked elements were determined.

Statistical analysis. The results of the elements determined in the samples were subjected to statistical evaluation using the Pearson correlation coefficients.

$$r^2 = \frac{\sum x_i y_i - \sum x_i \sum y_i}{[n\sum x_i^2 - (\sum x_i)^2][\sum y_i^2 - (\sum y_i)^2]}$$

Results and Discussion

Industrial areas from where samples were collected together with their sampling sites and designations are presented in Table 1. Results of the quality assurance of metals for the algal and sediment samples are presented in Table 2. The percentage recovery of the spiked elements in algae was between 93.8 to 96.0% while that of the sediments was in the range of 91.7 to 95.8%. The concentration of metals in algae and sediments for both the dry and rainy seasons at Oshodi/Isolo, Iganmu and Ikeja industrial areas are presented in Fig. 1a, b; 2a, b; and 3a, b, respectively.

Variation patterns of the metal values in the algal and sediment samples are shown in Fig. 4a, b; 5a, b; and 6a, b for Oshodi/Isolo, Iganmu and Ikeja industrial areas, respectively. The high percentage recoveries obtained from the spiked algal and sediment samples indicate the reliability of the analytical processes employed in this study.

The range and mean concentrations of Cd, Pb, Cu and Zn in algae at Oshodi/Isolo industrial area for the two seasons was 0.04-0.15 μg/g, 0.09 μg/g; 0.32-1.86 μg/g, 0.98 μg/g; 0.42-1.52 μg/g, 1.02 μg/g; and 0.10-1.80 μg/g, 1.12 μg/g, respectively. The range and mean concentrations of the elements in sediments in this area, in the same metal order were not detectable (ND)-0.10 μg/g, 0.05 μg/g; 0.12-1.32 μg/g, 0.78 μg/g; 0.21-2.65 μg/g, 1.27 μg/g; and 0.18-1.74 μg/g, 0.73 μg/g, respectively. The mean concentrations of metals at the residential areas (Table 3) for the two seasons were 0.02, 0.03, 0.06 and 0.12 μg/g in algae; 0.03, 0.07, 0.09 and 0.34 μg/g in sediments for the elements Cd, Pb, Cu and Zn in that order.

The range and mean concentrations of the metals (Cd, Pb, Cu and Zn) in algae at Iganmu industrial area were ND-0.21 μg/g, 0.08 μg/g; 0.20-1.84 μg/g, 1.12 μg/g; 0.17-1.90 μg/g, 0.93 μg/g; and 0.05-1.87 μg/g, 0.87 μg/g, respectively. The range and mean concentration in sediments for this area, also in the same metal order, were 0.04-0.50 μg/g, 0.13 μg/g; 0.22-1.85 μg/g, 0.99 μg/g; 0.15-1.78 μg/g, 0.88 μg/g; and 0.48-2.86 μg/g, 1.42 μg/g. The mean metal concentrations for the two seasons in algae at the residential areas were 0.02, 0.07, 0.05 and 0.07 μg/g for Cd, Pb, Cu and Zn, respectively, while those in sediments, in the same order were 0.05, 0.07, 0.08 and 0.08 μg/g.

Table 2. Percentage recoveries* of trace metals from spiked algae and sediment samples

Metals	% Recovery (algae)	% Recovery (sediments)
Pb	95.6 ± 0.4	92.4 ± 0.2
Cd	93.8 ± 0.2	91.7 ± 0.3
Cu	95.2 ± 0.3	95.8 ± 0.1
Zn	96.0 ± 0.1	94.2 ± 0.2

*average of three replicate analyses; ±: standard deviation

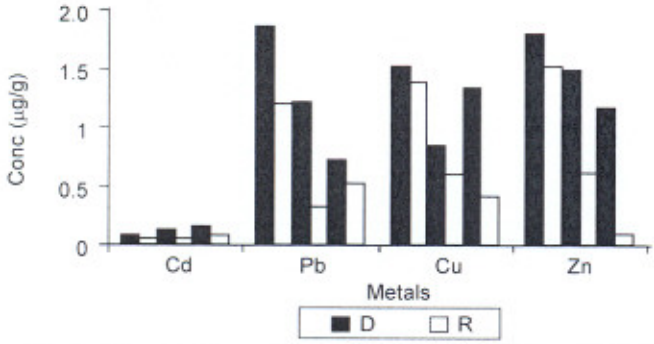

Fig. 1a. Concentration of heavy metals in algae (dry weight) at Oshodi/Isolo industrial area; D: dry season, R: rainy season.

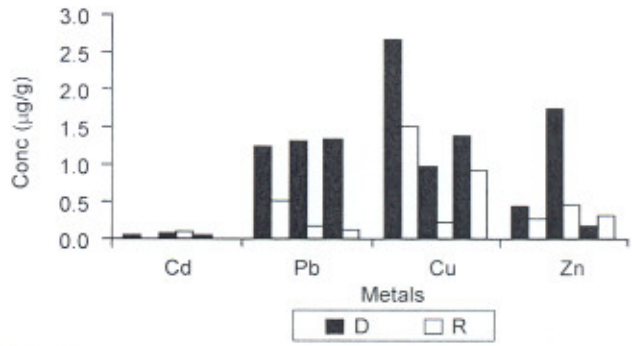

Fig. 1b. Concentration of heavy metals in sediments (dry weight) at Oshodi/Isolo industrial area; D: dry season, R: rainy season.

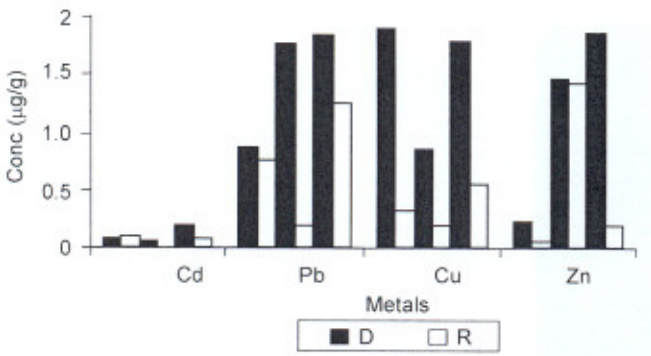

Fig. 2a. Concentration of heavy metals in algae (dry weight) at Iganmu industrial area; D: dry season, R: rainy season.

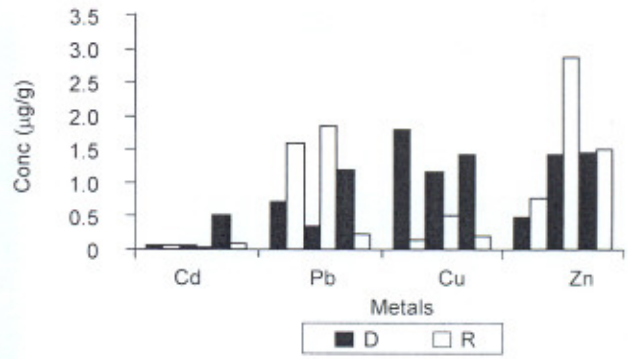

Fig. 2b. Concentration of heavy metals in sediments (dry weight) at Iganmu industrial area; D: dry season, R: rainy season.

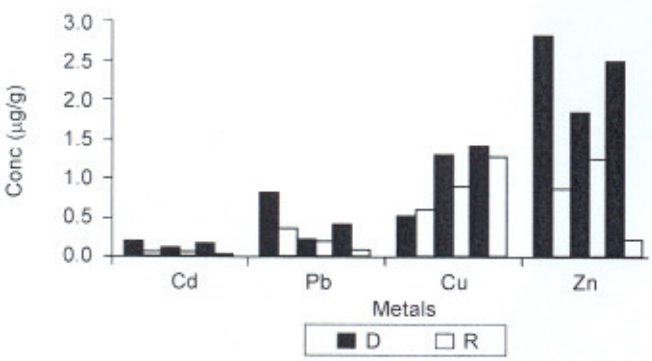

Fig. 3a. Concentration of heavy metals in algae (dry weight) at Ikeja industrial area; D: dry season, R: rainy season.

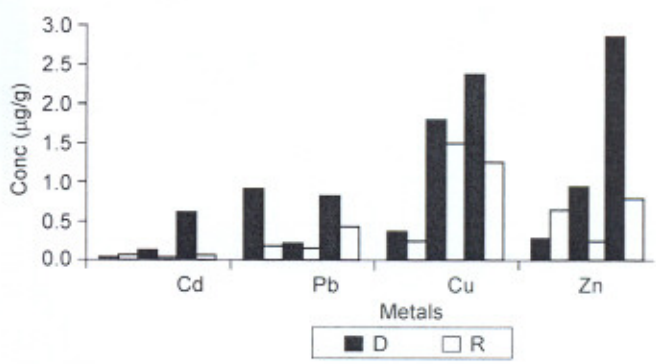

Fig. 3b. Concentration of heavy metals in sediments (dry weight) at Ikeja industrial area; D: dry season, R: rainy season.

At Ikeja industrial area, the range and mean concentrations of the metals (Cd, Pb, Cu and Zn) in algae for the sites and seasons were 0.05-0.18 µg/g, 0.10 µg/g; 0.08-0.82 µg/g, 0.40 µg/g; 0.51-1.40 µg/g, 1.00 µg/g; and 0.24-2.80 µg/g, 1.58 µg/g, respectively. Those for sediments in the same metal order were 0.04-0.60 µg/g, 0.15 µg/g; 0.16-0.90 µg/g, 0.50 µg/g; 0.24-2.35 µg/g, 1.24 µg/g; and 0.23-2.84 µg/g, 0.95 µg/g.

Samples from the residential areas at Ikeja recorded the presence but low concentrations of all the analysed metals when compared to values from the industrial areas. The mean concentration of metals for the two seasons following the above order in algae were ND, 0.14, 0.30 and 0.15 µg/g, while those in sediments were 0.07, 0.05, 0.33 and 0.46 µg/g.

Generally, highest concentrations of 0.21, 1.86, 1.90 and 2.80 µg/g of Cd, Pb, Cu and Zn, respectively, were obtained in algae while those in sediments were 0.60, 1.85, 2.65 and 2.86 µg/g, respectively, in the same metal order. There were significant differences in the mean metal concentrations

Table 3. Mean concentrations of metals (μg/g) in samples at residential areas for the two seasons

Metals	Samples					
	Algae			Sediments		
	Oshidi	Iganmu	Ikeja	Oshodi	Iganmu	Ikeja
Cd	0.02	0.02	ND	0.03	0.05	0.07
Pb	0.03	0.07	0.14	0.07	0.07	0.05
Cu	0.06	0.05	0.30	0.09	0.08	0.33
Zn	0.12	0.07	0.15	0.34	0.08	0.46

*three replicate analyses; ND: not detected

found in the algal and sediment samples from the three industrial areas when compared with their respective residential areas.

Although there were instances where individual metal level found in a sample from an industrial area was lower than those obtained from the residential areas at Oshodi/Isolo industrial area, relatively higher concentrations of Cu and Zn were detected in the samples than those of Cd and Pb. This is most likely due to the effluents coming from textile industries in this area which are released into the sewers, since Cu and Zn salts are known to be used in textile manufacturing processes.

Seasonal variation pattern of the metals revealed that their concentrations were higher during the dry seasons for algal samples with the exception of site III (Fig. 4a). Similar pattern was also shown by the sediment samples at this site with the exception of Cd at site II and Zn at site III (Fig. 4b).

At Iganmu industrial area, higher concentrations of all the elements were recorded in algae during the dry season with the exception of Cd at site IV (Fig. 5a). Similar distribution was shown by cadmium, copper and lead (except at site IV) in the sediment samples (Fig. 5b). A reverse distribution pattern was, however, displayed by Zn with higher concentration of the element during the rainy season.

At Ikeja industrial area, higher metal concentration was observed in algal samples at all sites during the dry season with the exception of Cu at site VII (Fig. 6a), while in sediment samples (Fig. 6b), only Cd and Zn at site VII showed higher concentrations during the rainy season.

Sediments are known to be the sink for heavy metals (Stephens *et al.*, 2001; Ankley *et al.*, 1996; Luoma 1989) where they concentrate according to the level of pollution. Algae that inhabit the sewers are also directly exposed to these trace metals from the effluents. Algae have been reported as accumulators of metals (Wong *et al.*, 2000; Cetinkaya *et al.*, 1999; de la Taboada *et al.*, 1998). The higher metal values, therefore, found in them, relative to that in the surrounding sediments are the possible indication of their accumulating capacity of heavy metals.

The higher values of the metals found in this lower plant during the dry season could be attributed to the nature of effluents being discharged into the sewers. During this period, effluent colours are usually deep in intensity and likely to contain higher concentrations of metals. Moreover, the volume of water flowing through the sewers during this season is usually lower than that during the rainy season. This possible dilution factor of the effluents might be responsible for the lower metal values obtained for the algal samples and some of the sediment samples during the rainy season. Generally, Cu and Zn were detected in both the algal and sediment samples in all the samples analysed.

Lead was also detected in most of the samples with the exception of samples from residential areas at the Oshodi/Isolo

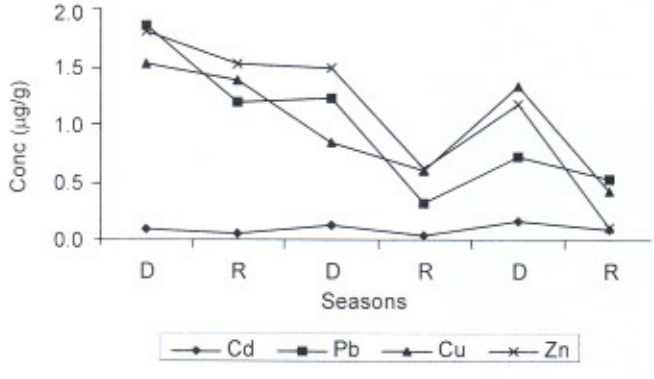

Fig. 4a. Seasonal variation of heavy metals in algal samples at Oshodi/Isolo industrial areas; D: dry season, R: rainy season.

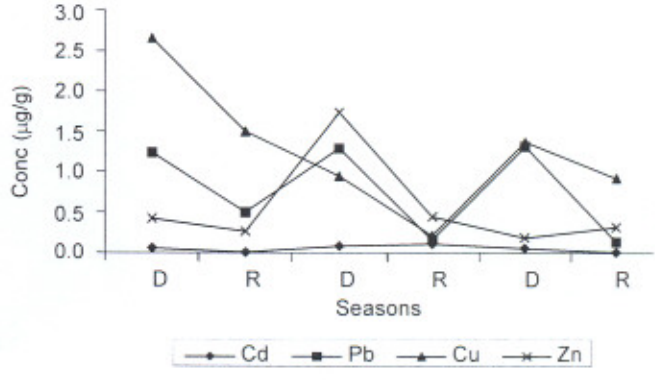

Fig. 4b. Seasonal variation of heavy metals in sediment samples at Oshodi/Isolo industrial areas; D: dry season, R: rainy season.

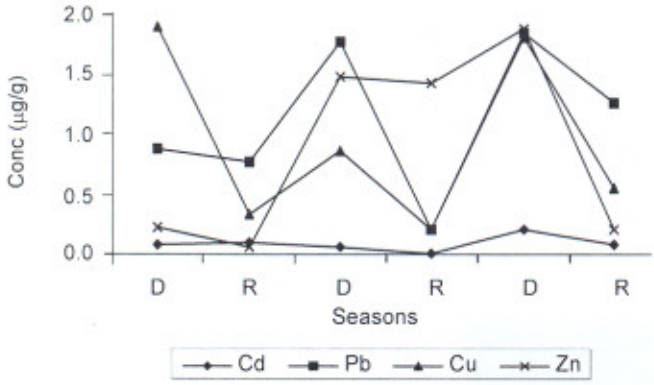

Fig. 5a. Seasonal variation of heavy metals in algal samples at Iganmu industrial areas; D: dry season, R: rainy season.

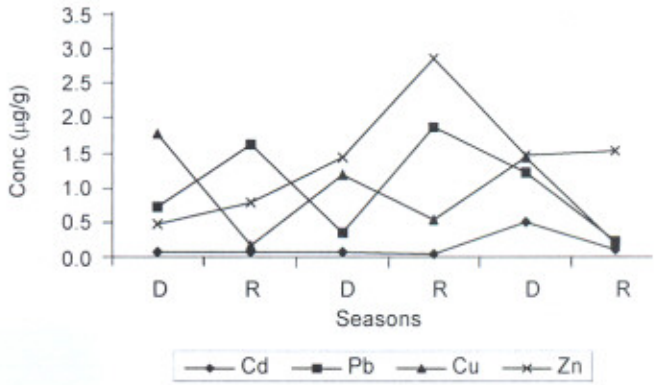

Fig. 5b. Seasonal variation of heavy metals in sediment samples at Iganmu industrial areas; D: dry season, R: rainy season.

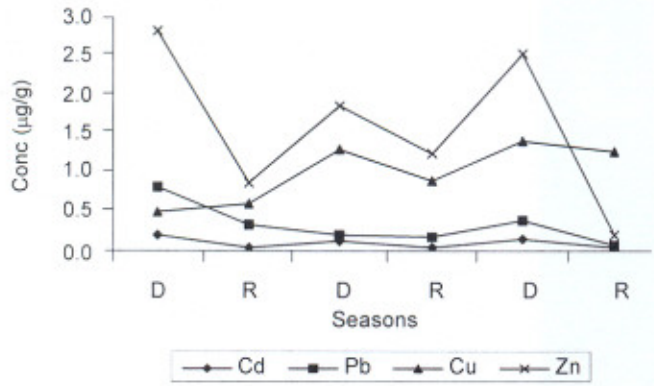

Fig. 6a. Seasonal variation of heavy metals in algal samples at Ikeja industrial areas; D: dry season, R: rainy season.

