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7-Azaindole Derivatives as Potential Antibacterial Agents

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Abstract. Azaindole analogues, as antimicrobial agent, have shown significant response against a number of gram positive and gram negative bacteria. In the present work, synthesis of novel derivatives of 7- azaindole and their antibacterial and cytotoxic activities are reported.

Keywords: azaindole, antibacterial agent, cytotoxicity

Introduction

Much work has been rendered on the antimicrobial activity of azaindole derivatives (Mushtaq *et al*., 2008; Saify *et al*., 1994a; Saify, 1986, 1984) which showed significant response against a number of microorganisms (Minakala *et al*., 1992). The aza-isatogens synthesized by Hooper *et al*. (1965) were effective against gram positive organisms. Also the 2-pyridyl-6-aza-indoles showed a broad spectrum of antibacterial activity and were generally more effective than the analogoue of indoles.

Bayomi *et al*. (1985a) described the synthesis of various pyrrolo (3,2-b) pyridine-6-carboxylic acid derivatives as potential antimicrobial agents against several gram positive and gram negative organisms. The results of microbial evaluation *in vitro* of most of the compounds exhibited moderate activity. Remarkable compounds, active against *Shigella sonnei*, are 1, 4-dimethyl-3-carbomethoxy, 7-oxo-pyrrolo (3,2-b)pyridine-6-carboxylic acid and 1-methyl, 4-ethyl, 3-carbomethoxy 7-oxo-pyrrolo (3, 2-b)pyridine-6-carboxylic acid.

In their next communication, Bayomi *et al*. (1985b) reported the synthesis of a series of 1, 4-dihydro-4-oxo-pyrrolo (3, 4-b) pyridine-3-carboxylic acid as an extension of the interest in fused pyrrolopyridines as potential antimicrobial agent. Few compounds of this series were found to exhibit a relatively broad spectrum of activity.

During the last decade a considerable attention has been focused on azaindole analogues as antimicrobial agents. A series of derivatives of 7-dihydro-4-oxo-7-azaindole-5-carboxylic acid were synthesized by Toja *et al*. (1986).

One compound of this series, 4,7-dihydro-4-oxo-1,2-dimethyl-7-ethyl-7-azaindole-5-carboxylic acid, was found to be the most potent antibacterial agent.

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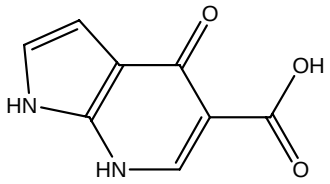
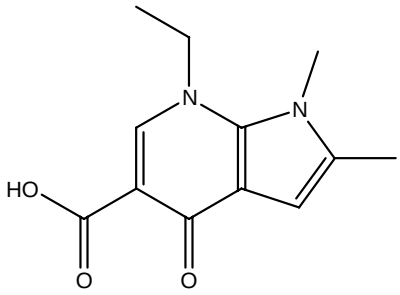


7-Dihydro-4-oxo-7-azaindole-5-carboxylic acid

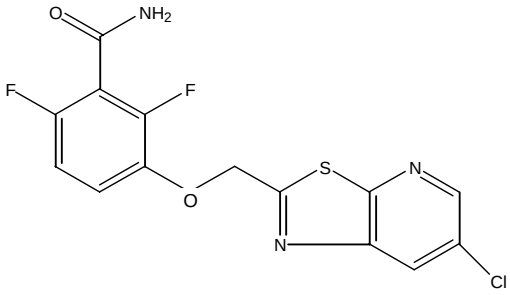


4,7-Dihydro-4-oxo-1, 2-dimethyl-7-ethyl-7-azaindole-5-carboxylic acid

Mohamed (1992) introduced some new azaindole derivatives as antimicrobial agents. These compounds had high moderate and weak inhibitory effect against the tested gram positive and gram negative bacteria. In a similar attempt, some more azaindole derivatives were prepared showing satisfactory antibacterial activity on the basis of related biological activity (Saeed *et al*., 1997, Saify *et al*., 1994b).

Drug resistance to antibiotics and related natural and synthetic drugs poses a great challenge to the chemists, in general, and the drug designers, in particular. Haydon *et al*., 2008, report the discovery of a class of small synthetic antibacterials,

PC190723, which inhibits FtsZ and prevents cell division. PC190723 has potent and selective *in vitro* bactericidal activity against staphylococci, including methicillin- and multi-drug-resistant *Staphylococcous aureus*.



PC190723

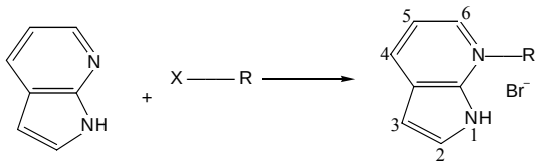
Flouro pharmaceuticals have shown significant antibacterial activity and have been drugs of choice against antibiotic-resistant strains. The flouro compounds synthesized during the course of our project have shown significant activity. Further research is in progress to find out their efficacy as potential antifungal agents.