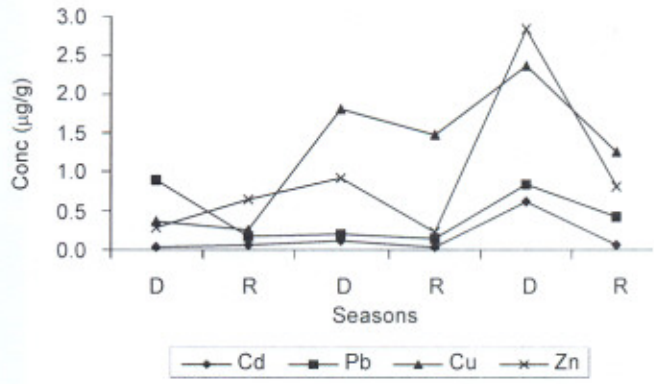

Fig. 6b. Seasonal variation of heavy metals in sediment samples at Ikeja industrial areas; D: dry season, R: rainy season.

industrial areas. Most probable sources of this metal were from effluents discharged into sewers by paint, battery, plastic and chemical industries within the sampled areas. Copper and zinc salts are known to be used in textile manufacturing processes. Effluents discharged by textile industries from the sampled areas are, therefore, most likely to contain these metals. Other sources include copper wire, plastics, and copper alloy industries with respect to copper, and electroplating, smelter, waste combustion and steel processing with respect to zinc. Although possible atmospheric deposition of metals from sources such as roadsides, automobile exhaust, etc., as contributing factors to the amount obtained, may not be ruled out (Harrop *et al.,* 1990). Copper and zinc are regarded as essential elements, however, their concentrations above the threshold levels could be toxic.

Recorded amounts of Cd were lower when compared to other analysed metals and was not detected in some of the algal and sediment samples. Possible sources of Cd and Pb in effluents are most likely from battery, paint, metal smelter and other chemical industries from the sampled areas. The metal values obtained in both the algal and sediment samples in each industrial area were subjected to statistical evaluation using the Pearson correlation coefficient. This was with a view to checking possible relationship between the metals in the samples. Average correlation of Pb ($r = 0.64$) was observed between algal and sediment samples, while a relatively significant correlation of Cu was obtained ($r = 0.81$) between the two samples at Oshodi/Isolo industrial areas. At Iganmu industrial area, Cd and Cu were highly correlated in the two samples with $r = 0.88$, and $r = 0.91$, respectively, while Zn and Pb were averagely correlated with values of 0.60 and 0.50, respectively. Significant correlation was also obtained with Pb and Cu at Ikeja industrial areas with values of 0.69 and 0.91, respectively. These correlations are indications of common sources of the metals in both samples. Generally, lower mean values of the analysed metals in sediments were obtained when compared with concentration range of 0.1-1.4 µg/g Cd, 9.0-61.9 µg/g Pb, 4219-15182 µg/g Cu, and 18.8-

126 µg/g Zn, reported in harbour sediments (Fatoki and Mathabata, 2001), 0.07-3.83 mg/kg Cd, 4.47-420 mg/kg Pb, 2.30-107 mg/kg Cu, and 9.75-2050 mg/kg Zn, reported in river sediments (Ouyang *et al.,* 2002) and mean concentration of 4.82 µg/g Cd, 28.1 µg/g Pb, 7.21 µg/g Cu, and 11.3 µg/g Zn reported in mangrove sediments (Shriadah, 1999).

The lower metal values obtained in sediments might be due to the continuous flow of effluents through the sewer which tend to "wash" off the sediments, only to be deposited in the larger fresh and marine environment. Consequently, accumulation and concentration of the metals within the sediment core in the sewers might not be significant compared to the high rate of sedimentation and lower undercurrent flow associated with sediments in the larger water bodies.

The results showed significant differences in the metal load in both algal and sediment samples analyzed from the industrial areas and those from their respective residential areas. The detection of metals such as Pb and Cd at residential areas might be due to atmospheric deposition of metal particulates possibly from automobile exhausts and Cd based waste materials such as batteries in some of the sewers. It is not uncommon to find wastes of various types of metal based materials such as batteries, iron rods, food cans, etc., in sewers at residential areas in Lagos.

Comparative assessment of the amount of metals obtained in sediment samples in all the sites was also made with standard guidelines. The Canadian Environmental Protection Authority guidelines for trace elements in sediments specifies maximum concentration of 3, 8, 22 and 40 µg/g for Cd, Pb, Cu and Zn, respectively (CEPA, 1976). The proposed South Africa guidelines as used by Maritz and Swanepool (1998), in the interpretation of their results on dredged silt suggested an amount of 10, 500, 500 and 750 µg/g in the same metal order as above. Both the individual and mean values of analysed metals in sediments were lower than those specified by both guidelines. Consequently, metal concentrations in sediments from sewers are not expected to be of environmental concern, however, regular monitoring of the metal load of the effluents from point sources is recommended. This is with a view to ensuring adequate protection and safety of the aquatic environment.

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Short Communication

Screening of Fused Pyrimidines as Antimicrobial Agents: Inhibitory Activities of Some Tetrahydrobenzothieno-Pyrimidines

M. M. H. Bhuiyan* and M. Fakruddin

Department of Chemistry, University of Chittagong, Chittagong-4331, Bangladesh

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Abstract. Seven synthetic tetrahydrobenzothieno fused pyrimidine derivatives were investigated for their antibacterial and antifungal activities. Their comparative ability to inhibit growth of bacterial species *Bacillus subtilis*, *B. megaterium, Staphyllococcus aureus*, *Salmonella typhi* and *Escherichia coli* in comparison with the commercial antibiotic brand Ampicillin, and of fungal species *Verticillium* sp., *Fusarium solanae, Aspergillus* sp., and *Penicillium* sp., in comparison with the commercial antifungal brand Nystatin is reported.

Keywords: fused pyrimidines, thienopyrimidines, antimicrobial activity

Pyrimidine derivatives, which constitute a partial structure of the purine base and many biologically active compounds, are involved widely in living organisms and have attracted much attention from the view point of medicinal chemistry. The soporific and hypnotic barbiturates and a number of antibacterial and antimalarial drugs also contain pyrimidine rings (Burger, 1960). Some of thienopyrimidine derivatives are reported as potential chemotherapeutic agents (Ram *et al.,* 1981). In a programme to obtain new potent antimicrobial agents, the synthesis of some tetrahydrobenzothieno fused pyrimidine derivatives has been reported earlier (Rahman *et al.,* 2000). The present work describes their antimicrobial activity.

Compounds for antimicrobial screening. Seven compounds studied for the purpose were: 4-amino-5,6,7,8-tetrahydrobenzothieno[2,3-d]pyrimidine(**1**), m.p. 226-227 °C; 8,9,10,11-tetrahydrobenzothieno[3,2-e]imidazo[1,2-c] pyrimidine (**2**), m.p.187-190 °C; 4-amino-2-phenyl-5,6,7,8-tetrahydrobenzothieno[2,3-d]pyrimidine (**3**), m.p. 224-225 °C; 5-phenyl-8,9, 10,11-tetrahydrobenzothieno[3,2-e]imidazo[1,2-c] pyrimidine (**4**), m.p. 183-185 °C; 5,6,7,8-tetrahydrobenzothieno[2,3-d] pyrimidine-2,4 (1*H*,3*H*)-dithione (**5**), m.p. 250 °C (decomposed); 4-amino-5,6,7,8-tetrahydrobenzothieno[2,3-d] pyrimidin-2(1*H*)-thione (**6**), m.p. 228-230 °C; and 3-tosyl-5, 6,7,8-tetrahydrobenzothieno[2,3-d]pyrimidine-4(3*H*)-one (**7**), m.p. 110-112 °C (Fig. 1).

Antimicrobial activity trials. The fused pyrimidine compounds (**1**-**7**) were screened for antibacterial activity (Table 1) against three gram-positive bacteria, *Bacillus subtilis, B. megaterium, Staphylococcus aureus* and two gram-negative bacteria *Salmonella typhi* and *Escherichia coli* using disc diffusion method (Bauer *et al.,* 1966). These compounds (**1**-**7**) were also screened for antifungal activity (Table 2) against four phytopathogenic fungi, *Verticillium* sp., *Fusarium solane, Aspergillus* sp., and *Penicillium* sp., using poisoned food technique (Grover and Moore, 1962). Commercial antibacterial and antifungal brands, respectively, Ampicillin and Nystatin were also tested under similar conditions for comparison.

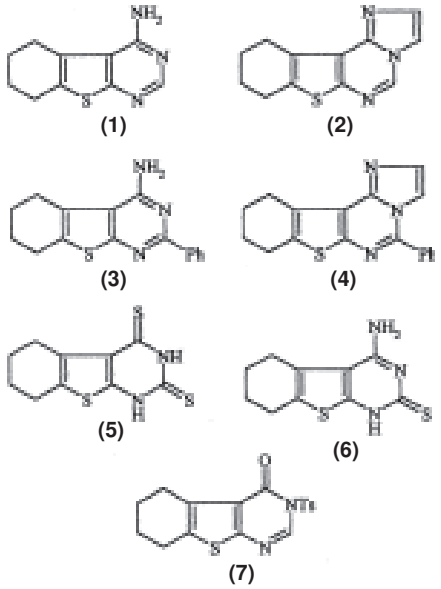

Fig. 1. Structures of fused pyrimidine compounds **(1-7).**

Compound **(5)** showed the highest antibacterial activity against *B. subtilis, Staph. aureus, Salm. typhi* and *E. coli*. Compound **(1)** showed the highest activity against *B. megaterium.* The other compounds also showed weak to moderate activity against all the tested bacteria. The antibacterial activities of

* Author for correspondence

Table 1. Antibacterial screening of the fused pyrimidine compounds **(1-7)**

Bacterial species	Diameter of zone of inhibition in mm (100 µg (dw)/disc)							
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7)**	Ampicillin 25 µg (dw)/disc
Bacillus subtilis	--	8	--	9	16	14	8	21
B. megaterium	10	6	8	7	8	8	--	20
Staphylococcus aureus	6	9	7	8	10	6	7	19
Salmonella typhi	8	--	6	--	11	7	11	24
Escherichia coli	6	7	6	7	9	6	6	12

--: no inhibition

Table 2. Fungicidal screening of the fused pyrimidine compounds **(1-7)**

Fungal species	% Inhibition of mycelial growth (100 µg (dw)/ml PDA)							
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7)**	Nystatin
Verticillium sp.	29	42	49	61	22	13	92	41
Fusarium solane	43	51	41	65	23	26	66	49
Aspergillus sp.	37	36	50	55	17	35	59	45
Penicillium sp.	57	67	67	70	22	46	97	52

all the compounds studied were, however, appreciably less than the commercial brand Ampicillin. For the antifungal activity all the compounds showed good to excellent activity against the tested fungi. Most of the tested compounds were, furthermore, significantly better antifungal agents than the commercial brand Nystatin.

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Determination of Trace Metals in Silver Cat Fish (*Chryssichthys nigrodigitatus*) Associated with Water and Soil Sediments from Beach-Line Fish Ponds

O. O. Ayejuyo[a]*, O. G. Raimi [b] and O. R. Moisili [a]

[a]Chemistry Department, Lagos State University, PMB 1087, Apapa Lagos, Nigeria
[b]Biochemistry Department, Lagos State University, PMB 1087, Apapa Lagos, Nigeria

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Abstract. Levels of cadmium, lead, zinc, copper and chromium were determined in the head, middle and tail regions of the silver cat fish (*Chryssichthys nigrodigitatus*). Water and sediments from three neighbouring man-made fresh water ponds were also analyzed using atomic absorption spectrophotometer. The mean concentration of these metals were found to be more in the soil, followed by the water and then the fish. The highest concentrations of zinc between 33.05-33.19 μg/ml and 39.72-40.13 μg/g, were found in the water and soil, respectively. Chromium concentration in water and soil ranged from 0.220-0.254 μg/ml and 0.335-0.347 μg/g, respectively. In the fish parts, zinc and chromium were found to be more in the head with values ranging from 19.05-19.23 μg/g and 0.210-0.215 μg/g, respectively. Cadmium, copper and lead were found to be more in the middle region, having values ranging from 0.260-0.261 μg/g, 4.60-4.62 μg/g and 0.320-0.321 μg/g, respectively. All metals investigated were consistently low in the fish tail. There was no significant difference in the mean concentration of all the metals in the three ponds at $p \leq 0.05$, while the distribution of the metals in the fish parts and between the fish and the water was significantly different at $p \leq 0.05$.

Keywords: trace metals, silver cat fish, soil sediments, atomic absorption spectrophotometer, *Chryssichthys nigrodigitatus*

Introduction

The advancement in technology and the growth in population have led to high levels of industrialization and urbanization which in turn have led to environmental pollution arising from the indiscriminate discharge of industrial effluents. These effluents may contain most common heavy metals, such as Hg, Zn, Cu, Co, Sb, Cd, Pb and Cr (Ibok *et al.*, 1989). It has been pointed out that industrial manufacturers may endanger public health by discharging toxic substances, including heavy metals into water, which may cause taste and odour problems, contaminating irrigated food crops, and killing fishes and other natural life in rivers (Oni, 1987). Water pollution by heavy metals has become a health hazard in recent years (Sastry and Tyaji, 1982), while human activities have increased the quantity and distribution of heavy metals in the atmosphere, on land, in rivers, lakes, and the seas (Warren, 1981). The extent of this widespread, but generally diffused contamination, has caused a great concern about its possible effects on plants, animals and human beings. Heavy metals are common components of natural waters, though some are essential for living organisms, these may be toxic when present beyond tolerant limits (Lehninger *et al.,* 1993; Volesky, 1990). Generally, these metals remain for a long time in seafoods, and through a series of reaction mechanisms accumulate in them. These are subsequentely transported in large concentrations through the food chain to animals or human beings when consumed.

*Author for correspondence; E-mail: walex_3k@yahoo.com

Description of site. The beach-line fishponds in Lagos, Nigeria are constructed in such a way that the Ojo river is allowed to flow freely in and out of the ponds. The ponds have been in existence for more than 10 years and the harvested fish are sold to the nearby consumers. The river is open to the sea at the south-east (Bar-beach) and also at the south-west (Cemebeach) through Badagry, Lagos. Human activities such as dumping of refuse, sewage discharge, excavation of soil and the discharge of industrial waste into the river have increased over the years. The data of this work will be helpful in determining the level of pollutants entering into the ponds through this river.

Materials and Methods

Description of the ponds. The ponds are square-shaped, each with a dimension of approximately 20 by 20 m by 1.3 m deep.

Sampling. ***Collection of fish.*** Fish samples of the same species were collected from three ponds (P1, P2 and P3) by hand-net. Later, each specimen was cut into three parts, head, trunk and tail, with a plastic knife and then stored at 4 °C prior to analysis.

Collection of water. The water samples were collected in plastic containers, acidified with 0.01N HNO_3 for preservation

(Holm *et al.*, 1995) and stored in polyethylene bottles prior to analysis.

Collection of the soil. Samples of bottom soil of the ponds were collected by soil sampler (Adeniyi, 1996), air-dried and kept in polyethylene bags prior to analysis.

Digestion. *Water.* The water samples were digested using the method of Holm *et al.* (1995).

Soil. Five g of sieved, air dried soil sample was digested with 2 N HNO_3 as described by Adeniyi (1996) and Abdul-Rida (1996).

Fish. The defrozen fish samples were rinsed with deionized water and each fish part was dried at 105 °C for 20 h and blended. 2 g of the blended fish part was weighed and digested by following the method described by Ibok *et al.* (1989).

Instrumentation. Zinc, lead, copper, chromium and cadmium were determined in the three samples (water, sediments and fish parts) using Perkin Elmer and Oak Brown atomic absorption spectrophotometer. The instrument settings and operational conditions were done in accordance with the manufacturer's specifications.

Statistical analysis. The results obtained were subjected to ANOVA to compare the mean levels of the metals in the three ponds and fish parts.

Results and Discussion

The results of this study are shown in Table 1, which indicate the presence of heavy metals in all the fish parts (head, middle region and tail). Presence of metals in fish has been described as an indication of the level of metal pollution of the water from which the fish was caught (Atta *et al.*, 1997). Lead, iron and copper was found to be highly concentrated in fish organs showing some likely evidence of bioaccumulation (Adeyeye, 1994). Among all the metals determined, zinc was found to be in the highest concentration with an average of 39.95 μg/g and 33.10 μg/ml in the soil and water, respectively in all the three ponds, followed by copper with 7.05 μg/g and 8.50 μg/ml, and chromium 0.34 μg/g and 0.24 μg/ml in the soil and water, respectively (Table 1). These results are in agreement with the results of Ibok *et al.* (1989) in which zinc, lead and copper were found to be more in the streams investigated. The high level of zinc and copper in the present study could be due to the discharge of untreated industrial wastewaters and human activities, including smelting of iron around the Ojo River, and subsequently in the receiving ponds. The average values of these metals are far above the United States Environmental Protection Agency (USEPA) recommended maximum limit values in drinking water (Table 2).

Table 2. US Public health services drinking water standards*

Metals	Cd	Cr	Cu	Pb	Zn
Recommended limit (ppm)	0.02	0.05	1.0	0.05	5.0

*USEPA (1976)

In the fish parts, zinc concentration ranged from 19.05-19.23 μg/g in the head, 18.18-18.20 μg/g in the middle region and 17.19-17.21 μg/g in the tail (Table 1). Concentrated copper was found in the middle region with values ranging from 4.60-4.62 μg/g followed by the head with a range of 3.00-3.15 μg/g. Lead was also found to be more in the middle region with a range of 0.320-0.321 μg/g followed by cadmium with concentration level of 0.260-0.261 μg/g, while the chromium range of 0.208-0.215 μg/g was the highest in the head. The high levels of these metals in the head are as a result of the gills, which help in respiration and filtration of water. This

Table 1. Mean metal concentrations in fish (μg/g), soil (μg/g) and water (μg/ml) in the three ponds, P1, P2, P3

	Cd			Pb			Zn			Cu			Cr		
Samples	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
Head	0.202 ± 0.01	0.205 ± 0.01	0.203 ± 0.01	0.300 ± 0.03	0.290 ± 0.02	0.295 ± 0.03	19.23 ± 0.09	19.05 ± 0.09	19.20 ± 0.10	3.00 ± 0.25	3.09 ± 0.25	3.15 ± 0.27	0.210 ± 0.01	0.215 ± 0.01	0.208 ± 0.01
Middle	0.261 ± 0.01	0.260 ± 0.01	0.261 ± 0.01	0.320 ± 0.04	0.321 ± 0.05	0.321 ± 0.05	18.20 ± 0.21	18.20 ± 0.22	18.18 ± 0.21	4.60 ± 0.11	4.62 ± 0.12	4.62 ± 0.12	0.190 ± 0.01	0.191 ± 0.01	0.191 ± 0.01
Tail	0.177 ± 0.01	0.178 ± 0.02	0.176 ± 0.01	0.273 ± 0.07	0.275 ± 0.06	0.276 ± 0.06	17.19 ± 0.21	17.20 ± 0.21	17.21 ± 0.22	2.73 ± 0.01	2.75 ± 0.02	2.74 ± 0.02	0.160 ± 0.01	0.160 ± 0.01	0.162 ± 0.01
Water	0.660	0.669	0.670	2.900	3.090	3.040	33.05	33.19	33.06	8.50	8.48	8.48	0.243	0.254	0.220
Soil	0.730	0.710	0.721	3.140	3.250	3.200	40.13	39.72	40.00	7.05	7.05	7.05	0.335	0.335	0.347

±: standard deviation

result agrees with the findings of Ayejuyo *et al.* (2003) in which zinc was found in the highest amount in the gills of *Clarias lazera* as compared with all the other body parts studied. In the present study, the gills were not separated from the head. Relatively high amount of metals were found in the middle portion. Visceral muscles are found within the middle portion of the fish and these muscles are known to concentrate toxic metals (Atta *et al.*, 1997). The concentration of the metals determined in the fish parts followed the order Zn > Cu > Pb > Cd > Cr. Fish head has become a delicacy (Okoye, 1991) and the highest levels of zinc and copper are recorded in the head and the middle portions of the fish analyzed. The presence of these metals in all the samples may be taken as an indicator of pollution of the ponds and the Ojo River water, which freely flows in and out of the ponds (Fig. 1). These metals are known to be toxic to fishes and other aquatic animals and also to humans, who subsequently consume them.

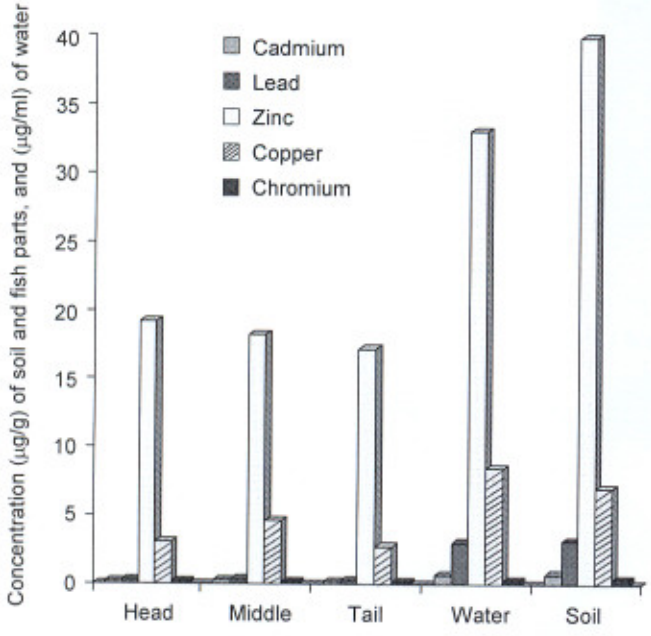

Fig. 1. Histogram of mean metal distribution in samples from the three ponds.

Conclusion

Since Nigerians depend on the fish caught locally to supplement their protein requirement, the research on levels of heavy metals in fish including their sources could be seen as one bold step towards preserving the Nigerian natural habitat. Therefore, human activities such as dumping of refuse, digging of sand and discharging of industrial effluents into rivers and streams should be discontinued in order to make fishes and other aquatic animals safe in rivers and for their subsequent human consumption.

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Development of a High Yielding Wheat Variety "Bahawalpur-97" for Southern Punjab, Pakistan

Mushtaq Ahmad, Lal Hussain Akhtar*, S. Z. Siddiqi, Manzoor Hussain, A. Rashid, Ghulam Hussain, M. Aslam, M. Safdar, M. Masood Akhtar, M. Arshad and A. H. Tariq

Regional Agricultural Research Institute, Bahawalpur, Pakistan

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Abstract. Studies were conducted to develop and release new improved wheat (*Triticum aestivum* L) varieties that can yield better and resist the diseases. On the basis of performance under field conditions, a line, MLT'S' (Metaltail)= ORE F1 158/FDI/Kl/BB/3/Nac, was selected from the Bread Wheat Observation Nursery MRA (1985-86), received through the courtesy of CIMMYT (Mexico) and given the No. V-7222. This line was tested/evaluated in 36 yield trials at different locations in Preliminary and Advanced Yield Trials (1986-89), Micro Wheat Yield Trials (1989-90) and National Uniform Wheat Yield Trials (1990-91 and 1991-92). On the average of 36 yield trials, Bahawalpur-97 gave 2.14, 5.94 and 1.22% higher yield than Inq-91, Pwz-94 and Pb-96, respectively. Its production technology was also developed. Its best sowing time was November to December. It gave maximum yield when NPK @ 125-100-50 kg/ha was applied. It was resistant to all foliar diseases. Its yield potential was 7200 kg/ha. This variety was approved and released by the Punjab Seed Council, Lahore, as a general-purpose variety for Southern Punjab in the name of "Bahawalpur-97" during 1998.

Keywords: wheat variety, disease resistance, Southern Punjab

Introduction

Wheat is the staple food in most countries of the world.The ever growing population pressure keeps the wheat breeders busy to develop sustainable new and better wheat varieties. Among the most widely cultivated crops in the Punjab, in terms of area and production, wheat ranks the highest covering more than 15 million acres. Inspite of the biggest cereal crop, yield per acre is low, only slightly more than one ton per hectare. Consequently, a huge amount of foreign exchange is being spent on its import every year. Research efforts have resulted in increasing the yield per hectare from 849 kg (APCOM, 1987) to 2300 kg in 1997. Dwarf gene has resulted in the green revolution in the world. Genetic yield improvement is almost non-significant. On the other hand, the removal of agronomic plant constraints, better response to fertilizer and resistance against foliar diseases has resulted in a jump in the yield.

Southern Punjab of Pakistan, although cotton zone, contributes about 44% to the total wheat production of the province. Cotton, being the major crop of the region, has a long stay in the field. So, about 80% of the wheat crop is being planted under late conditions. This cropping pattern demands wheat varieties that are of medium duration and can be successfully grown after the harvest of cotton. At present, wheat variety Inqlab-91 is covering maximum area under normal and late sowing. There is a need therefore for a high yielding, widely adapted, disease resistant variety, which can fetch more area under wheat cultivation and increase genetic diversity in the wheat fields.

The new wheat strain V-7222 developed at the Regional Agricultural Research Institute, Bahawalpur has a potential to share the only adapted wheat variety Inqlab-91(Inq-9) for sowing after cotton harvest in Southern Punjab. Furthermore, the strain carries blood of the world famous cross "Metaltail", which will be a new addition to the existing wheat genetic make up in the country. The cross "Metaltail" is famous for its high yield potential and resistance to foliar diseases. Therefore, the new variety V-7222 has the high yield potential and resistance to foliar diseases. It has more tillers compared to Inq-91. The food products prepared from this variety have better dietary qualities. Its 'bhoosa' (chopped wheat shaft) is cream-white in colour and is liked by the animals as fodder.

Materials and Methods

On the basis of performance under filed conditions, a line, MLT'S' (Metaltail) = ORE F1 158/FDI//Kl/BB/3/Nac was selected from the Bread Wheat Observation Nursery MRA (1985-86), received through the courtesy of CIMMYT (Mexico) and given the No. V-7222 (Bahawalpur-97; Bwp-97). This line, having desirable traits, was evaluated in Preliminary Yield Trials (1986-87), Advanced Yield Trials

*Author for correspondence; E-mail: lhakhtar@yahoo.com

(1987-1989), Micro Wheat Yield Trials (1989-90) and National Uniform Wheat Yield Trials (1990-92). Sowing date and fertilizer trials were also conducted to ascertain its production technology during 1993-94 to 1996-97. The line V-7222 was also tested against diseases, such as rusts, loose smut and karnal bunt at the Regional Agricultural Research Institute, Bahawalpur; Wheat Research Institute, Faisalabad; and Crop Disease Research Institute, NARC, Islamabad during 1990-1997 in comparison with the then existing standards. The Coordinator Wheat, NARC, Islamabad also studied the quality characteristics of the line during 1990-1992. The Federal Seed Certification and Registration Department, Islamabad studied its plant characteristics. The yield data were subjected to analysis of variance by computer using MSTAT statistical programmes and means were compared using Duncan's multiple range test (Steel and Torrie, 1980).

Results and Discussion

Yield performance. ***Station yield trials.*** The variety was tested in preliminary and advance yield trials at the Regional Agricultural Research Institute, Bahawalpur during 1986-87 and 1988-89 in normal planting, and in late planting during 1991-92 and 1992-93 in comparison with the national checks, i.e., Pak-81, Pb-85 and Inqlab-91. The performance of the variety Bahawalpur-97 is given in Table 1and 2. On the basis of average for 3 years, the variety Bahawalpur-97 gave 6.59% and 10.34% higher yield than Pak-81 and Pb-85 in normal trial, and 14.5% and 6.90% higher yield than Pak-81 and Inqlab-91, respectively, in short trials.

Microwheat yield trial. The Director, Wheat Research Institute, Faisalabad also evaluated the performance of Bahawalpur-97 during 1989-90 at various locations throughout Punjab in replicated yield trials under coded numbers. The results given in Table 3 show that 5.20% and 6.18% higher yield was obtained from Bahawalpur-97 as compared to Pak-81 and Pb-85, respectively, on the basis of average of 10 locations.

Table 1. Normal station trials

Trial year	Yield (kg/ha)		
	V-7222	Pak-81	Pb-85
1986-87 (A8)	3079a	2628a	2950a
1987-88 (B3)	3750a	3917a	
1988-89 (C2)	4520a	4103a	3937b
Average-I	*3783	3549	
Average-II	3800		3444
Increase (%)		6.59	10.34

a, b: values with different alphabets significantly different from each other at p = 0.05 (Duncan's multiple range test)

Table 2. Late station trials

Trial year	Yield (kg/ha)		
	V-7222	Pak-81	Inq-91
1991-92 (B3)	3521a	2146b	
1992-93 (C7)	3875a	3438a	3625a
Average	3198	2792	
Increase (%)		14.5	6.90

a, b: values with different alphabets significantly different from each other at p = 0.05 (Duncan's multiple range test)

Table 3. Microwheat yield trial (normal) 1989-90

Locations	Yield (kg/ha)		
	V-7222	Pak-81	Pb-85
Wheat Res. Inst., Faisalabad	4025a	4247a	3740b
Govt. Agric. Farm, Jhang	3426a	2592b	2778b
Agric. Res. Farm, Sheikhupura	4120a	4028a	3935a
Kot Mubarak, Gujranwala	5048a	4630b	4491c
Shamki Bhattian, District Lahore	5554a	5556a	5926a
Potato Seed Centre, Khanewal	5185a	4722a	4490b
Regnl. Agric. Res. Inst., Bahawalpur	5594a	4372c	4583b
Agric. Res. Station, Rahimyar Khan	3824a	4144a	3958a
Mr. Hanif Gujar, Farmer, Muzaffargarh	3704a	3704a	3935a
Ch. Liaqat Ali, Farmer, Bahawalpur	3833a	4120a	3889a
Average	4431	4214	4173
Increase (%)		5.20	6.18

a, b, c: values with different alphabets significantly different from each other at p = 0.05 (Duncan's multiple range test)

National uniform wheat yield trial. Coordinator Wheat, Islamabad, also evaluated the variety Bahawalpur-97 in a replicated trial called NUWYT under normal and short conditions throughout Pakistan during 1990-91 and 1991-92. The performance of Bahawalpur-97 in this trial is given in Table 4 and 5, which reveals that Bahawalpur-97 gave 8.91% and 10.54% higher yield than Pak-81 and Pb-85, respectively, on the national level on the basis of 10 locations of cultivation during 1990-91, while it was 9.66% and 2.87% higher than the above said checks during 1991-92.

Varietal characteristics. Various varietal characteristics recorded by the Federal Seed Certification and Registration Department, Islamabad, in comparison with Inqlab-91, are given in Table 6.

Table 4. National uniform wheat yield trial (normal), 1990-91

Locations	Yield (kg/ha)		
	V-7222	Pak-81	Pb-85
Wheat Res. Inst., Faisalabad	5359a	4861b	3414c
Govt. Agric. Farm, Jhang	4552a	4479a	4511a
Potato Seed Centre, Khanewal	4906a	4511b	4188c
Govt. Agric. Farm, Sahiwal	5177a	5229a	4969b
Wahan Adam, Kasur	3958a	3479b	4063a
Rice Res. Inst., Kala Shah Kaku	3729a	3729a	3896a
Univ. Agric., Faisalabad	3496a	2841b	
Chak No.249/G.B,Toba Tek Singh	2885a	2251b	
Regnl. Agric. Res. Inst., Bahawalpur	4313a	4125a	
Khanpur, Rahimyar Khan	2938a	2429a	
Average-I	4131	3793	
Average-II	4614		4174
Increase (%)		8.91	10.54

a, b, c: values with different alphabets significantly different from each other at p = 0.05 (Duncan's multiple range test)
Source: Mustafa *et al*. (1991)

Table 5. National uniform wheat yield trial (normal), 1991-92

Locations	Yield (kg/ha)		
	V-7222	Pak-81	Pb85
Food Res. Inst., Sargodha	5531a	5042b	5083b
Chak No.195/G.B., Faisalabad	3735a	3594a	
Wheat Res. Inst., Faisalabad	5121a	5352a	5315a
Renala Khurd, Dist., Okara	4656a	4313b	4625a
Wahan Adam, Dist., Kasur	6000a	5031b	5813a
Agric. Res. Farm, Sheikhupura	4771a	4563a	4313b
Hafizabad, Gujranwala	3613a	3417b	3758a
Regnl. Agric. Res. Inst., Bahawalpur	3771a	3177b	
Chak No.75/4-R, Haroonabad	3292a	2521b	
Layyah Agric. Res. Farm, Karore	5042a	4115b	4719a
Potato Seed Centre, Khanewal	5167a	5104a	5167a
Average-I	4609	4203	
Average-II	4988		4849
Increase (%)		9.66	2.87

a, b: values with different alphabets significantly different from each other at p = 0.05 (Duncan's multiple range test)
Source: Mustafa *et al*. (1992)

Agronomic studies. Six trials were conducted at the Regional Agricultural Research Institute, Bahawalpur during 1991-1995 to ascertain its package of production technology. The final findings are given as under:

•sowing time: November to December;

•seeding rate: 125 kg/ha;

•fertilizer requirements: 125-100-50 NPK (kg/ha); and

• irrigation: 5-6 times.

Pathological studies. The response of the variety Bahawalpur-97 to various foliar diseases studied at Crop Diseases Research Institute, NARC, Islamabad; Wheat Research Institute, Faisalabad; and Regional Agricultural Research Institute, Bahawalpur are given in Table 7-11. The perusal of the data shows that the variety is resistant/tolerant to yellow rust, leaf rust, loose smut, *Fusarium,* and karnal bunt.

Table 6. Varietal characteristics

Characteristic	Bwp-97 (V-7222)	Inq-91 (Check)
Days to heading	118 days	114 days
Days to maturity	145-158 days	135 days
Plant height	92-102 cm	98 cm
Lodging resistance	resistant	resistant
Tillers per meter row	155	132
Thousand kernel weight	40-42 g	44 g
Protein percentage	13.07%	10.51%
Disease reaction	resistant	resistant
Grain shape	long-rounded	round-elongated
Maturity status	long-duration	medium-duration
Growth habit	semi-erect	drooping
Yield potential	7200 kg/ha	6900 kg/ha

Table 7. Disease reaction at Regional Agricultural Research Institute, Bahawalpur

Year	Leaf rust		Yellow rust		
	V-7222	Pak-81	Pb-96	V-7222	Pb-96
1992-93	20MR	10S		0	
1993-94	0	20MS		0	
1994-95	20MR, MS	50S		0	
1995-96	10R	50MS		0	
1996-97	10MR	90S	10MS	0	10S

MR: moderately resistant; MS: moderately susceptible; S: susceptible

Table 8. Disease reaction report of Crops Diseases Research Institute, National Agricultural Research Centre, Islamabad

Research station	Trial year 1990-91				Trial year 1991-92			
	V-7222		Pak-81		V-7222		Pak-81	
	LR	YR	LR	YR	LR	YR	LR	YR
Wheat Res. Inst., Faisalabad	T, MR, MS		5MS		30MR, MS		50S	
Cereal Res. Inst., Pirsbak				T, MS				
Agric. Res. Station, Gujrat					T, MS			
National Agric. Res. Cent., Islamabad								T
Agric. Res. Station, Mardan					15MS			

LR: leaf rust; YR: yellow rust; MR: moderately resistant; MS: moderately susceptible; S: susceptible; T: traces
No disease was detected on trials at Gov. Agric. Farm, Jhang; Wheat Res. Station, Rawalpindi; Univ. Agric. Faisalabad; Agric. Res. Farm, Sheikhupura

Table 9. *Fusarium* disease reaction in national uniform wheat yield trial during 1991-92

	Site	*Fusarium* infection
V-7222	Mardan	0
PR-38	Mardan	6

Source: Mustafa *et al*. (1992)

Table 10. Loose smut data in national uniform wheat yield trial during 1990-91

Variety	Baha-walpur	Khane-wal	Jhang	Faisal-abad	Univ. Agic. Faisal-abad	Chakwal
V-7222			TS			
86038		TS		TS	TS	TS

T: traces; S: susceptible
Source: Mustafa *et al*. (1991)

Table 11. Disease reaction at Wheat Research Institute, Faisalabad

Diseases	V-7222	Pak-81 (Check)	WL 711 (Check)
Yellow rust	0	40S	80S
Leaf rust	TMS	100S	100S

T: traces; S: susceptive; MS: moderately susceptible
Source: WRIF (1999)

Quality studies. The quality characters recorded by National Agricultural Research Centre, Islamabad, are given in Table 12, which reveal that the new variety is comparable if not better than the existing checks.