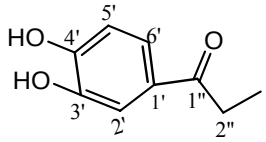
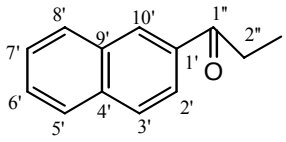
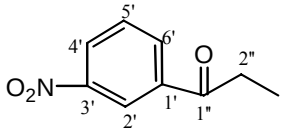
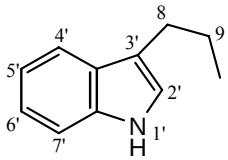
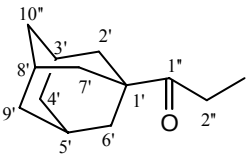
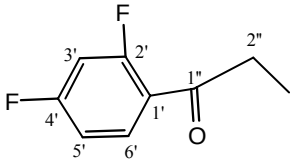
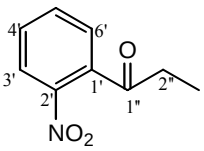
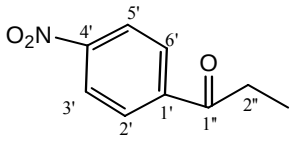
Materials and Methods

General method of synthesis. Equimolar quantities of 7-azaindole and substituted phenacyl halide and/or 2-(2-bromoethyl)-2, 5, 5-trimethyl-1, 3-dioxane were dissolved in approximately 25 ml of acetone in separate conical flasks and then mixed together in another conical flask. The reaction mixture was stirred at room temperature for 2 to 3 h. After completion of the reaction, solid precipitates were obtained. The reaction was monitored by TLC (solvent system of $CHCl_3$-MeOH in different proportions). The resultant compound was filtered and washed with acetone and/or mixture of acetone and ether to remove the unreacted starting materials. Crude precipitates were recrystallized several times from ethanol and/or the mixture of solvents to give pure crystals of the compound.

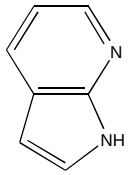
Confirmational techniques. All melting points were recorded on Gallenkamp melting point apparatus and are uncorrected. Silica gel type 60 P254 of E. Merck was used for preparing TLC plates. Spots on plates were detected by iodine using iodine tank. Ultraviolet (UV) spectra were recorded in methanol on Hitachi U-3200 spectrophotometer. Infra red (IR) spectra were measured on Shimadzu IR 460 spectrophotometer using KBr disc, mass spectra (MS) on Massen spectrometer MAT 311A Varian Bermen spectrometer and nuclear magnetic resonance (NMR) spectra, on Bruker AM-300 spectrometer at 300 MHz.

Synthesis scheme

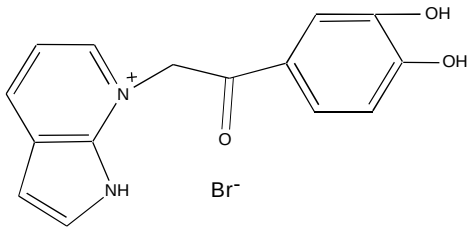


X	R	Compound no.
Br		**2**
Br		**3**
Br		**4**
Br		**5**
Br		**6**
Cl		**7**
Br		**8**
Br		**9**

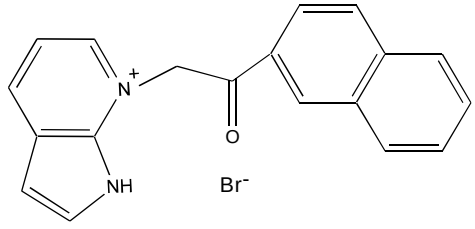
Spectral studies. ***Compound 1: 1H-prrolo[2,3-b]pyridine (7-azaindole).***



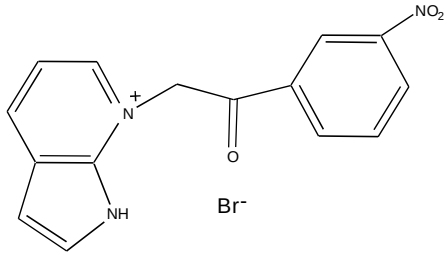
Compound 2: 1-[3-(3,4-dihydroxyphenyl) 3-oxoethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide. Molecular formula: $C_{15}H_{12}N_2O_3Br$, 348.011amu; Yield: 79%; UV(MeOH) λ_{max} nm: 289, 227 and 208; IR(KBr) ν_{max} cm^{-1}: 3100, 1670, 1595 and 1290; EIMS *m/z* (relative int., %): 268 (M^+-HBr, 4), 239(14), 193(3), 165(12), 137(15), 118(100) and 109(25); ^{1}H-NMR (D_2O, 300 MHz): δ 8.72 (1H, d, *J*=7.85 Hz, H-6), 8.24 (1H, d, *J*=6.80 Hz, H-4), 7.70 (1H, dd, *J*=8.46, 2.21 Hz, H-6'), 7.67 (1H, d, *J*=3.65 Hz, H-2), 7.62 91H, dd, *J*=7.85, 6.80 Hz, H-5), 7.55 (1h, d, *J*=2.19 Hz, H-2'), 7.06 (1H, d, *J*=8.46 Hz, H-5'), 6.97 (1H, d, *J*=3.65 Hz, H-3) and 6.48 (2H, s, H-2").