Conclusion

The variety Bahawalpur-97 (V-7222) is not only a high yielder and tolerant/resistant to all diseases, but is also best suited in wheat-cotton-wheat rotation. Due to its better adaptability, it has the potential to replace the previously approved wheat varieties, especially in Southern Punjab. This variety was accordingly approved and released by Punjab Seed Council, Lahore for general cultivation.

Table 12. Quality characteristics of V-7222 versus checks

Year	Variety	Test weight	Flour yield (kg/hl)*	Flour ash (% d b)**	Protein (% d b)**	1000 kernel	Gluten (%)	Chapati quality
1990-91								
	V-7222	78.0	65.5	0.59	13.07	45.7	8.9	good
	Inq-91	78.1	65.5	0.57	10.51	42.0	9.6	good
1991-92								
	V-7222	76.6	65.1	0.53	8.71	40.9	6.85	good
	Pak-81	76.1	67.0	0.58	8.00	41.7	6.56	good

*hl: hectolitre; **d b: on dry wt basis
Source: Mustafa *et al*. (1991; 1992)

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Studies on the Lipolytic Enzymes of *Carica papaya* Seed Powder

M. Akhtar Javed*, Muhammad Naeem and Rana Amjad
PCSIR Laboratories Complex, Lahore-54600, Pakistan

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Abstract. The lipolytic enzymes (lipase and phospholipase) extracted from the defatted seeds of *Carica papaya* showed optimum activity at 40 °C and pH 7 in aqueous media. *n*-Heptane was found to be the most satisfactory solvent to maximize activities of lipase and phospholipase. The activity of lipase extracted from germinated seeds increased with the stage of seed development, but the phospholipase activity was noted to decrease.

Keywords: *Carica papaya*, lipase/phospholipase activity, pH/temperature optima, triglycerides

Introduction

The enzymatic studies of lipase and phospholipase of *Carica papaya* have been carried out using different temperatures, pH, aqueous media and organic solvents. The objective was to establish optimum conditions for the hydrolysis of simple triglycerides and phosphoglycerides by lipase and phospholipase so that these conditions can be applied both in the laboratory and industry.

Carica papaya (papaya) locally known as "papita", of the family Caricaceae is used as a common fruit in the Indo-Pakistan sub-continent and also in other countries of the world. The medicinal usefulness of the papaya fruit is well-established for different ailments (Kirtikar and Basu, 1984; Nadkarni, 1982), especially in the treatment of digestive system due to the presence of enzymes. Enzymes *in vivo* play an important role both in the synthesis and metabolism of a number of organic compounds in the animal and plant kingdoms. Review of the literature reveals that *C. papaya* has been studied for its papain, latex, lipase catalyst, flavonoids, proteolytic activity, sugar content, chitinase, cysteine proteinases, pectinesterase and lipids (Caro *et al.,* 2000; Rakhimov, 2000; Mangos *et al.,* 1999; Nguyen and Thanh, 1999; Askari and Qadri, 1998; Esperanza *et al.,* 1998; Albert and Philippe, 1997; Fayyaz *et al.,* 1993; Raie *et al.,*1992; Azarkan *et al.,* 1977), respectively, but studies related to the lipase and phospholipase of *C. papaya* seeds have not been previously reported. In the present studies, these enzymes have been extracted from mature and germinated seeds to determine their optimum activities on purified triglycerides of olive oil and egg lecithin, respectively, under different conditions. Such type of investigations have also been carried out on corn, wheat grains, oat grains and castor bean (Berner and Hammond, 1972; Banu and Serban, 1970; Ory 1969; Ferrigan and Geddes, 1958). The PCSIR Laboratories have carried out similar studies on *Citrullus* sp., *Carum capticum, Zea mays* and *Cassia* sp., of local origin (Javed *et al.,* 1999; Ahmad *et al.,* 1993; Aman and Akhtar, 1991; Zaka *et al.,* 1989). The present work on papaya seeds is thus an extension of the earlier studies but the present findings are being reported for the first time.

*Author for correspondence

Materials and Methods

Extraction of lipase and phospholipase. The dried seeds of papaya obtained from the fruit available in the local market, were ground to a fine powder and defatted in a Soxhlet extractor with diethylether. The defatted seed powder (50 g) was suspended in 200 ml citrate buffer (citric acid 0.1 M and disodium hydrogen phosphate 0.2 M) of pH 7 for 1 h at 40 °C. The supernatant containing enzymes was obtained by centrifugation (Karl Kolb, Germany) for 15 min at 12,000 rpm. The extract was diluted to 200 ml with citrate buffer and utilized to study the enzyme activities under different conditions (Blain *et al.,* 1976).

Preparation of substrates and determination of enzyme activities. Olive oil (origin: Italy, local market) was taken and its triglycerides were separated and purified by thin layer chromatography. The triglycerides (1 g) were emulsified by blending with 10% gum acacia suspension (aqueous medium) to determine lipase activity, whereas 10% egg lecithin (BDH, England) emulsion was used as substrate for the phospholipase activity (Javed *et al.,* 1999). Hydrolysis of the two substrates by enzymes (lipase and phospholipase), extracted from mature seeds under different parameters, is described below:

Effect of pH. The enzyme extract (15 ml) was incubated at 40 °C for 1 h in the presence of substrates (triglycerides or lecithin emulsion), separately with citrate buffer (pH 7) and calcium chloride (0.1 M). The released fatty acids, after extraction with 5 ml hexane:chloroform (1:1 v/v), were treated with

2.5 ml of Cu-TEA reagent in a test tube, shaken (linear Gallenkamp shaker) for 5 min and then centrifuged. The upper layer (3 ml) was reacted with 0.5 ml of 0.1% sodium diethyldithiocarbamate to develop a golden yellow colour whose absorbance at a fixed wavelength (440 nm) was recorded on a spectrophotometer (Beckman, model 24, England) against a blank prepared by boiled, denatured enzyme powder. A linear standard curve was drawn between the concentrations (80-800 µg/l) of palmitic acid against the absorbance (0.300-0.500) at a fixed wavelength (440 nm). The standard curve was used to calculate µ equiv of fatty acids released per g/h. The activity of lipase or phospholipase was calculated by using the method (Guven *et al.,* 1979) as follows:

$$\text{lipase/phospholipase activity } (\mu U) = \frac{\text{conc of fatty acids}}{1000} \times 80$$

Experiments were conducted with citrate buffer solutions of various pH (5.0-8.5) to determine the effect of pH on hydrolysis of the substrates. The results are reported in Table 1.

Effect of temperature. Experiments to determine the effect of temperature on the hydrolysis of substrates were conducted by changing the incubation temperature from 20-70 °C at 10 °C intervals under the same conditions as mentioned above. The results are reported in Table 2.

Effect of solvents. Defatted seed powder (1 g) was placed in a 50 ml stoppard conical flask containing 50 µl water and 5 ml liquid triglyceride:solvent (1:9) to determine the effect of various organic solvents on lipase activity (Table 3). Lecithin: solvent (1:9) was used to study the effect of solvents on phospholipase activity. The above mixtures were shaken for 2 h at 40 °C as reported earlier (Waheed *et al.,* 2002). The mixture was cooled to room temperature, additional 3 ml solvent was added and mixed thoroughly. The rest of the procedure was conducted as described above for the determination of the effect of pH.

Lipase and phospholipase activities in germinated seeds. Seeds of papaya were germinated in an incubator at 30±1 °C (Aman and Akhter, 1991). Seedlings with roots at root lengths of 5, 10, 15, 20, 25 and 30 mm were dried and crushed separately. The lipase and phospholipase extracted (see effect of pH) from the above mentioned various root lengths were assayed on substrates (triglycerides and lecithin) with buffer solution of pH 7 and an incubation temperature of 40 °C. The fatty acids released were determined from the standard curve and the enzyme activities were calculated.

Results and Discussions

The lipase and phospholipase activities were determined under different conditions of pH, temperatures and solvents. The conditions of pH and temperature, which gave maximum activity of lipase and phospholipase in mature dried seeds in aqueous medium were also applied to germinated seeds.

The lipase and phospholipase activities of defatted seeds in the pH range of 5.0 to 8.5 were studied by conducting the experiment for 1 h (Table 1). The data show that the activities of lipase and phaspholipase in neutral medium (pH 7) were the maximum (5.20 and 5.02 µU, respetively). Optimum pH 7 had also been reported for these enzymes in other seeds such as apple, apricot, and local cultivars of honeydew melon ('sarda' and 'garma') (Akhtar *et al.,* 1975). Other studies were carried out by adjusting the reaction media to pH 7 and varying the reaction temperatures and by changing the solvents in the media.

The activities of lipase and phospholipase in the defatted mature seeds were determined under various temperature conditions, i.e., 20-70 °C at pH 7 for 1 h. The maximum activities of lipase (5.10 µU) and phospholipase (4.89 µU) were observed at 40 °C for both enzymes (Table 2). The activities

Table 1. Lipase and phospholipase activities of mature papaya seeds at different pH

	Lipase			Phospholipase		
pH	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)
5.0	0.346	252	2.02	0.340	225	1.80
5.5	0.370	383	3.06	0.364	352	2.82
6.0	0.384	463	3.70	0.378	428	3.42
6.5	0.399	545	4.36	0.392	505	4.04
7.0	0.418	650	5.20	0.414	628	5.02
7.5	0.405	578	4.62	0.398	540	4.32
8.0	0.377	425	3.40	0.374	405	3.24
8.5	0.359	323	2.58	0.363	345	2.76

decreased when the temperature was increased or decreased from 40 °C. These observations show that these enzymes are more active at 40 °C, being in agreement with the studies on seed lipase of *Hibiscus cannabinus* (Kausar and Akhtar, 1979).

A set of experiments was also conducted at pH 7 and 40 °C in which different organic solvent suspensions were used in the media to determine the most appropriate solvent for hydrolysis of triglycerides and lecithin substrates by lipase and phospholipase of mature seeds. *n*-Heptane proved to be the best solvent for optimum enzyme activity for both the enzymes as compared to cyclohexane, di-isopropylether and cyclohexanol. The observed order of activity was *n*-heptane > cyclohexane > di-isopropylether > cyclohexanol: 4.80 > 3.84 > 2.62 > 1.62 µU for lipase while in the case of phospholipase it was 4.52 > 3.64 > 2.92 > 1.48 µU, respectively (Table 3). The higher activities of these enzymes in *n*-heptane may be due to its straight chain structure.

The parameters of temperature (40 °C) and pH 7, which showed maximum activities for the enzymes from mature seeds were also applied to germinated seeds at root lengths of 5 to 30 mm (Table 4). The activity of lipase, carried out in aqueous medium, was found to be directly proportional to the increase in root length of the germinated seeds. The maximum activity of the lipolytic enzyme was 6.78 µU at the root length of 30 mm. In contrast, the activity of phospholipase was inversely

Table 2. Lipase and phospholipase activities of mature papaya seeds at different temperatures

Temp (°C)	Lipase			Phospholipase		
	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)
20	0.368	375	3.00	0.362	338	2.72
30	0.407	590	4.72	0.396	527	4.22
40	0.416	638	5.10	0.411	612	4.89
50	0.404	572	4.58	0.394	517	4.14
60	0.385	465	3.72	0.371	388	3.10
70	0.373	401	3.21	0.349	268	2.14

Table 3. Lipase and phospholipase activities of mature seeds of papaya in the presence of different solvents

Solvents	Lipase			Phospholipase		
	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)
n-Heptane	0.409	600	4.80	0.403	565	4.52
Cyclohexane	0.387	480	3.84	0.383	455	3.64
Di-isopropylether	0.360	328	2.62	0.365	355	2.92
Cyclohexanol	0.337	202	1.62	0.334	185	1.48

Table 4. Lipase and phospholipase activities of germinated seeds of papya at different root lengths

Root length (mm)	Lipase			Phospholipase		
	Absorption at 440 nm	Conc of fatty acids (m equiv/g/h)	Activity (µU)	Absorption at 440 nm	Conc of fatty acids (m equiv/g/h)	Activity (µU)
5	0.419	655	5.24	0.444	796	6.37
10	0.422	673	5.38	0.431	724	5.79
15	0.427	700	5.60	0.395	522	4.18
20	0.438	763	6.10	0.386	475	3.80
25	0.444	796	6.37	0.373	401	3.21
30	0.453	848	6.78	0.359	325	2.80

proportional to the root length of germinated seeds. The maximum activity of phospholipase was 6.37 µU at the root length of 5 mm. Similar patterns were observed by other workers (Ahmad *et al.,* 1993; Aman and Akhtar, 1991) who worked on C*arum capticum* and *Zea mays,* respectively.

Conclusion

Lipase and phospholipase of mature and germinated seeds of *Carica papaya* exhibited maximum activities at pH 7 and 40 °C in aqueous medium. In the case of organic solvents, *n*-heptane showed the maximum activities for both the enzymes at pH 7 and 40 °C. The lipase activity was maximum at the maximum root length, but phospholipase activity was minimum at the maximum root length. It is concluded that multiple factors are involved for the lipase and phospholipase activities of mature and germinated seeds. The study provides useful information for further work on an industrial scale and on the resolution of the technical processing problems of papaya, and perhaps of other seed crops.

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Characterisation of Amidohydrolytic Activity of *Bacillus megaterium* in Submerged Fermentation

Quratulain Syed[a]*, Nasira Bashir[b] and Mohammad Akram Kashmiri[b]

[a]PCSIR Laboratories Complex, Lahore-54600, Pakistan

[b]Government College University, Lahore, Pakistan

(received January 7, 2004; revised August 5, 2004; accepted January 28, 2005)

Abstract. Cultural conditions for the production of penicillin amidohydrolase by *Bacillus megaterium* 5B were investigated in shake flasks. The extra-cellular amidohydrolytic activity of the strain after 24 h of incubation was 37 u/ml. The enzyme production was found to be affected by different carbon sources at different concentrations in the fermentation medium. The most suitable carbon source was sucrose at the concentration of 0.3% (w/v). The enzyme activity reached the maximum level (70 u/ml) with a cell mass of 3.1 g/l in 25 ml of the fermentation medium contained in 250 ml flask at pH 7 after 24 h of incubation.

Keywords: penicillin amidohydrolase, *Bacillus megaterium*, submerged fermentation

Introduction

Penicillin amidohydrolase is the enzyme that cleaves the acyl side chain of penicillin. This enzyme is used widely in pharmaceutical industries for the production of 6-aminopenicillanic acid (6-APA), which is processed further during the manufacturing of various clinically important semi-synthetic penicillins (Bruggink *et al.,* 1998). Penicillin amidohydrolase catalyses the hydrolysis of penicillin to 6-APA, which is widely distributed among bacteria, fungi and actinomycetes (Bernard *et al.,* 2002; Babu and Panda, 1991). Bacteria, like *Escherichia coli* and *Bacillus megaterium,* are known to produce penicillin amidohydrolase. The penicillin amidohydrolase activity of *B. megaterium* in the fermentation broth has been reported by Son *et al.* (1982) and Fumian *et al.* (1996) in the range of 11.0 and 45.0 iu/ml, respectively. Most of the reported work has been on the intracellular penicillin amidohydrolase activity of *E. coli,* despite the fact that it involves expensive cell disruption for the enzyme recovery (Isebel *et al.,* 1994; Sudhakaran *et al.,* 1991). Therefore, the present work deals with the optimization of cultural conditions in shake flasks for the production of extra-cellular penicillin amidohydrolase by the selected strain of *B. megaterium* 5B.

Materials and Methods

Microorganism. The strain of *B. megaterium* 5B was provided by the Pakistan Type Culture Collection (PTCC), Biotechnology and Food Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan. The cultures were maintained on nutrient agar slants at 4 °C and revived after every 2 weeks.

Fermentation technique. The 20 h old vegetative inoculum was developed in 250 ml flasks containing 25 ml of nutrient medium (g/l): yeast extract, 2.0; peptone, 5.0; sodium chloride 5.0 (Oxoid). Fermentation was carried out at 37 °C for 24 h in shake flasks. The fermentation medium consisted of (g/1): glucose, 2.0; polypeptone, 5; yeast extract, 5; K_2HPO_4,1; $MgSO_4$. $7H_2O$, 0.2; and benzyl penicillin, 0.1 at pH 7.0 (Sunaga *et al.,* 1976). The inoculum was transferred aseptically into 25 ml fermentation medium in 250 ml shake flasks at the rate of 3% v/v. After optimization of carbon source, glucose was replaced by sucrose at the rate of 0.3% (w/v) in the fermentation medium for further studies.

Assay of penicillin amidohydrolase. The penicillin amidohydrolase activity was determined by measuring 6-APA liberated from benzyl penicillin according to the ninhydrin method (Nam and Ryu, 1979). One unit of penicillin amidohydrolase activity was defined as the enzyme that formed 1 µmole of 6-APA per min under the procedure conditions.

Determination of cell mass. Cell mass was obtained from the culture broth by centrifugation at 10,000 rpm and dried at 105 °C to constant mass (Kim *et al.,* 1981).

Standard solutions. ***Phosphate buffer (0.5 mol/l, pH 7.0).*** Phosphate buffer was prepared by mixing 24.3 g potassium dihydrogen phosphate and 56.0 g di-potassium hydrogen phosphate in 1 litre of distilled water. Lower or higher pH values were obtained by adding hydrochloric acid (5 mol/l) or sodium hydroxide (5 mol/l).

*Author for correspondence; E-mail: naila_malik70@yahoo.com

Citrate buffer. Citrate buffer was prepared by dissolving 1 g of citric acid in 50 ml of sodium hydroxide (0.8 mol/l).

Ninhydrin solution. 0.5 gram of ninhydrin was dissolved in 100 ml of acetone.

Results and Discussion

Effect of carbon source on the production of enzyme. The composition of culture medium is known to influence the propagation of bacterial cultures and synthesis of enzymes or metabolites. Carbon sources and other growth factors are reported to particularly influence the fermentation pattern (Roshan and Ganapathi, 1960). It is known that some strains of B. *megaterium* produce penicillin amidohydrolase in appreciable quantities using different carbohydrate sources (Yang, *et al.*, 2003). Sunaga *et al.* (1976) have also evaluated different carbon sources and found 0.3% glycerol to be the best carbon source for enzyme production by alkalophillic *Bacillus* species. Five different carbon sources, namely, glucose, sucrose, starch, maltose and fructose were incorporated into the medium for maximum production of the enzyme by *B. megaterium* 5B. All the carbon sources were supplemented at the rate of 0.1%, 0.2% and 0.3%. Cell growth and enzyme activity was recorded after 24 h of fermentation. The experimental data so obtained are summarized in Table 1. All the sugars tested supported the cell growth. However, the most suitable carbohydrate source for enzyme production was found to be sucrose at the concentration of 0.3% (w/v). Glucose at a concentration of 0.3% also enhanced the enzyme production considerably, though lesser than in in the presence of sucrose. In the subsequent experiments, therefore, 0.3% sucrose was used as the carbon source; pH of the medium was adjusted to 7.0.

Table 1. Effect of carbon sources on cell growth and penicillin amidohydrolase production

Carbon source	Concentration (%, w/v)	Cell growth (g/l)	Activity (u/ml)
Glucose	0.1	1.7	26
	0.2	1.9	37
	0.3	2.2	55
Sucrose	0.1	2.4	52
	0.2	2.9	60
	0.3	3.1	70
Starch	0.1	1.7	36
	0.2	1.9	40
	0.3	2.0	49
Maltose	0.1	0.9	10
	0.2	1.5	17
	0.3	1.8	32
Fructose	0.1	0.9	11
	0.2	1.3	16
	0.3	1.5	21

Effect of pH on enzyme synthesis. The initial pH of the fermentation medium influenced significantly the synthesis and stability of the enzyme, as the extra-cellular release of the enzyme depended on the pH gradient on bacterial cell wall. In order to study this effect, initial pH of the fermentation medium was adjusted to 5, 6, 7 and 8. The maximum enzyme production was attained at pH 7.0 (Fig. 1). It is also evident that in acidic and slightly basic conditions, the synthesis of enzyme by *B. megaterium* was less than at the neutral pH. The decrease in enzyme activity above pH 7 may be due to

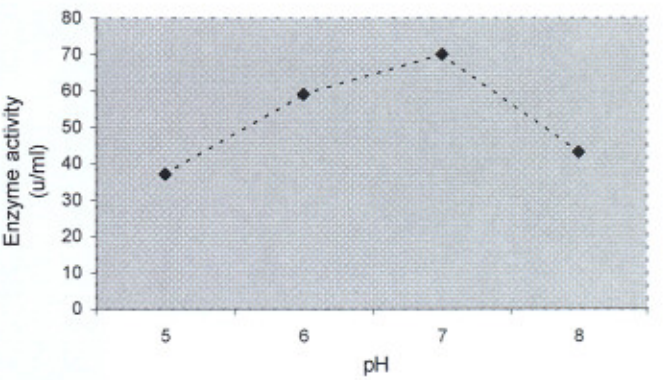

Fig.1. Effect of initial pH of the fermentation medium on the production of penicillin amidohydrolase.

alkaline degradation of penicillin amidohydrolase (Sunaga *et al.*, 1976). Meesschaert *et al.* (1991) have reported pH 8.0 to 8.5 to be optimum for enzyme production by some strains, whereas Nam and Ryu (1979) have reported pH 7.0 to 7.5 as the optimum for enzyme production.

Effect of aeration during fermentation. The supply of oxygen in aerobic fermentation is of importance since the supply of oxygen to the culture should be greater than the rate of consumption by the growing culture. The supply of oxygen in shake flask studies was varied by changing the volume of the culture medium (5-30 ml) in 250 ml conical flasks (Fig. 2). It was noted that increase in the culture medium volume resulted in increase in the enzyme activity. Similar observations were reported by Vojtisek and Slezak (1975) and Qadeer *et al.* (1992). Nam and Ryu (1979) also reported that an increase in the culture medium volume in shake flasks (low aeration) contributed to an increase in enzyme production. This increase was, however, limited only upto 100 ml in 500 ml flasks, while volume increase beyond that decreased aeration rate, further resulting in lower enzyme production. This result is consistent

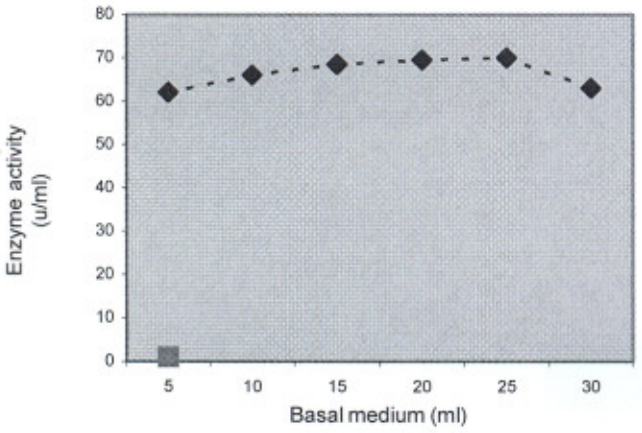

Fig.2. Effect of aeration on penicillin amidohydrolase production by *Bacillus megaterium* in shake flasks.

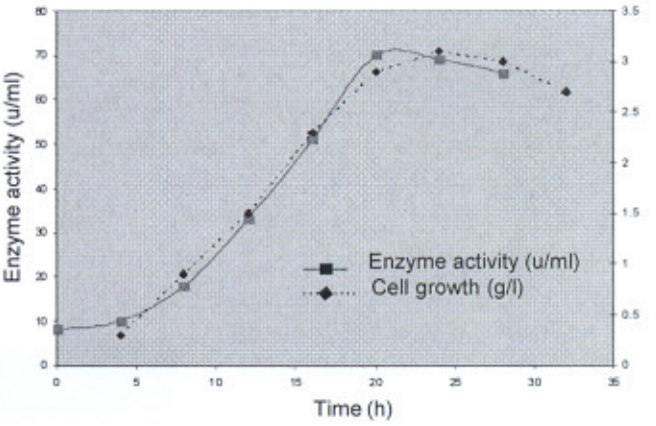

Fig.3. Time course of penicillin amidohydrolase activity and cell growth of *Bacillus megaterium* in shake flasks.

with the hypothesis of Lockhart and Squires (1963) that although sufficient oxygen must be provided to permit adequate metabolism, aeration above a certain level may induce an oxidation-reduction potential that may be unsuitable for enzyme formation or inhibit the function of essential sulfhydryl groups of the enzyme.

Effect of incubation temperature on the production of enzyme. Table 2 shows the biomass growth of *B. megaterium* 5B cultured at three different temperatures (35, 37 and 40 °C). The

Table 2. Effect of different temperatures on the production of amidohydrolase penicillin by *Bacillus megaterium* 5B

Incubation temperature (°C)	Cell mass (g/l)	Enzyme activity (u/ml)
35	2.7	62
37	3.1	70
40	2.0	59

cell mass after 24 h incubation was 2.7 mg/l at 35 °C. With the increase in temperature, the cell mass increased upto 3.1 g/l, yielding 70 u/ml of enzyme activity at 37 °C. Further increase in temperature resulted in the decrease of cell mass (2.0 g/l) as well as enzyme activity (59 u/ml). Similar temperature dependent behaviour was noted in the case of some strains of *E. coli*, which showed the maximum enzyme activity at 25 and 27 °C (Marancenbaum and Park, 1979).

Time course influence on cell growth and enzyme production in shake flask. Rate of fermentation was studied in 250 ml shake flasks. The enzyme production initiated slowly after the inoculation and increased gradually up to 8 h. A drastic increase was found between 8 and 24 h of incubation, which shows that the production of enzyme was related to cell growth. The cell growth was also monitored during the course of enzyme production. The cell mass at zero hour of incubation was 0.3 mg/ml. It increased exponentially during the course of fermentation and reached the maximum after 24 h of fermentation. Further incubation resulted in decrease in cell growth as well as the enzyme activity (Fig.3). Sunaga *et al.* (1976) reported that maximum enzyme activity occurred after 20 to 24 h of incubation of *B. megaterium* cultures. Similar growth behaviour of *B. megaterium* during the production of penicillin amidohydrolase in 500 ml shake flasks was reported by Son *et al.* (1982).

Conclusion

From the above studies it is concluded that the strain 5B of *B. megaterium* produced maximum activity (70 u/ml) of penicillin amidohydolase in 25 ml of the fermentation medium (pH 7.0) containing 3.1 g/l of sucrose after 24 h of incubation at 37 °C.

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Short Communication

Mechanism of Monocarpic Senescence of *Momordica dioica:* Source-Sink Regulation by Reproductive Organs

Ashis Ghosh
Saraswati Vidyamandir, P.O. Midnapore, Dist. Paschim Medinipur, 721 101, W.B., India

(received February 25, 2003; revised September 13, 2004; accepted November 20, 2004)

Abstract. Average chlorophyll levels of male (♂) plants alongwith defruited plants of *Momordica dioica* were higher than female (♀) plants or monoecious plants. The order of senescence was as follows: ♀ > monoecious > ♂ > defruited. Protein content in the leaves and dry weight of aerial plant parts remained higher in ♂ as compared to defruited, ♀ and monoecious plants. Evidently, the absence of fruit was noted to delay senescence.

Keywords: chlorophyll, defruited plant, monoecious, protein, dry weight, *Momordica dioica*

Most of the Cucurbitaceous plants are annuals and monoecious. In a strict sense, however, not all fruit bearing Cucurbitaceous plants are monoecious annuals showing monocarpic senescence. *Momordica dioica* seems to provide useful evidences for the determination of whole plant senescence in annuals. Though most of these plants are hermaphrodite, yet some are unisexual (dioecious). So, except a few, Cucurbitaceous members are monoecious. Due to peculiar male-female differentiation of *M. dioica,* it was selected for the study of mechanism of monocarpic senescence. *Momordica* has 3 types of plants (♂ , ♀ and ♂♀ plants) developed from the same lot of seeds in the same field under normal conditions. This paper aims at separating the role of male and female flowers in relation to their combined effect (monoecious plants) during correlative senescence of this plant.

Certified seeds of *M. dioica* were procured from Bhubaneswar, Government of Orissa, India. Seeds were surface sterilized in 0.1 % $HgCl_2$ for 1 min and then washed well in running water. *M. dioica* seeds were sown in the field in lines on the ridges (80 cm apart) at the advent of winter (November). Soil was moist lateritic, previously mixed with rotted farmyard manure. Watering was done as and when required. All the experiments were conducted in a net-house to avoid damage from pests. It was noted on seed germination that few plants were bisexual, some produced only male flowers, while maximum number of plants produced fruits. Twenty plants were defruited through excision of fruits. For the determination of senescence and source-sink relationship, chlorophyll and protein levels of leaves as well as dry weight of the aerial plant parts were determined at the plant age of 240 days.

Chlorophyll was extracted from 50 mg randomized samples of leaves with chilled acetone (-4 °C) and the values were determined at 660 nm in a Spectrochem spectrophotometer according to Arnon (1949). After removing chlorophyll, the leave samples were washed 3 times with trichloroacetic acid (18 %) and the residue dissolved in 1 ml of 0.5 M NaOH at 85 °C for 1 h. After removing the tissue debris, protein was determined with Folin-phenol reagent (Lowry *et al.,* 1951). For the determination of dry weight, the aerial plant parts were oven dried at 80 °C for 12 h. Each determination was done on 3 replicates and the entire experiment was repeated at least thrice. All the data were statistically analysed by taking the source of variance as days, replication and error. The critical difference (CD) values were calculated at the significant level P = 0.05 (Panse and Sukhatme, 1967).

The female plants senesced earlier than the male plants. The chlorophyll level (Table 1) of defruited and male plants remained higher than those of female and monoecious plants indicating the senescence pattern as follows: female > monoecious > male > defruited. Maximum deferment of senescence in both defruited and male plants may possibly be due to the absence of fruits, which may be the initiator of the senescence signal. The senescence signal developed in the fruits and migrated downwards for the induction of leaf senescence as reported earlier (Ghosh, 2002; Ghosh and Biswas, 1995;

Table 1. The levels of chlorophyll, protein and dry weight of plants at the plant age of 240 days (just prior to harvest)

Plant category	Chlorophyll (mg/g FW)	Protein (mg/g FW)	Dry weight (g/plant)
Male (♂)	1.04	41.10	32.20
Defruited	1.07	40.80	32.00
Female (♀)	0.67	22.40	30.10
Monoecious	0.84	20.10	31.00
CD at 5 %	0.15	4.25	00.66

CD: critical difference; FW: fresh weight

Biswas and Mandal, 1987; Nooden, 1984; 1980). The level of protein in the leaves of defruited and male plants remained higher than those of female and monoecious plants (Table 1). Greater protein level in the leaves of the defruited and male plants may be due to the lack of fruits. Maximum increase in the dry weight of aerial plant parts was found in male plants, which again indirectly supports the predominant role of fruits for the early onset of senescence in plants.

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Short Communication

Status of Grain Smut *Sphacelotheca sorghi* and Long Smut *Tolyposporium ehrenbergii* of Sorghum in Sindh and Balochistan, Pakistan

A. A. Hakro* and A. Khan
Crop Diseases Research Institute, SARC, PARC, Karachi University Campus,
Karachi-75270, Pakistan

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Abstract. Survey of various parts of Sindh and Balochistan was conducted to determine the presence and distribution of *Sphacelotheca sorghi,* the causal organism of grain smut of sorghum. The percentage of disease in different localities of Sindh was 2% in Dadu, 0.5% in Piyaro Goth, 3% in Mehar, 3% in Nasirabad, 4% in Larkana, 8% in Kamber, 4% in Shahdadkot, 5% in Jacobabad, 5% in Thull, 6% in Kandhkot, 7% in Lakhi, 7.5% in Jehan Khan, 8.3% in Sukkur, 20% in Rohri, 15% in Ghotki, 15% in Mirpurmathelo, 15% in Ubaro, 20% in Daherki, 20.5% in Khairpur (Mir) and 17.1% in Karoondhi, and in localities of Balochistan was 7% in Nasirabad and in 7% Usta Mohammad. The incidence of long smut, *Tolyposporium ehrenbergii,* in Sindh was recorded at 0.2% in Dadu, 0.1% in Piyaro Goth, 0.5% in Mehar, 0.03% in Nasirabad, 0.6% in Larkana, 0.3% in Kamber, 1% in Shahdadkot, 1.2% in Jacobabad, 1.5% in Thull, 1.1% in Kandhkot, 1% in Lakhi, 1.3% in Jehan Khan, 1.5% in Sukkur, 3.5% in Rohri, 5% in Ghotki, 8.1% in Mirpurmathelo, 7.5% in Ubaro, 8.9% in Daherki, 9.1% in Khairpur (Mir) and 8% in Kroondhi, and in Balochistan was1.3% in Nasirabad and 1.4% in Usta Mohammad.

Keywords: grain smut, *Sphacelotheca sorghi*, long smut, *Tolyposporium ehrenbergii*, sorghum

Sorghum (*Sorghum vulgare*) is cultivated for grain, forage and juice in the drier climate regions of Pakistan, on limited acreage because of its low yield and susceptibility to diseases. Davies (1978) reported that sorghum is grown in 55% semi-arid tropical countries of the world. Sorghum crop in Pakistan occupies a major position in Rawalpindi, Sargodha, Multan, Bahawalpur, D.I. Khan, Sukkur, Hyderabad and Sibi Divisions (Hafiz, 1986). Sattar and Hafiz (1952) reported that the grain smut *Sphacelotheca sorghi* and the long smut *Tolyposporium ehrenbrgii* cause considerable loss in the production of sorghum in all sorghum growing areas of Pakistan. Reed (1923) recorded grain smut in USA and other sorghum growing countries. Hafiz (1958) reported that the long smut disease of sorghum caused appreciable loss in the districts D.G. Khan, Muzaffargarh, Sukkur, Jacobabad, Larkana, Nawabshah and Khairpur. Kamal and Mughal (1968) and Hakro *et al.* (1990) reported that grain smut is more prevalent in all sorghum growing areas of Sindh. In Sindh and Balochistan provinces of Pakistan the old/susceptible varieties are still being grown for grain and fodder. An extensive survey of sorghum crop was thus carried out to determine the latest position of the prevalence and distribution of the sorghum smut diseases (grain smut and long smut) in Sindh and Balochistan as no recent studies had been conducted in these two provinces.