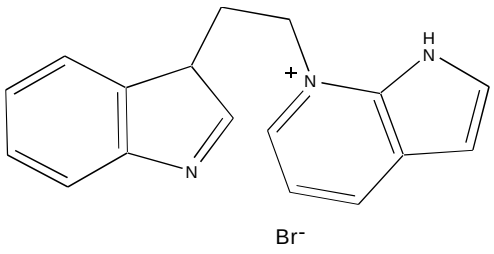
Compound 3: 1-[3-(2-naphthyl)3-oxoethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide. Molecular formula: $C_{19}H_{15}N_2O$ Br, 367.23 amu; Yield: 86.2%; UV(MeOH) λ_{max} nm: 340, 293, 250, 224, 203; IR(KBr)ν_{max} cm^{-1}: 3755, 2923, 1687, 1361, 987, 823.5, 783, 545, 474.5; EIMS *m/z* (relative int, %): 286 (M^+, 7.4) 259(8.2), 258(62), 257(52), 155(15), 81(18), 77(22); ^{1}H-NMR(CD_3OD, 300 MHz): δ 8.82 (1H, dd, *J*=7.96,1.82 Hz, H-6), 8.67 (1H, d, *J*=6.12Hz, H-4), 8.16 (2H, d, *J*=7.99 Hz, H-2,8′), 8.09 (1H, d, *J*=3.93 Hz, H-2), 8.05(2H, d, *J*=7.57H,H-3′,9′), 7.73(1H,dd, *J*=7.57,4.00 Hz, H-4), 7.65 (2H,m,H-5′,8′), 7.01(1H,d, *J*=3.49 Hz, H-3), 6.65 (2H,s, H-2″).



Compound 4: 1-[3-(3-nitrophenyl)3-oxoethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide. Molecular formula: $C_{15}H_{12}N_3O_3$ Br, 362.178 amu; Yield: 80%; UV(MeOH) λ_{max} nm: 299.6, 228, 200; IR(KBr)ν_{max} cm^{-1}: 3232, 2916, 1965, 1797, 1697, 1529, 1475, 1356, 1224.9, 1172.8, 1091.5, 930, 883, 799, 598, 519, 481.2; EIMS *m/z* (relative int., %): 282(M^+, 9.8), 252(100), 236(41), 206(57), 193(6.1), 150(15.6), 131(53.5), 118(13.3), 90(6.2), 77(17.1), 63(6.6); ^{1}H-NMR (CD_3OD, 300 MHz): δ 8.93 (1H, s, H-2′), 8.81 (1H, d, *J*=7.79 Hz, H-6), 8.69 (1H, d, *J*=7.71,Hz, H-2′), 8.64 (1H, d, *J*=4.73 Hz, H-4′), 8.52 (1H, d, *J*=8.85 Hz, H-4), 8.42 (1H, t, H-6′), 8.39 (1H, S, H-5′), 7.93 (1H,dd,*J*=8.02, 3.35 Hz H-5)7.77 (1H, d, *J*=3.35 Hz, H-3) 7.67(1H, d, *J*=6.5 Hz, H-6) 7.01 (1H, d, *J*=3.50 Hz, H-2),6.9(2H,s, H-2″).

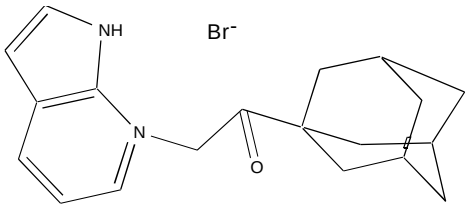


Compound 5: 1-[2-(1H-indol-3-yl) ethyl]-7H-pyrrolo[2,3-b] pyridine-1-ium;bromide. Molecular formula: $C_{17}H_{15}N_3Br$ 340.01 amu; Yield: 75%; UV(MeOH) λ_{max} nm: 200, 221, 283, 289; IR(KBr)ν_{max} cm^{-1}: 3437, 3375, 3217, 2917, 2074, 1837, 1616, 1462, 1360, 1297, 1102, 1005, 800.5, 726, 604, 543, 428.3; EIMS *m/z* (relative intensity, %): 261(M^+, 2) 260(1), 225(1), 143(100), 142(20) 115(37), 103(5), 91(12), 82(10), 63(7); ^{1}H-NMR (CD_3OD, 300 MHz): δ 8.53 (1H, d, *J*=7.74 Hz, H-6), 7.91 (1H, d, *J*=6.03 Hz, H-4), 7.74 (1H, d, *J*=3.5 Hz, H-5), 7.27 (2H, dd, *J*= 4.84,3.51 Hz, H-4',6'), 7.06(1H, s, H-5'),6.85 (1H,s, H-2'), 5.02 (1H,t,H-2), 4.83(2H,s,H-2"),3.50 (2H,s,H-1"),3.28 (1H,t,H-3).

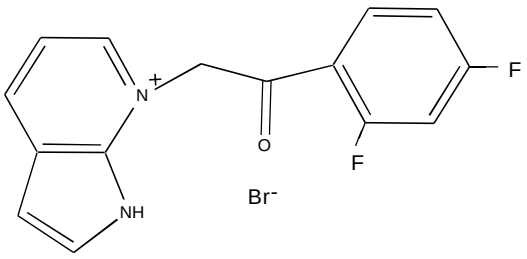


Compound 6: 1-[3-(1-adamantyl)3-oxoethyl]-7H-pyrrolo [2,3-b]pyridine-1-ium;bromide. Molecular formula: $C_{19}H_{23}N_2O$ Br, 375.30 amu; Yield: 81.5%; UV(MeOH) λ_{max} nm: 299.6, 226.0; IR(KBr)ν_{max} cm^{-1}: 3409, 2918, 2850, 2773, 1712, 1620, 1458, 1357,

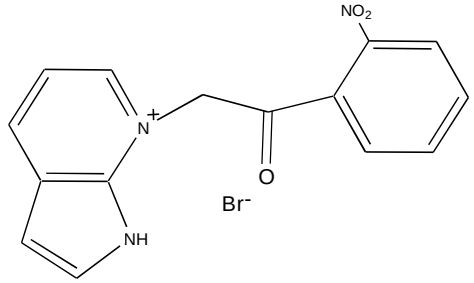
1164, 1097, 887, 779, 727, 661, 536, 476; EIMS *m/z* (relative intensity; %): 295 (M^+, 7.3), 294(35), 237(2.6), 176(3), 159(15.5), 131(100), 93(18.4), 79(27); ^{1}H-NMR(DMSO-d_6, 300 MHz): δ 8.77 (1H, d, *J*=8.68 Hz, H-6), 8.48 (1H, d, *J*=6.15 Hz, H-4), 7.95(1H,d, *J*=3.48Hz, H-2), 7.66 (1H, dd, *J*=7.8,1.5 Hz, H-5), 6.97 (1H, d, *J*=3.57, H-2), 6.06(2H,s,H-2"), 1.97 (3H,s,H-4',7',8'),1.73(3H,s , H-2',3',7',10').



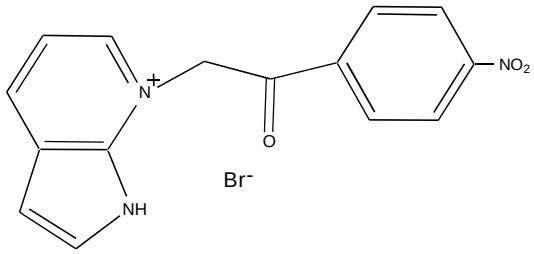
Compound 7: 1-3-[(2,4-diflorophenyl) 3-oxoethyl]-7H-pyr-rolo[2,3-b]pyridine-1-ium;bromide. Molecular formula: $C_{15}H_{10}F_2N_2O$ Cl, 352.0026 amu; Yield: 32; UV(MeOH) λ_{max} nm: 301, 276 and 198; IR(KBr)ν_{max} cm^{-1}: 3400, 2800, 1610, and 1560; EIMS *m/z* (relative int., %): 272 (M^+-HBr, 53), 235 (6), 141(100), 132 (70), 118 (12) 113 (48) and 77(45); ^{1}H-NMR (D_2O, 300 MHz): δ 8.79 (1H, dd, *J*=7.85, 1.96 Hz, H-6), 8.42 (1H, d, *J*=8.85 Hz, H-4), 8.21 (1H, dd, *J*=7.94, 5.42 Hz, H-6'), 7.96 (1H, d, *J*=3.40 Hz, H-2), 7.72 (1H, dd, *J*=8.85, 7.85 Hz, H-5), 7.41 (1H, m, H-3'), 7.32 (1H, d, *J*=3.40 (Hz, H-3), 7.21 (1H, dd, *J*=11.96, 7.94 Hz, H-5') and 6.18 (2H, s, H-2").



Compound 8: 1-[3-(2-nitrophenyl)3-oxoethyl]-7H-pyrrolo [2,3-b]pyridine-1-ium;bromide. Molecular formula: $C_{15}H_{12}N_3O_3Br$, 817.1410 amu; Yield: 48%; UV(MeOH) λ_{max} nm: 290, 252 and 202; IR(KBr)ν_{max} cm^{-1}: 3385, 2930, 1695, 1610 and 1340; EIMS *m/z* (relative int., %): 282 (M^+-Br, $C_{15}H_{12}N_3O_3$, 10), 224(30), 164(10), 144 (4), 132(10) and 118(80); ^{1}H-NMR (CD_3OD, 300 MHz): δ 9.22 (1H, dd, *J*=8.32, 1.21 Hz, H-6), 9.01 (1H, dd, *J*=6.18, 1.21 Hz, H-4) 8.96 (1H, dd, *J*=8.32, 6.18 Hz, H-5), 8.32 (1H, d, *J*=3.26 Hz, H-2), 8.32 (1H, dd, *J*=7.90, 1.68 Hz, H-6'), 8.31 (1H, ddd, *J*=8.30, 7.48, 1.68 Hz, H-4'), 7.93 91H, ddd, *J*=8.30, 7.90, 1.64 Hz, H-5'), 7.85 (1H, dd, *J*=8.30, 1.64 Hz, H-3'), 7.64 (1H, d, J=3.26 Hz, H-3) and 6.25 (2H, s, H-2").