*Author for correspondence

An extensive survey was carried out during 2002 to determine the incidence and distribution of sorghum smut diseases in Sindh and Balochistan. The prevalence of grain smut and long smut, in the farmers' fields in the two provinces, was recorded for their incidence and intensity on the head count basis according to the procedure of Rodenhiser and Holton (1945). Diseased and healthy heads were counted at three random localities of each field in one square metre area. It was observed that the grain smut and long smut were prevalent in all sorghum growing areas of Sindh and Balochistan. Sorghum grain smut and long smut data and the infection level at individual sites are presented in Table 1.

Out of the 22 sites of sorghum crop fields visited, the lowest grain smut (0.5%) was recorded at Piyaro Goth and the highest (20.5%) was recorded at Khairpur. In the remaining 20 sites, i.e., Dadu, Mehar, Nasirabad, Larkana, Kamber, Shahdadkot, Jacobabad, Thall, Kandhkot, Lakhi, Jehan Khan, Sukkur, Rohri, Ghotki, Mirpurmathelo, Ubaro, Daherki, and Karoondhi in Sindh, and Nasirabad and Usta Mohammad in Balochistan, grain smut was recorded from 2 to 20%. The lowest (0.1%) and highest (8.9%) infections of long smut were recorded at Piyaro Goth and Daherki, respectively. The long smut infection ranged from 0.2 to 8.1% at Dadu, Mehar, Nasirabad, Larkana, Kamber, Shahdadkot, Jacobabad, Thull, Kandhkot, Lakhi, Jehan Khan, Sukkur, Rohri, Mirpurmathelo, Ubaro, Khairpur and Karoondhi in Sindh, and Nasirabad and Usta Mohammad in Balochistan. These results are comparable with the earlier reports (Hakro *et al.,* 1990; Hafiz, 1986;

Table 1. Grain smut and long smut of sorghum in Sindh and Balochistan

Locality	District (Province)	Grain smut (%)	Long smut (%)
Dadu	Dadu (Sindh)	2.0	0.2
Piyaro Goth	Dadu (Sindh)	0.5	0.1
Mehar	Dadu (Sindh)	3.0	0.5
Nasirabad	Larkana (Sindh)	3.0	0.3
Larkana	Larkana (Sindh)	4.0	0.6
Kamber	Larkana (Sindh)	8.0	0.3
Shahdadkot	Larkana (Sindh)	4.0	1.0
Jacobabad	Jacababad (Sindh)	5.0	1.2
Thull	Jacobabad (Sindh)	5.0	1.5
Kandhkot	Shikarpur (Sindh)	6.0	1.1
Lakhi	Shikarpur (Sindh)	7.0	1.0
Jehan Khan	Sukkur (Sindh)	7.5	1.3
Sukkur	Sukkur (Sindh)	8.3	1.5
Rohri	Sukkur (Sindh)	20.0	3.5
Ghotki	Ghotki (Sindh)	15.0	5.0
Mirpurmathelo	Ghotki (Sindh)	15.0	8.1
Ubaro	Ghotki (Sindh)	15.0	7.5
Daherki	Ghotki (Sindh)	20.0	8.9
Khairpur	Khairpur (Sindh)	20.5	7.1
Karoondhi	Khairpur (Sindh)	17.1	8.0
Nasirabad	Nasirabad (Balochistan)	7.0	1.3
Usta Mohammad	Nasirabad (Balochistan)	7.0	1.4

Kamal and Mughal, 1968). From these observations it is concluded that there is a dire need to provide high yielding and disease resistant varieties to the farmers of Sindh and Balochistan for enhancing the yield of sorghum as proposed already (Hakro *et al.,* 1990; Hafiz, 1986).

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The Dyeability Potential of Cellulosic Fibres Using African Yellow Wood *(Enantia chlorantha)*

A. O. Adetuyi*, A. V. Popoola, L. Lajide and M. O. Oladimeji
Department of Industrial Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria

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Abstract. The dye from African yellow tree, *Enantia chlorantha*, extracted by solvent extraction using acetone at a solute-solvent ratio of 1:25 was studied for its dyeability potential on cellulosic fibres. A golden yellow dye having a melting point ca 146-149 °C with 6.2% recovery was obtained. The dye was soluble in hydroxyl organic solvents. The cellulosic fibre has a greater dye uptake (26.0-23.2 mg/g) when dyed at a temperature of 80 °C than at 60 °C (22.0-21.6 mg/g). Its optimum dye-uptake, at both the temperatures, was achieved 90 min after the commencement of dyeing. However, the dyeability potential of the dye on unmordanted cellulosic fibres showed less substantivity as revealed by its low mean fastness ratings of 1.5 and 1.0 to washing and light, respectively. The tensile properties of the dyed cellulosic fibres, nevertheless, were greatly enhanced.

Keywords*:* Enantia dye, dye-uptake, *Enantia chlorantha,* African yellow wood

Introduction

African yellow wood, *Enantia chlorantha* (locally called, oso pupa/yaru), family Annonaceae, recognised by its bright yellow slash and conspicuous black fruits, is a fairly sized forest tree usually grown in dense shade (Dalziel, 1955). It is found in Southern Nigeria, West Cameroun, Gabon, Angola and Zaire. The wood is yellow, uniform throughout, fairly fine-grained, splitting easily and rather soft, turning brown after long exposure. It is used locally for house building and furniture work. Native caps are made from the fibrous bark in South Nigeria (Dalziel, 1955). The bark is intensely bitter to taste and has a lot of medicinal uses (Burkill, 1985). The bitterness is attributed to the presence of berberine, known to be haemostatic and febrifuge, which is present in all the species of *Enantia* (Dalziel, 1955). In comparison, *Morinda lucida* (locally called, awopa/oruwo), family Rubiaceae, having a similar texture, and outer bark blackish with deeper inner yellow, is also used as the source of a yellow dye (Adetuyi *et al.,* 2002).

An attempt has been made in this study to examine the dyeability potential of Enantia dye, closely related to Morinda yellow dye, on cellulosic fibres and its fastness properties to light, washing, perspiration and ironing.

Materials and Methods

Sample collection and treatment. The bark of the African yellow wood *E. chlorantha* was purchased from Alade Market in Idanre, Ondo State, Nigeria. The outer part of the wood bark (dark grey) was removed using pen-knife leaving only the inner yellow part which was cut into smaller pieces and oven dried at 110 °C for 6 h to reduce its moisture content considerably before extraction. The sample was finely ground and stored.

Extraction. Pulverized sample (10 g) was extracted with acetone in a Soxhlet extractor for 2 h at a solute-solvent ratio of 1:25. The solvent was removed from the extract by evaporatien (atmospheric). The colourant was transferred to an evaporating dish to which 20 ml petroleum ether (60-80 °C) was added and heated to dryness to remove the sticky nature of the extract. It was dried in an oven at 60 °C for 30 min, cooled, weighed and stored.

Determination of physicochemical properties. The following physicochemical properties were determined for the colourant.

Melting point. The melting point of the dye extract was determined using the capillary tube electrothermal method (Furniss *et al.,* 1978).

Solubility test. The solubility of the dye was tested in the following selected solvents: acetone, groundnut oil, hydrochloric acid (5%), methanol, petroleum ether, palm kernel oil (PKO), sodium hydroxide, sulphuric acid (conc), sodium hydrogen carbonate and water. The test was carried out by adding 10 mg of the colourant to each of the above solvents in a test tube, and in case the colourant was not soluble in the cold, gentle heat was applied by placing the test tube inside a heated water bath.

* Author for correspondence

Lassaigne test. The colourant was tested for the presence of nitrogen, sulphur and halogens by Lassaigne test (Furniss *et al.,* 1978).

Phenolic hydroxyl group test. This was carried out using ferric chloride-ethanol solution (1:10 w/v). A developed TLC plate was sprayed with the test solution. The presence of blue-black colour on the plate thereafter indicated the presence of phenolic hydroxyl group in the sample.

Dyeing of the cellulosic fibres. Scoured and bleached white cellulosic fibres (yarn and fabric) were used for dyeing without prior application of a mordant. The dyeing was done at two different depths, 2% and 5% for cotton yarn and fabric, respectively, carried out in an infinite dye bath in which the concentration of the colourant was maintained constant. The recipes followed were proportional to the weight of the goods (White, 1980; Giles, 1974). Dyeing was done in a single-bath dyeing machine MBMK II.

2 % Dyeing of cellulosic yarn for dye-uptake measurement. Two dyebaths, each containing 4 g/l of the dye extract, 30% sodium chloride and 3% sodium hydroxide in a liquid-to-goods ratio of 100:1, and a blank bath of the same liquid-to-goods ratio without the dye were prepared for 2% dyeing of 500 mg cotton yarn. One dyebath served as a standard solution from which various aliquots were taken for serial dilutions. Their optical density was measured on Biochron-4060 spectrophotometer at λ_{max} 390 nm of the pure dyestuff solution. The other dyebath and blank were transferred into the dye tubes mounted inside the dyeing machine and set at the dyeing temperature of 60 °C or 80 °C. On attainment of the temperature, the pre-soaked cellulosic yarn at the same temperature was introduced into the dye tube to commence dyeing . Sampling of the spent dye-liquor, about 2 ml, was done at intervals of 30 min for a period of 3 h. Prior to sampling, dyed yarn was removed from the dye-liquor. Equivalent aliquot from the blank liquor was introduced into the dye liquor to replace the dye assistants that had been removed by the dyed yarn. The dyed yarn was returned and dyeing continued till the next sampling period. The sampled solutions were diluted and their optical density measured. The dye-uptakes were determined using equations 1 and 2 (Popoola, 1983):

$$\frac{1000.C_{Df}}{W_{Df}} \qquad (1)$$

where:

W_{Df} : weight of dyed cellulosic yarn (0.5 g)

C_{Df} : the concentration of dye in yarn (% w/v), which is as given in equation (2) below

$$C_{Df} = 0.02 - C_{Ds} \qquad (2)$$

where:

0.02 (% w/v): a constant (initial concentration of dye-liquor)

C_{Ds}: the concentration of spent dye-liquor at various time intervals of dyeing, deduced from the calibration curve

After the dyeing operation, the yarn was removed from the dyebath and properly washed in a mild soap solution before being rinsed in cold distilled water and dried.

5 % Dyeing of cellulosic fabric for fastness properties test. The dyebath for 5% dyeing of the dye extract on cellulosic fabric (6 g) in the dye-liquor ratio of 30:1 contained 3 ml of the dye solution (1 g/100 ml), 30% sodium chloride and 5% sodium hydroxide. The dyeing operation was allowed to proceed for 3 h at 60 °C after which the fabric was removed from the dye bath and treated as previously described for dyed yarn. It was then fixed with hot iron.

Assessment of fastness properties. Four fastness properties, namly, washing, perspiration, hot pressing (heat treatment) and light were determined on the dyed fabric according to the International Standards Organisation (ISO) procedures (SDC, 1992). For each test, 10 specimens of the dyed article were tested and the mean value of the results obtained was taken as the fastness rating for the test.

Tensile properties of the dyed cellulosic yarn. The load-elongation at break of the dyed cellulosic yarn of various lengths was done at the constant rate of elongation of 500 per min and 500 N per tension on Instron tensile tester T5000 at Yaba College of Technology, Lagos, Nigeria.

Results and Discussion

Physicochemical properties. The results of the physicochemical properties carried out on the Enantia colourant and the colour of the dyed cellulosic fibres are summarized in Table 1. It was of low yield (6.2%) when compared with Morinda yellow dye of 16.5% yield (Adetuyi *et al.,* 2002). The solubility test results shown in Table 2 indicate that the Enantia dye was less soluble in the tested polar organic solvents than the Morinda dye. However, it was found soluble in concentrated sulphuric acid as is Morinda, which was an indication of the presence of an acidic group or electron withdrawing groups such as hydroxyl, carbonyl or nitro-groups (Mohring and Nechers, 1979).

Dyeing and dye-uptake. All the cellulosic dyed fibres, in both yarn and fabric, were uniformly and evenly coloured yellow (Table 1). Fig. 1and 2 show the calibration and the dye uptake curves, respectively, of the dyed cellulosic yarn at 60 °C and 80 °C. The dye-uptake of the yarn in the Enantia dye extract

Table 1. The physicochemical parameters obtained for colourant from African yellow wood (*Enantia chlorantha*)

Tests performed	Observations
Appearance of crystals	golden yellow
Yield	6.2%
Melting point	146-149 °C
Lassaigne's sodium fusion test	only nitrogen present
Phenolic hydroxyl group test	negative
Shade of dyed articles:	
cotton yarn	light yellow
cotton fabric	yellow

Table 2. Solubility of Enantia dye in selected solvents

Solvents	Observation
Acetone	+
Distilled water	+
Groundnut oil	-
Hydrochloric acid (5%)	-
Methanol	+
Palm kernel oil (PKO)	-
Petroleum ether	-
Sodium hydrogen carbonate (5%)	-
Sodium hydroxide (5%)	+
Sulpuric acid (conc.)	+

+ soluble; - insoluble

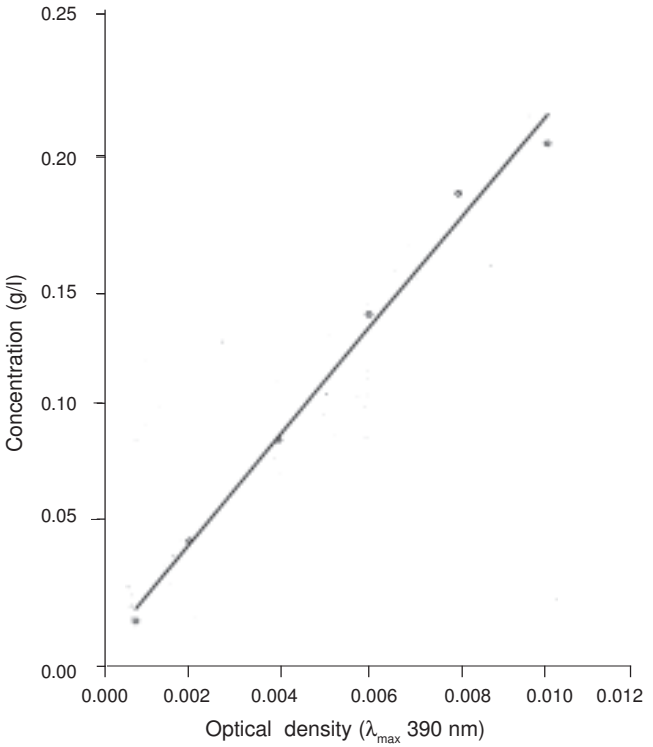

Fig. 1. Optical density versus concentration (g/l) calibration curve for Enantia dye in alkaline medium.

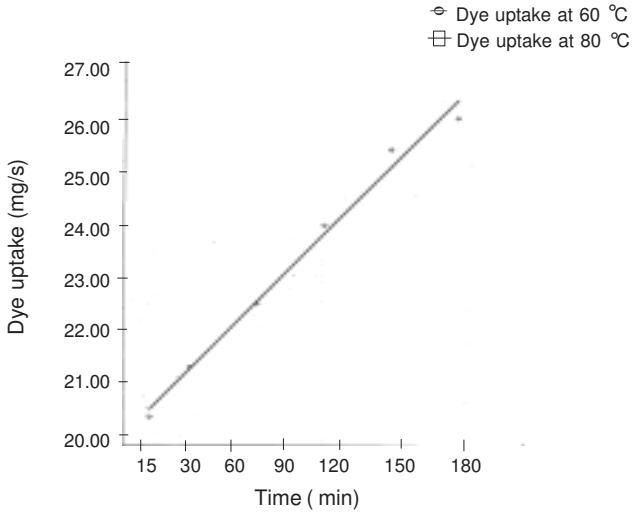

Fig. 2. Dye uptake of cotton yarn in Enantia dye at various time intervals (min) at 60 °C and 80 °C.

increased porgressively with time at the two dyeing temperatures. After 90 min of dyeing, a sudden fall in the dye uptake was noticed in the cellulosic yarn dyed at 80 °C, while that at 60 °C was fairly constant (Fig. 2). However, the dye uptake of the dyed yarn at 80 °C was higher than that at 60 °C. This observation was contrary to the expected behaviour of a direct colourant on cellulosic fibres (Giles, 1974). The deviation may be attributed to the purity of the Enantia colourant and the fibre morphology. It has been reported that changes in fibre morphology, as a result of high dyeing temperature with time, affects the dye-uptake of fibre (Burdett, 1975), as heat treatment of the fibre or yarn affects the amorphous content of the fibre, which is the region accessible to dyestuffs. The fibre at 80 °C presented a more accessible region for dye absorption than at 60 °C and these regions were more for the first 90 min of dyeing commencement. Thereafter, the colourant bleeds out of these sites. Thus, increasing dyeing time at this particular temperature probably affects the mobility of the chain segments of the fibre molecules, thereby increasing its crystallinity and decreased its dye-uptake.

Fastness properties of the dyed cellulosic fabric. The results obtained for the fastness ratings of the dyed fabric are given in Table 3. The dyed fabrics have poor mean fastness ratings of 1.0 and 1.6 on a Grey scale of 8 and 5 to light and washing, respectively. This shows that the dye structure itself was not stable (fugitive) to high energy radiation, such as ultraviolet. It also suggested a less extensive conjugation or a straight chain for the dye molecule (Kemp, 1991). Its low fastness rating to washing suggested that the dye was less substantive to cotton fabric and the substantivity can be improved with

Table 3. Fastness ratings of *Enantia chlorantha* dyed cellulosic fabric

Specimen no.	Washing	Perspiration	Hot pressing	Light
1	2	3	4	1
2	1	3+	4	1
3	1+	3	4	1+
4	2	3	4	1
5	1	3+	4+	1+
6	1+	3	4	1
7	2+	3	4+	1
8	1	2	4	1
9	2	3	4	1
10	1	3	4	1
Mean fastness rating	1.6	3.0	4.1	1.0

Grey scale rating: 1-2 low fastness; 3-4 moderate fastness ; 5-6 high fastness

the use of a suitable mordant, either natural or synthetic. However, the dyed fabric had good fastness ratings of 3.0 and 4.0 based on a scale of 5 in respect of perspiration and hot pressing, respectively. This is an indication of the stability of the dye factor to alkaline/acidic liquid treatment (perspiration solution) and dry heat that the material may be exposed to during usage.