Compound 9: 1-[(4-nitrophenyl)3-oxoethyl]-7H-pyrrolo [2,3-b]pyridine-1-ium;bromide. Molecular formula: $C_{15}H_{12}N_3O_3Br$; Yield: 61%; UV(MeOH) λ_{max} nm: 270, 248 and 201; IR(KBr)ν_{max} cm^{-1}: 3390, 2920, 1690, 1600 and 1340; EIMS *m/z* (relative int., %): 282 (M^+-Br, $C_{15}H_{12}N_3O_3$, 8), 164(4), 160(12), 132 (11), 122(18), 118(100) and 77 (23); ^{1}H-NMR (CD_3OD, 300 MHz): δ 8.81(1H, d, *J*=7.84, 1.08 Hz, H-6), 8.84 (2H, d, *J*=8.02, Hz, H-3',5') 8.26 (1H, d, *J*=8.99 Hz, H-4), 7.78 (2H, d, *J*=8.02 Hz, H-2',6'), 7.71 (1H, d, *J*=3.51 Hz, H-2), 7.68 (1H, dd, J=8.99, 7.84 Hz, H-5), 7.02 (1H, d, *J*=3.51 Hz, H-3) and 6.02 (2H, s, H-2").



Antibacterial activity. ***Method.*** Antibacterial activitiy of all compounds was studied using disc diffusion assay method of Bauer *et al*. (1996). Stock solution of test compound (20,000 µg/ml) was prepared by dissolving 20 mg of test compound in 1 ml of DMSO. Filter paper disc of about 6 mm were sterilized by autoclaving at 15lb/in^2 pressure for about 30 min. Each disc was soaked in 10 µl of the stock solution of compound extract in order to achieve a final concentration of 200 µg/disc.

Sterile petri plates were poured with about 18-20 ml of auto-claved Muller hinton agar (Mueller and Hinton, 1941) and were pre-incubated at 37 °C for 18-24 h. The test cultures were inoculated in about 4-5 ml of Mueller hinton broth, incubated overnight at 37 °C. Next day inoculated cultures were vortexed and a uniform lawn of culture was made on Mueller hinton agar plate after streaking sterile cotton swab in overnight broth culture. Plates were air dried for 10-15 min then filter

paper discs soaked in the test compound solution, were placed at different places on the plate. Plates were then incubated at 37 °C for 18-24 h. Next day, the zone of inhibition around each disc was measured in millimeter.

Cytotoxicity evaluation using 3T3 cell. *Method.* Cytotoxic activity of compounds was evaluated in 96-well flat-bottom micro plates by using standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay. For this purpose, 3T3(mouse fibroblast) were cultured in Dulbecco's modified Eagle's medium, supplemented with 5% of foetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin in 25 cm^3 flask and kept in 5% CO_2 incubator at 37 °C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of $1x10^5$ cells/ml was prepared and introduced (100 µl/well) into 96-well plates. After overnight incubation, medium was removed and 200 µl of fresh medium was added with different concentrations of compounds (1-100 µm). After 72 h, 50 µl MTT (2 mg/ml) was added to each well and incubated for further 4 h. Subsequently, 100 µl of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a microplate ELISA reader (Spectra Max plus, molecular devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition for 3T3 cells.

Results and Discussion

Antibacterial activity of compounds **1-9** against gram positive and gram negative organisms are presented in Table 1 and 2, respectively.

Table 1. Results of compounds **1-9** against gram positive organisms

Compound no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Staphylococcous aureus AB188	28	10	7	8	-	7	7	-	-
Staphlococcous epidermidis	-	-	9	7	-	7	-	-	-
Methicillin resistant *Staphlococcous aureus 3*	-	13	9	7	7	7	-	-	-
Micrococcous luteus	7	20	15	20	7	11	11	-	-
Micrococcous luteus ATCC 9341	-	21	-	-	-	13	7	-	-
Bacillus subtilis ATCC	9	12	11	12	15	14	10	-	-
Bacillus cereus ATCC	8	12	11	7	8	11	9	-	-
Corynebacterium diphtheriae	8	15	9	8	15	14	10	-	-
Corynebacterium hofmanii	-	-	-	-	9	14	10	-	-
Corynebacterium xerosis	8	15	8	7	7	15	-	-	-
Listeria monocytogene	7	-	9	7	-	7	-	-	-
Streptococcous feacalis	7	-	8	7	7	10	-	-	-
Microbacterium seregmotis	-	-	-	-	7	15	-	-	-

- = no activity

Table 2. Results of compounds **1-9** against grams negative organisms

Compound no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Salmonella typhi ATCC	-	10	9	9	10	7	-	-	-
Salmonella paratyphi A	7	7	9	12	-	7	-	-	-
Salmonella paratyphi B	-	17	10	11	-	-	-	-	-
Shigella dysenteriae	20	11	8	8	-	7	-	-	-
Proteus micrabilis	7	7	7	-	-	7	-	-	-
Enterobecter sp.	7	17	7	8	7	8	10	-	-
Escherichia coli ATCC	-	18	10	12	7	-	-	-	-
Escherichia coli MDR	-	9	11	12	7	-	-	-	-
Klebsiella pneumoniae	17	15	10	12	7	8	11	-	-
Pseudomonas aerugonosa	8-	7	7	7-	-	9	-	-	-

- = no activity

The parent compound **1,** 1H-pyrrolo[2,3-b]pyridine(7-azaindole) showed negligible activity except against *Staphylococcous aureus* AB 188 among gram positive strains and *Shigella dysenteriae* and *Klebsiella pneumonia* among gram negative strains.

Compound **2** is a 3,4 dihydroxy derivative of compound **1** which showed significant activity against methicillin resistant *Staphylococcous aureus 3*, *Micrococcous luteus, Micrococcous luteus* ATCC, *Bacillus cereus* ATCC among the grain positive strains while against *Salmonella paratyphi B, Enterobacter* sp., *Escherichia coli* ATCC and *Klebsiella pneumonia* among the gram negative bacteria.