Tensile properties of the dyed cellulosic yarn. The tensile properties of the dyed cellulosic yarn at the dyeing temperatures of 60 ˚C and 80 ˚C are presented in a load-elongation curve shown in Fig. 3. The dyed yarn was less extensive than the undyed yarn (control). The dyed cellulosic yarn at 80 ˚C

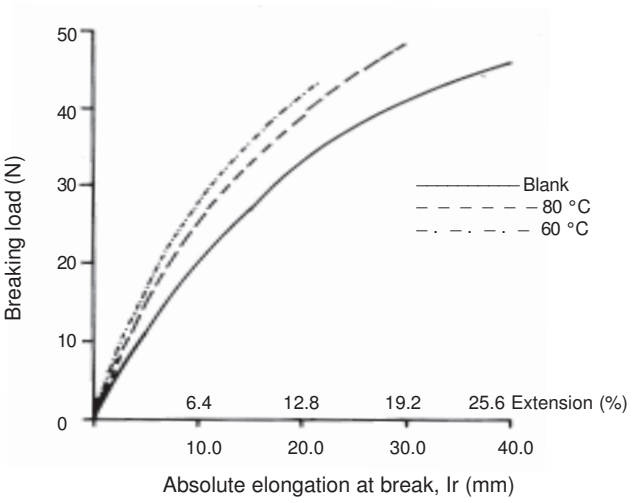

Fig. 3. Load-elongation curves for 15-cm Enantia dyed cotton yarn at 60 ˚C and 80 ˚C.

was stronger than that dyed at 60 ˚C, corroborating maximum dye-uptake at this temperature (80 ˚C), which contributed to the yarn strength and the subsequent fabric to be produced from them.

In conclusion, based on the performance of the colourant in this study, and with a little modification to the dyeing processes to increase its substantivity as earlier suggested, the Enantia yellow colourant may be best used for cottage cellulosic textile industry (because of low yield) at 80 ˚C for a dyeing period of 90 min.

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The Effect of Local Materials (Fillers) on the Crosslink Density, Hardness, Resilience and Hysteresis of Natural Rubber

B. F. Adeosun[a] and O. Olaofe[b]*

[a] Science Technology Department, Federal Polytechnic, PMB 5351, Ado-Ekiti, Nigeria

[b] Chemistry Department, University of Ado-Ekiti, PMB 6353, Ado-Ekiti, Nigeria

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Abstract. This work reports the influence of local clay, charcoal, silica-sand, limestone and carbon black on the crosslink density, hardness, resilience and hysteresis of the natural rubber compound. The results revealed that all the fillers enhanced the crosslink density and hardness of the gum stock. Charcoal showed higher values of crosslink density, hardness and hysteresis than the other local fillers. At relatively low loading, local fillers showed appreciably higher resilience and slightly lower hysteresis than carbon black charcoal, being the least resilient and most hysteric. The present work suggests that the denser is the crosslink of the composite, the harder, less resilient and the more hysteric the composite becomes.

Keywords: crosslink density, hardness, resilience, hysteresis, carbon black, natural rubber composite

Introduction

Reports show that fillers are used in rubber vulcanization to achieve two purposes, namely: (i) to reinforce the rubber in order to improve the mechanical properties of the resultant composite, and (ii) "beef up" the volume of rubber compounds (Billmeyer, 1984; Maurice, 1981; Studebaker and Beatty, 1978; Garvey, 1970). Reinforcing fillers improve the quality of the final vulcanizate, while inert fillers add little or nothing to the reinforcing characteristics of the final composite. However, these inert fillers are added just to reduce cost and sometimes better processing properties are achieved.

Properties of impregnated natural rubber composites have been investigated extensively (Adu, 1991; Bristow, 1986; Elliot, 1986; Bernard *et al.,* 1985). Our earlier work reported the effect of some agricultural wastes on the mechanical and rheological properties of natural rubber (Adeosun, 2000; Adu *et al.,* 2000). Recently, the characterization of natural rubber impregnated with some locally available materials such as clay, limestone, charcoal and silica-sand was investigated and reported (Adeosun and Olaofe, 2003; 2002). The aim was to examine the reinforcing properties of these local materials when incorporated into natural rubber with a view to finding industrial applications for them.

The thermal and electrical conductivities of natural rubber impregnated separately with local clay, limestone, charcoal and silica-sand have been reported (Adeosun and Olaofe, 2002). In the present work, the crosslink density, hardness, resilience and hysteresis of natural rubber, filled with these local materials, have been determined as a function of filler loading.

*Author for correspondence; E-mail: ojolo48@yahoo.com

Materials and Methods

Source of materials. Twenty-two conventional accelerator/sulphur compounds were tested as indicated in Table 1and 2. The natural rubber used was a Nigerian standard rubber grade 10 (NSR10) produced at Michelin plantation, Araromi-Obu, Ondo State, Nigeria. Clay was collected from Afao-Ekiti, limestone from Arimogija, silica-sand from Igbokoda, and wood charcoal was purchased from Erekesan Market in Akure, all in Nigeria.

Elemental analysis of materials. Elemental analysis was done using atomic absorption spectrophotometer (AAS) and colourimeter following the experimental procedures of Vogel (1961) and AOAC (1981).

Table 1. Formulation of the composites examined

Composite	Content in parts per hundred rubber (pphr)
Natural rubber	100
Zinc oxide	5
Stearic acid	3
MBT[a]	1
Sulphur	3
Filler	variable[b]

[a]mercaptobenzothiazole; [b]filler loading in parts per hundred rubber (pphr)

Table 2. Formulated composites as a function of filler loading

Composite code	Filler(pphr)	Filler loading
N1	0	00
NCB1	carbon black	28
NC1	clay	12
NC2	"	28
NC3	"	36
NC4	"	48
NC5	"	72
NL1	limestone	12
NL2	"	28
NL3	"	36
NL4	"	48
NL5	"	72
NCH1	charcoal	12
NCH2	"	28
NCH3	"	36
NCH4	"	48
NCH5	"	72
NS1	silica-sand	12
NS2	"	28
NS3	"	36
NS4	"	48
NS5	"	72

N: gum compound without filler (control); NL: limestone filled composite; NCB: carbon black filled composite; NCH: charcoal filled composite; NC: clay filled composite; NS: silica-sand filled composite; pphr: parts per hundred rubber

Mixing. Mixing was done on a two-roll mill at 70 °C and maximum speed of 24 rpm. The mixing cycle per batch varied between 8-10 min. After milling, the composites were cooled immediately in chilled water and covered with cellophane paper (Adeosun and Olaofe, 2003).

Rheometer test. Milled composite was received, cut and weighed (5.0-5.05 g). The sample was inserted into the rheometer which was operated at 185 °C for complete measurement. The rheometer was connected to a plotter, which produced torque-time plots (rheographs). The rheographs showed maximum torque (M_H) and minimum torque (M_L), the difference of which is a measure of the crosslink density of the composite (Adeosun and Olaofe, 2003).

Hardness measurement. For the measurement of hardness, circular shaped test pieces were cured at 152 °C for 15 min, dropped into chilled water and allowed to cool for 48 h. The test piece was then tested for hardness using rubber hardness tester (BSO, 1975).

Resilience and hysteresis test. Resilience cuboids test piece was cured at 142 °C for 35 min using an electric press. The vulcanizate was allowed to mature for 48 h after being dropped into chilled water. The matured sample was then aged at 50 °C for 2 h. The aged sample was fixed into the Wallace resilience test equipment operated at 30 °C (BSO, 1975). The disc was raised to 45 °C and released. The disc hit the sample and rebound. The maximum angle of rebound was noted and resilience calculated by using equation (1) mentioned below:

$$R = \frac{hr}{hd} \times \frac{100}{1} = \frac{1 - \cos\phi}{1 - \cos 45} \times \frac{100}{1} \qquad (1)$$

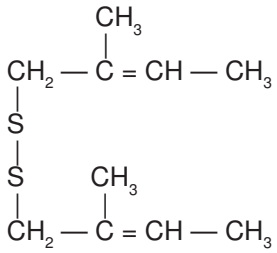
where:
R: resilience
hr: height of rebound
hd: height of release
ϕ: angle of rebound

Hysteresis was calculated as the reciprocal of resilience (i.e., hysteresis = 1/resilience).

Results and Discussion

The unloaded compound has a crosslink density of 7.3 dN-m which increases to 13.35 dN-m on the addition of 12 pphr (parts per hundred rubber) of conventional carbon black filler. The increase in crosslink density on the addition of clay, limestone, silica-sand and charcoal within the concentration range of 12 pphr and 72 pphr was observed to fall, respectively, within 7.99 and 9.65 for clay, 7.64 and 9.64 for limestone, 8.50 and 12.65 for silica-sand, and 11.16 and 19.36 for charcoal. These results suggest that charcoal had better ability to improve crosslinkage than clay, limestone, silica-sand, and even the conventional carbon black.

Noting that even if the entire residue of clay, limestone and silica-sand was carbon (Table 3), charcoal had the highest percentage of carbon. Crosslink in natural rubber vulcanization is of the sort C-S-S-C, as in the structure below:



It is therefore reasonable to infer that high carbon content in the matrix would lead to increased linkage in the presence of

Table 3. Chemical composition of the local materials

Filler	SiO_2	SO_2	Al_2O_3	K_2O	Na_2O	CaO	MgO	Fe_2O_3	CO_2	Residue
Clay	48.83	23.81	10.09	5.91	0.59	1.34	1.02	4.91	nd	3.50
Limestone	4.26	1.49	1.16	6.21	0.70	52.92	10.36	4.02	nd	18.88
Silica-sand	78.13	2.80	1.06	0.24	0.18	0.34	0.02	2.09	nd	15.14
Charcoal	22.44	2.40	0.17	0.34	0.29	0.22	0.09	0.07	51.72	22.29

nd: not determined

sulphur. This explains why charcoal with the highest carbon content shows denser crosslinkage than the other local fillers (clay, limestone and silica-sand). A critical look at Table 3 revealed that charcoal contained sulphur in addition to carbon which could be made available for C-S-S-C linkage. This could be an explanation for charcoal's superior ability over carbon black (which is close to 100% carbon content) to form C-S-S-C linkage. For all the fillers examined, crosslink density increased with increasing filler content (Fig. 1).

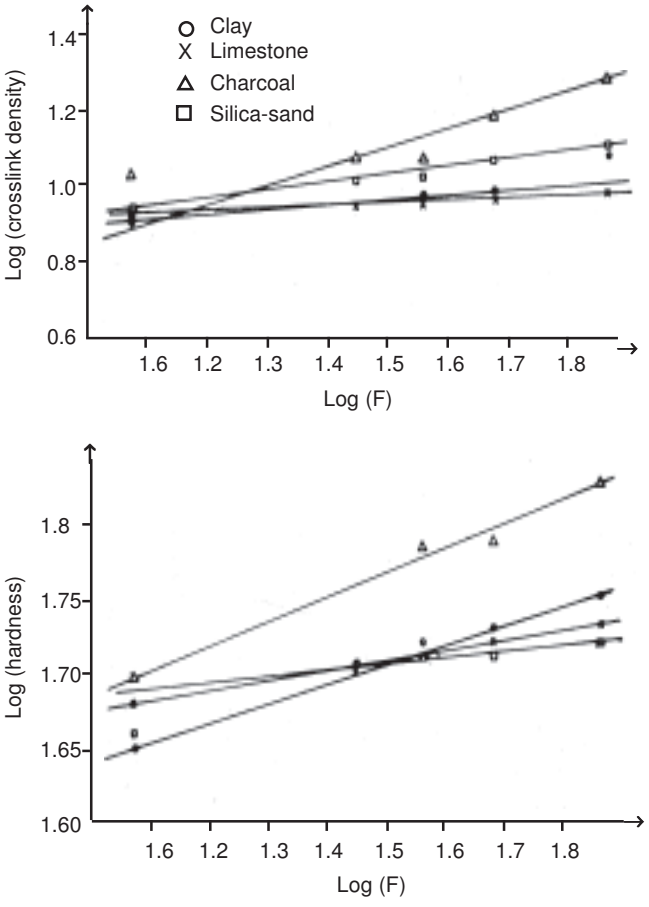


Fig. 1. Log crosslink density and log hardness versus log filler (F) content in the composites.

The unloaded compound had a hardness value of 43.5 IHRD. This value increased to 59.6 IHRD on the addition of 12 pphr of conventional carbon black filler. On the addition of clay, limestone, silica-sand and charcoal, within the concentration range of 12 pphr and 72 pphr, hardness of composites fell within 44.8 and 55.1 for clay, 48.2 and 55.1 for limestone, 46.0 and 52.9 for silica-sand and 50.3 and 66.9 for charcoal. These results indicate that charcoal composites were as hard as the carbon black composites. Also, charcoal composites were harder than the composites of clay, limestone and silica-sand. This trend agrees with the trend observed for crosslink density and the reasons adduced for crosslink density could also be advanced for hardness. The denser the crosslink of the composite, the harder is the composite. Hardness increased with increasing filler loading (Fig. 1). This observed increase in hardness with increasing filler loading agrees with the observation of Parkinson (1946). A similar trend was reported by Adu (1991) for spent paper, wood dust, animal charcoal and coconut fibre.

The unloaded compound showed a resilience of 95.7%, while the resilience of the carbon black composite was observed to be 91.1%. Composites of clay, limestone, silica-sand and charcoal showed maximum resilience values of 94.7%, 95.6%, 94.7% and 93.3%, respectively. These results suggest that clay, limestone and silica-sand composites showed comparable resilience to that of the unloaded compound, which was slightly higher than that of charcoal composite. The carbon black composite was the least resilient out of all the fillers examined. This is the reverse of the trend shown by crosslink density and hardness. The trend of resilience decreasing with increasing filler loading has been reported for carbon black, which agrees with the observed trend in this work (Fig. 2) and with the observations reported for spent paper, wood dust, animal charcoal and coconut fibre (Adu, 1991). This trend is the reverse of the trend observed for crosslink density and hardness. It appears that the denser the crosslink of a composite, the harder and the less resilient the composite becomes.

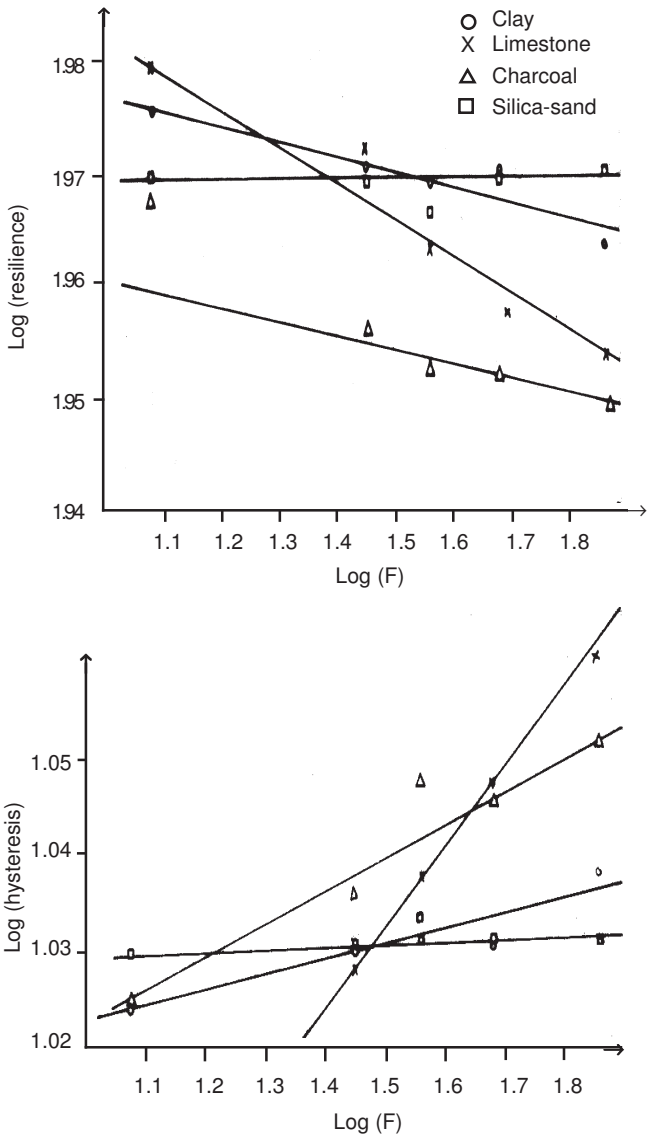


Fig. 2. Log resilience and log hysteresis versus log filler (F) content in the composites.

Hysteresis is a measure of the heat build-up by a composite during usage. All the fillers enhance the value of hysteresis slightly. Hysteresis increased with increasing filler content in the mix (Fig. 2). At relatively low loading (12 pphr), charcoal, clay and limestone showed hysteresis comparable to the gum stock slightly lower than for carbon black. The trend observed for hysteresis agrees with the trend reported for carbon black, wood dust, spent paper, coconut fibre and animal charcoal (Adu *et al*., 2000; Adu, 1991).

Conclusion

Clay, limestone, silica-sand, charcoal and carbon black enhance the crosslink density of the gum stock. Charcoal shows higher crosslink density than clay, limestone and silica-sand. Hardness was also enhanced by all the fillers examined. The charcoal loaded composites further showed higher hardness values as compared to clay, limestone and silica-sand.