Compound **3** is an acetonaphthone derivative which showed good activity against *Micrococcous luteus* among the gram positive strains but no reasonable activity against the gram negative strains.

Compound **4** is a 3 nitro acetophenone derivative and showed significant results against *Micrococcous luteus* and *Bacillus* ATCC among the gram positive strains and among the gram negative bacteria, it showed better activity against *Salmonella typhi* A, *Escherichia coli* ATCC and MDR, and *Klebsiella pneumonia* strains.

Compound **5** is a bromoethyl indole derivative of compound **1** and demonstrated better activity against gram positive strains including *Bacillus subtilis* ATCC, *Corneybacterium diphtheriae* while it was inactive against all the tested gram negative strains.

Compound **6** is an adamantyl derivative which displayed significant results against the gram positive *Micrococcous luteus* ATCC, *Bacillus subtilis* ATCC, *Corneybacterium hofmanii, Corneybacterium diphtheriae, Corneybacterium xerosis* and *Microbacterium seregmotis* while it was inactive against the gram negative strains.

Compound **7**, a diflouro-acetophenone derivative showed weak activity against both the tested strains.

Compound **8** and **9** are *ortho* and *para*-nitroacetophenone derivatives which did not display any activity against all the tested strains.

Regarding SAR, all the derivatives of 7-azaindole derivative except **3**, **5** and **6** have different functional groups at the phenyl ring. Compounds **4**, **8** and **9** are nitro derivatives; the difference is only in the position of nitro group at phenyl ring. Compound **4**, a *meta* nitro derivative, showed reasonable inhibitory activity while compounds **8** and **9** are *ortho* and *para* nitro derivatives, respectively, which were devoid of any inhibitory activity.

Hydroxyl, naphthalene containing group also enhanced the activity of parent compound **1** while bromoethyl indole and diflouro-acetophenone binding to 7-azaindole (**1**) decreased the activity against both the gram positive and the gram negative bacteria.

From the above discussion it can be concluded that binding and position of different functional groups in 7-azaindole have influence on the antibacterial activity.

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Synthesis of Some New Substituted Quinazolin-4-*3H*-Ones as Potent Anticonvulsant Agents

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Abstract. A new series of 3-(4-(2-(6,8-dibromo-3-(substituted phenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)methyl) hydrazinyl)thiazol-2-yl)-2-phenylthiazolidin-4-ones were synthesized and their structures were elucidated on the basis of elemental analyses and spectroscopic studies (IR, ^{1}H-NMR). All the synthesized compounds **1-32** were screened for their anticonvulsant activity at a dose of 30 mg/kg. The compound **31** was found to be the most potent compound of this series showing 90% protection against MES.

Keywords: benzylidenoquinazolinones, thiazolylquinazolinones, thiazolidinoylquinazolinone, anticonvulsant activity, toxicity

Introduction

Quinazolinone derivatives have evoked considerable attention in recent years as these are endowed with a range of pharmaceutical activities. 3H-quinazoline-4-one represents a useful nucleus for preparation of some new anticonvulsant agents, since quinazolines exhibited interesting pharmacological properties like anticonvulsant activity (Georgey *et al.*, 2008; Guan *et al.*, 2007; El-Helby and Wahab, 2003; Zappala *et al.*, 2003) and anti-inflammatory activity (Alagarsamy *et al.*, 2006). Thiazoles and thiazolidinones having different heterocyclic nuclei were found to possess anticonvulsant activity (Shekarchi *et al.*, 2005; Arachana *et al.*, 2003; 2002). In the present study, a new series of 3-(4-(2-(6,8-dibromo-3-(substituted phenyl)-4-oxo-3,4-dihydroquinazolin-2-yl) methyl)hydrazinyl)thiazol-2-yl)-2-phenylthiazolidin-4-ones were synthesized and structures of these compounds were elucidated on the basis of elemental analyses and spectroscopic studies (IR, ^{1}H-NMR). All the synthesized compounds **1-32** were screened for their anticonvulsant activity at a dose of 30 mg/kg.

Materials and Methods

Compound **1** (3,5 dibromoanthranitic acid) synthesized according to the method of Wheeler and Oates (1910) and its reaction with acetic anhydride (Bogert and Seli, 1907) yielded compound **2** (6, 8-dibromo-2-methyl-*4H*-benzoxazin-4-one). Reaction of the latter with *P*-hydroxy amline furnished compounds 3-4. Bromination of 6, 8-dibromo-3(substitute diphenyl)-2-methylquinazolin-4 (*3H*)-ones i.e., compounds **3-4** yielded 6,8-dibromo-2-bromomethyl- 3-(substituted phenyl) quinazolin-4(*3H*)-ones i.e., compounds **5-6**. These brominated products on treatment with 99% hydrazine hydrate afforded 6,8-dibromo-2-hydrazinylmethyl-3-(substituted phenyl) quinazolin-4-(*3H*)-ones i.e., compounds **7-8**, which on reaction with chloroacetylchloride gave 2-chloro-(6,8-dibromo-3-(substituted phenyl)-4-oxo-3, 4-dihydroquinazolin-2-yl) methyl) acetohydride compounds **9-10;** these were converted to thiazole congeners i.e., 2-(2'-aminothiazol-4'-yl) hydrazinyl) methyl)-6,8-dibromo-3-(substituted phenyl) quinazolin-4(*3H*)-ones (compounds **11-12**) by the reaction of thiourea. The compounds **11-12** reacted with different aromatic aldehydes to give 2-(2'-(benzylideneamino-thiazol-4'-yl) hydrazinyl)-6, 8-dibromo-3-(substituted phenyl) quinazolin-4(*3H*)-ones (compounds **13-22**). Substituted benzylidene congeners **13-22** were cyclized on reacting with thioglycolic acid in the presence of a pinch anhydrous $ZnCl_2$ to yield 3-(4-(2-(6, 8-dibromo-3-(substituted phenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)hydrazinyl)thiazol-2-yl)-2-phenyl thiazolidin-4-ones (compounds **23-32**).

The melting points of compounds were determined in open capillaries and are uncorrected. Homogeneity of the synthesized compounds was routinely checked by thin layer chromatography on silica gel-G plates. The eluent was a mixture of different polar and nonpolar solvents in different proportions and spots were located in iodine chamber. The IR spectra were recorded on Bruker IFS-66 V FT IR (ν_{max} in cm^{-1}). The ^{1}H NMR spectra were recorded by Brucker DRX-400 FT NMR instrument using $CDCl_3$ and DMSO-d_6 as solvent and tetramethyl silane (TMS) as internal reference standard. All chemical shift (δ) values were recorded in ppm. Elemental analysis (CHN) of these newly synthesized

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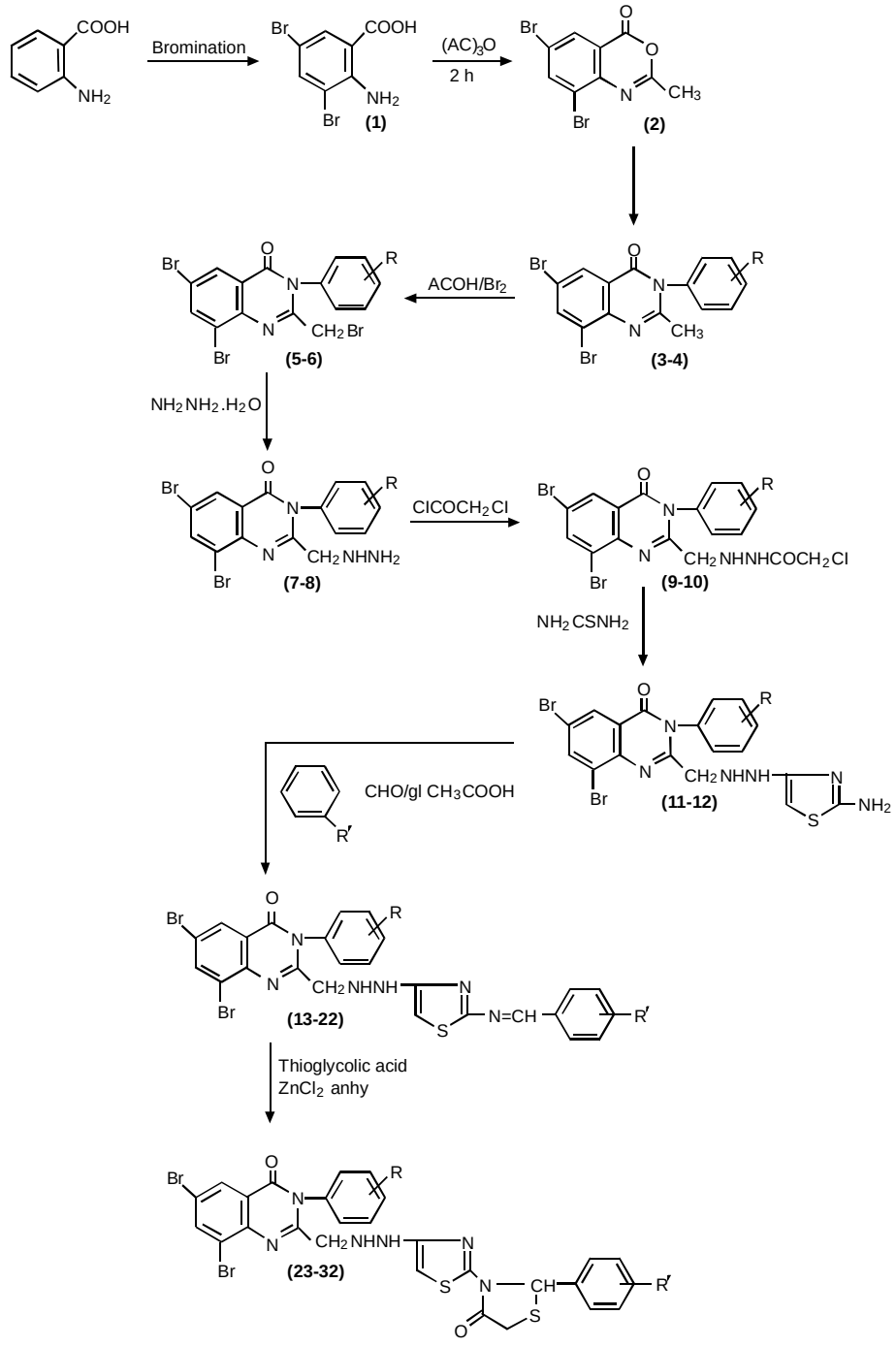


Scheme

compounds were performed on a Carlo Erba-1108 elemental analyzer.