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Preparation and Characterisation of Alkyd Resins Using Crude and Refined Rubber Seed Oil

E. U. Ikhuoria* and F. E. Okieimen
Industrial Agriculture Products Research Laboratory, Department of Chemistry, University of Benin, Benin City, Nigeria

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Abstract. Six different alkyds were formulated with crude rubber seed oil 45% **(1)**, 50% **(II)** and 55% **(III)**; refined rubber seed oil 45% **(IV)**, 50% **(V)** and 55% **(VI)**; phthalic anhydride and glycerol. All the alkyds were formulated to alkyd constant of about 1.0. The alcoholysis method was used. Refining enhanced the quality of rubber seed oil in alkyd resin manufacture. The properties of the finished alkyd resins such as viscosity, number average molecular weights, drying schedule, chemical resistance and film hardness were determined. The intrinsic viscosity (η) was observed to be proportional to the number average molecular weight of the two sets of alkyd resins. However, samples **I-III** exhibited higher intrinsic viscosity in toluene than samples **IV-VI**. On the contrary, the films of samples **IV-VI** were harder, dried faster, and were more chemically resistant than those of samples **I-III**. The practical implications of these results are discussed.

Keywords: alkyd resins, rubber seed oil, refining, surface coatings, coating binders

Introduction

Generally, surface coatings are composed of binders, solvents and pigments. Binders are film-forming components of the coatings. They are responsible for the performance quality of the coatings such as the rate of drying, gloss, durability of the dry film, and resistance of the dry film to abrasion and chemicals. Alkyds are examples of binders. They constitute over 70% of binders currently used in surface coatings due to their unique qualities like gloss and gloss retention, exterior durability, and compatibility with other film formers for purposes of cost reduction (Bajpai and Seth, 2000). They are essential products of condensation reaction between polyols and polybasic acids modified with fatty acids or triglyceride oils. Glycerol and phthalic anhydride, respectively, are examples of polyols and polybasic acids commonly employed in the preparation of alkyds because of their relative abundance and low cost. They form the main alkyd backbone chain to which fatty acids are attached thereby terminating the growth of the alkyd chain in that direction. Vegetable oils are now most commonly employed in alkyd formation due to their low cost. Drying oils are among the oldest binders used in coating formulations because of their ability to form hard and solid films upon exposure to air (Patel *et al.,* 2000).

The nature of the drying oil used governs the physical and chemical properties such as rate of drying, film hardness, colour and gloss retention, flexibility, and adhesiveness of the alkyd film, thereby making drying oil the most important component of alkyd resins (Wick *et al.,* 1999). Natural forms of the oils seldom fulfil the technical requirements for film properties like resistance to weathering, chemicals, acid, water, alkali and abrasion (OCCAA, 1983). Thus, to enhance their initial quality, several physical and chemical modifications of the oils have been evolved over the years. Examples of such modification techniques include acrylation (Akintayo and Adebowale, 2004a), catalytic and thermocatalytic polymerization (Patel *et al.,* 2000), interesterification (Athawale and Joshi, 2001), phosphorylation, expoxidation (Zhong *et al.,* 2001), copolymerization (Trumbo *et al.,* 2001), dehydration (Thames *et al.,* 1997), and chlorination (Akintayo and Adebowale, 2004b).

Various physical and chemical modifications of rubber seed oil, such as heat treatment (Aigbodion and Pillai, 2001) and monomer-modification (Aigbodion *et al.,* 2003) have been previously reported. In this study, we report on the quality of alkyd resins modified with crude rubber seed oil (RSO) compared with the quality of those modified with refined rubber seed oil. Rubber seed oil has been found to have strong potential for use in the production of alkyd resins with quality comparable to that of commercial alkyd resins (Aigbodion *et al.,* 2001; Aigbodion and Okieimen, 1995). Like other vegetable oils, crude rubber seed oil is composed of a mixture of triglycerides and minor constituents like free fatty acids, which are extraneous to the neutral oil and contaminants. It was then hoped that removal of these minor constituents would enhance the quality of rubber seed oil in the manufacture of alkyd resins.

*Author for correspondence; E-mail: esyikhuoria@yahoo.com

Materials and Methods

Materials. Rubber seeds were collected from the plantation of Rubber Research Institute of Nigeria, Iyanomo, Benin City. The seeds were dried in oven at 50 °C for 72 h and shelled. Kernels were ground and oil was extracted from the milled kernels with *n*-hexane (40-60 °C) using Soxhlet extractor.

Refining. Rubber seed oil was refined according to the method adopted by Protein Oil Starch Pilot Plant Corporation, Canada (Cocks and Rede, 1966). In a typical batch, a known weight of the pre-heated oil (60 °C) was treated with 10% (w/w) of 0.8 M sodium hydroxide by stirring for 5 min, after which it was allowed to stand or centrifuged to separate the aqueous layer. The oil phase was washed with water for about 3 times until neutral. The physicochemical properties of refined rubber seed oil and those of the crude rubber seed oil are compared with those of linseed and soybean oils in Table 1.

Preparation of alkyds. Six different alkyd samples were prepared with crude and refined rubber seed oil, glycerol, and phthalic anhydride using lead (II) oxide as catalyst according to the recipe in Table 2. The preparation was carried out in a three-necked round bottom flask fitted with a motorized stirrer, a Dean and Stark trap carrying water cooled condenser and nitrogen inlet tube. Reaction temperature ranged from 230-250 °C. Xylene was employed as the azeotropic solvent (Aigbodion *et al.,* 2003). Following two stages were involved.

***Stage 1. Alcoholysis*.** In the alocholysis stage, the mono-glyceride was first prepared by reacting glycerol with rubber seed oil at a temperature of 230-250 °C (Aigbodion and Pillai, 2000; Athawale *et al.,* 2000). Alcoholysis was completed when one part of the reaction mixture dissolved in three parts of anhydrous methanol and formed a clear solution. The reaction was cooled to 140 °C.

Stage 2. Calculated amount of phthalic anhydride was added to the reaction mixture and temperature was quickly raised to about 230 °C and maintained at a range of 230-250 °C. The water of condensation was drained into a Dean and Stark trap where water of condensation was removed and the xylene returned to the reaction flask through an overflow point. The reaction was monitored by periodic determination of the acid value of the mixture until acid value dropped to below 10.

Physicochemical characterisation of crude and refined rubber seed oil. Physicochemical properties of the crude and refined rubber seed oils (colour, specific gravity, refractive index, acid value, free fatty acids, saponification value, iodine value and solid content) were determined according to ASTM standard methods. These properties were compared with those of conventional drying oils (soybean and linseed oils) commonly used in surface coatings (Table 1).

Physicochemical characterisation of alkyd resins. The physicochemical properties of the alkyd resins (colour, specific gravity, refractive index, acid value, free fatty acids, saponfication value, iodine value and non-volatile matter) were determined according to ASTM standard methods (ASTMD 1541-50, 1979; 1639-90, 1963-4, 1979; 1959-69, 1979).

Viscosity measurements. Viscosity measurements were carried out with solutions of the alkyds in toluene using Ubbelohde 50 viscometer. Three additional dilutions were made in the viscometer, allowing reflux time to be measured at concentration of 2.5, 2.0, 1.5, 1.0 and 0.5 g/100 cm^3. Viscosity measurements were carried out at temperature of 30±0.5 °C. Another 10 ml of the solution was evaporated to dryness and weighed to determine the exact concentration of the solution. Intrinsic viscosities were determined by extrapolation of the plots of η_{sp}/C against concentration to zero concentration, where C is the concentration (g/100 ml) and η_{sp} is the specific viscosity. Fig. 1 shows typical plots of η_{sp}/C versus concentration (C). The values of the Huggin's constant, K_H, were calculated as follows from the Huggin-Kreamer viscosity relationship.

$$\eta_{sp}/C = [\eta] + K_H[\eta]^2C \qquad (1)$$

Table 1. Physicochemical properties of crude and refined rubber seed oils (RSO) compared with soybean and linseed oils

Properties	Crude RSO	Refined RSO	Soybean oil*	Linseed oil**
Colour	brown	yellow	yellow	yellow
Specific gravity (at 30 °C)	0.930	0.920	0.932	0.931 – 0.980
Refractive index	1.477	1.472	1.475	1.479 – 1.480
Acid value (KOH, mg/g)	19.18	8.96	4.78	1
Free fatty acids (%, as oleic acid)	9.54	4.48	2.39	
Saponification value (KOH, mg/g)	181.14	178.75	190.00	185.00 – 194.00
Iodine value, Wijs (I_2, g/100 g)	136.2	148.6	140.0	188.0

*Cocks and Rede (1966); **Majumder (1990)

Table 2. Recipe of the alkyd resins prepared with crude (samples **I-III**) and refined (samples **IV-VI**) rubber seed oils

Ingredients	(I)	(II)	(III)	(IV)	(V)	(VI)
Rubber seed oil (g)	136.18	151.13	164.87	136.18	151.13	164.87
Phthalic anhydride (g)	101.02	91.28	83.31	101.40	91.28	83.31
Glycerol (g)	62.78	57.60	51.60	62.78	57.60	51.60
Alkyd constant	1.00	1.00	1.00	1.00	1.00	1.00

Determination of molecular weights. Molecular weights of the alkyds were determined using the Rast method (Furniss *et al.,*1978). The technique involves melting point depression of camphor in the presence of the alkyd sample.

Performance characteristics of the alkyd resins. The drying schedule and resistance of the alkyd films to different service media were determined using the methods described in ASTM 1647-89 and 1640-83 (1994) , respectively. Pencil hardness was also determined according to ASTM (1979).

Results and Discussion

Table 1 compares the properties of crude and refined rubber seed oils with those of soybean and linseed oils commonly used in alkyd production. The colour of the crude rubber seed oil was observed to be brown. Although colour is a useful characteristic of oils and an important parameter commonly employed in quality grading of oils and their sales appeal (Wick *et al.,* 1999), it is not necessarily the sole determinant of the potential end-use of the oil in industrial applications. However, refining was observed to lighten the colour of rubber seed oil, thereby enhancing its utilization in the formulation of light coloured coatings. The yellow colour observed with the refined oil was found to be the same as that of soybean and linseed oils commonly used in surface coatings. The specific gravity of rubber seed oil of 0.930 compares well with the value of 0.932 for soybean oil and 0.931 for linseed oil. The refractive index of 1.478 obtained for rubber seed oil in this study compares well with the values of 1.475 and 1.470 reported for soybean and linseed oils, respectively. The acid value of rubber seed oil (19.18 mg KOH/g) is relatively high compared to the value of (1.0 mg KOH/g) for linseed oil and (4.78 mg KOH/g) for soybean oil. A comparatively high acid value had earlier been reported for rubber seed oil (Aigbodion and Pallai, 2000). The saponification value of rubber seed oil (181.14 mg KOH/g) was found to be close to the value of soybean seed oil (190 mg KOH/g) and linseed oil (185-194 mg KOH/g). The iodine value of rubber seed oil (136 g I_2/100 g) was found to be somewhat lower than that of soybean oil (140 g I_2/100 g) and much lower than linseed oil (188 g I_2/100 g). Refining also increased the iodine value of rubber seed oil to 148.6 g I_2/100 g.

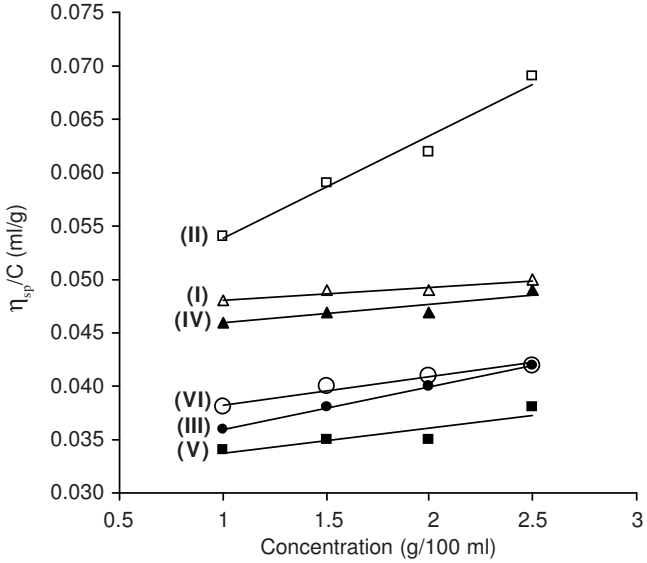

Fig. 1. Plots of reduced viscocity (η_{sp}/C) against concentration (C) of alkyd samples from crude rubber seed oil of 45% **(I)**, 50% **(II)**, 55% **(III)** oil content and refined rubber seed oil of 45% **(IV)**, 50% **(V)**, 55% **(VI)** oil content.

Physicochemical properties of the finished alkyds are shown in Table 3. All the alkyd resins were of good colouration with acid value below 4 mg KOH/g. Saponification values of the alkyd samples **I-VI** were relatively higher than those of unrefined and refined rubber seed oils. This is probably so, as they are essentially polyesters. Generally, the iodine value of the finished alkyd resins increases with the oil length of the samples. However, samples **I-III** modified with unrefined rubber seed oil were found to have lower iodine values than the corresponding alkyd samples modified with the refined oil. This implies that samples **IV-VI** were of higher degree of unsaturation. The percent solid content of samples **IV-VI** was higher than that of samples **I-III**. This may imply that refined rubber seed oil could be suitable in formulating high solid alkyd resins for use as binders in environmentally friendly coatings. Scratch/gouge pencil hardness tests for samples **I-III**, respectively, were HB/4H, HB/3H and H/4H, and for

Table 3. Phyiscochemical properties of finished alkyd resins

Properties	(I)	(II)	(III)	(IV)	(V)	(VI)
Specific gravity (at 30 °C)	0.945	0.938	0.933	0.943	0.037	0.930
Colour	brown	brown	dark brown	brown	ash brown	ash brown
Acid value (KOH, mg/g)	2.41	1.11	0.74	2.60	1.95	3.61
Saponification value (KOH, mg/g)	345.10	326.29	322.60	335.14	337.04	315.37
Iodine value (I_2, g/100 g)	65.64	72.21	83.30	53.84	60.34	72.59
Solid content (%)	62	60	64	76	85	82
Pencil hardness (scratch)	HB	HB	H	H	H	H
(gouge)	4H	3	H	H	4H	3H 4H

samples **IV-VI,** respectively, were 2H/4H, 3H/4H and HB/2H. The films of samples **IV-VI** formulated with refined rubber seed oil were observed to be the hardest films.

Table 4 shows the drying schedule of the alkyd resins in terms of the times of set-to-touch and dry-through. The result showed that the time of set-to-touch and dry-through were about 30 min and 1440 min, respectively, for the alkyd resins from the crude rubber seed oil samples **I-III**. In the case of refined alkyd resin samples **IV-VI**, the time of set-to-touch and dry-through was found to be about 20 min and 1200 min, respectively. The results indicate that alkyd samples formulated with refined oil showed faster drying rates than the resins formulated with crude oil. This trend is in agreement with the level of unsaturation as indicated by the iodine values of the samples as shown in Table 3. The higher the level of unsaturation, the faster was the rate of drying. Drying is believed to occur through the process of auto-oxidation, which involves the adsorption of oxygen at the double bonds of the unsaturated fatty acids (Wick *et al.,* 1999).

Table 5 shows the chemical resistance of the resins. All the alkyd samples seemed to be highly resistant to brine while they were poorly resistant to alkali. This poor resistance to alkali was probably due to alkaline hydrolysis. Samples **IV-VI** were virtually unaffected by water and acid while samples **I-III** can be said to be fairly resistant to water and acid. Based on these results, it can be deduced that refining improved the chemical resistance of alkyd film.

Table 6 shows the intrinsic viscosity and number average molecular weight determined for the alkyd sample. Dilute solution properties such as intrinsic viscosity and number average molecular weight are important for characterizing alkyds (Okieimen and Aigbodion, 1998; 1997). The intrinsic viscosity was observed to be proportional to the molecular weight, whereas intrinsic viscosity decreased with increase in oil length for the two sets of alkyds samples **I-III** and samples

Table 4. Drying schedule of the alkyd resins

Alkyd sample	Set-to-touch time (min)	Dry-through time (min)
I	30	1440
II	30	1440
III	31	1440
IV	20	1200
V	20	1200
VI	22	1200

Table 5. Chemical resistance of the alkyd resins

Alkyd sample	Medium			
	Distilled H_2O	NaCl (50% solution)	H_2SO_4 0.1 M	KOH 0.1 M
I	c	a	b	d
II	b	a	b	d
III	c	a	b	d
IV	a	a	a	e
V	a	a	a	e
VI	a	a	a	e

[a]no effect; [b]whitening; [c]shrinkage of film; [d]shortening of film; [e]removal of film

Table 6. Estimated solution parameters of the alkyd samples

Alkyd sample	Intrinsic viscosity [η] (cm^3/g)	Huggin's constant (K_H)	Number average molecular weight (M)
I	0.0469	0.546	899.87
II	0.0442	4.914	412.06
III	0.0320	3.906	316.97
IV	0.0441	0.926	486.52
V	0.0313	2.450	500.17
VI	0.0359	2.040	571.63

IV-**VI,** except for sample **V**. Values of the Huggin's constant (K_H) do not show regular order of variation.

Conclusion

Alkyd resins from crude samples **I-III** and refined samples **IV-VI** of rubber seed oils have been prepared. In addition to the yield of bright coloured oil, refining also decreased the level of acidity by over 50%, increased the level of unsaturation as estimated by iodine value, and increased the solid content of the finished alkyds. The alkyd samples formulated with the refined oil were observed to produce harder films. The drying time and chemical resistance of the alkyd films were greatly enhanced by refining. Therefore, refining of rubber seed oil intended for alkyd production may be explored further.

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Erratum

The Coden: PSIRAA of the Pakistan Journal of Scientific & Industrial Research was inadvertantly printed as PJSIRAA in vol. **47**. The inconvenience caused to readers is deeply regretted.

Editor-in-Chief

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All correspondence should be addressed to:

Executive Editor, Pakistan Journal of Scientific and Industrial Research, Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

Phone: (92-21)-4651739, 4651740, 4651741-3

Fax: (92-21)-4651738

E-mail: pcsirsys@super.net.pk; pcsir-sic@cyber.net.pk