Synthesis of 3,5-dibromoanthranilic acid (1). It was prepared according to the method of Wheeler and Oates (1910). Bromine (0.8 mol) in acetic acid (20 ml) was added dropwise to the solution of anthranilic acid (0.4 mol) in absolute ethanol (60 ml).The solid product thus crystallized out was washed with hot water and dried. It was recrystallized from methanol to afford compound **1**, m.p. 219 °C (reported m.p. 218 °C).

Synthesis of 6,8-dibromo-2-methyl-*4H*-benzoxazin-4-one (2). It was prepared according to the method of Bogert and Seli (1907). A mixture of compound **1** (3,5-dibromoanthranilic acid, 0.01 mol) and acetic anhydride (0.02 mol) were refluxed

for 2-3 h with constant stirring. The excess of acetic anhydride was distilled off on cooling a solid separated out which was filtered, washed with petroleum ether (60-80 ºC) and dried to give compound **2**, m.p. 182 °C (reported m.p. 184 °C).

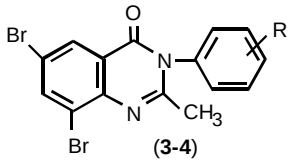
Synthesis of 6,8-dibromo 3-(*p*-hydroxyphenyl)-2-methyl-quinazolin-4(*3H*)-one (3). A mixture of compound **2** (0.2 mol) and *p*-hydroxy aniline (0.2 mol) was heated on free flame for 10-20 min in conical flask. After the disappearance of water droplets in conical flask, it was kept at room temperature. On cooling a jelly like mass was obtained which was dissolved in methanol, refluxed and poured into water. The solid thus obtained was filtered, dried and finally recrystallized from ethanol to give compound **3**, m.p. 202 °C; yield 62%; molecular formula $C_{15}H_{10}N_2O_2Br_2$; IR(KBr)ν_{max} cm^{-1}: 610 (C-Br), 1635 (C=N), 1550 (C=C of aromatic ring), 1720 (C=O of quinazolin ring), 1734 (OH): ^{1}H-NMR ($CDCl_3$): δ 2.30 (s, 3H, CH_3), 7.25-7.90 (m, 6H, Ar-H), 9.30 (s, 1H, Ar-OH exchangeable). Compound **4** was prepared by employing the aforementioned method. Physical and analytical data are shown in Table Ia.

Synthesis of 6,8-dibromo-2-bromomethyl-3-(*p*-hydroxyphenyl) quinazolin-4(*3H*)-one (5). Bromine (0.4 mol) in acetic acid (20 ml) was added drop wise to the solution of compound **3** (0.2 mol) in acetic acid (50 ml).The reaction mixture was poured onto crushed ice then left overnight at room temperature. The precipitate thus obtained was filtered, washed with water, dried and recrystallized from ethanol to afford compound **5**, m.p. 214 °C; yield 70%; molecular formula $C_{15}H_9N_2O_2Br_3$; IR(KBr) ν_{max} cm^{-1}: 608 (C-Br), 1550 (C=C of aromatic ring), 1632 (C=N), 1715 (C=O of quinazolin ring), 3425 (OH); ^{1}HNMR ($CDCl_3$): δ 2.75 (s, 2H, CH_2), 7.20-7.85 (m, 6H, Ar-H), 9.86 (s, 1H, Ar-OH exchangeable). The compound **6** was prepared by employing the aforementioned method. Physical and analytical data are shown in Table Ia.

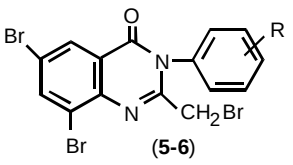
Synthesis of 6,8-dibromo-2–hydrazinylmethyl-3-(*p*-hydroxyphenyl)quinazolin-4(*3H*)-one (7). A mixture of compound **5** (0.1 mol) and hydrazine hydrate (99%) (0.2 mol) in methanol was refluxed for 10 h the excess of solvent was distilled off and the reaction mixture was poured onto ice. The solid thus obtained was filtered, washed with water, dried and recrystallized from methanol to afford compound **7**, m.p. 222 ºC; yield 64%; molecular formula $C_{15}H_{12}N_4O_2Br_2$; IR(KBr)ν_{max} cm^{-1}: 610 (C-Br),1270 (N-N), 1300 (C-N), 1550 (C=C of aromatic ring),1720 (C=O of quinazolin ring), 1620 (C=N), 3425 (OH), 3300 (NH, NH_2): ^{1}H-NMR ($CDCl_3$: δ 2.64 (s, 2H, CH_2N), 6.45 (s, 2H, -NH_2 exchangeable with D_2O), 7.22-7.82 (m, 6H, Ar-H), 9.42 (s, 1H, $NHCH_2$), 9.85 (s,1H, Ar-OH exchangeable). The compound **8** was prepared by employing the afore mentioned method and their physical and analytical data are shown in Table Ia.

Synthesis of 2-chloro-(6,8-dibromo--3-(*p*-hydroxyphenyl) -4-oxo-3, 4-dihydro quinazolin -2-yl) methyl) acetohydride (9). To the solution of compound **7** (0.01 mol) in dry benzene chloroacetylchloride (0.02 mol) was added gradually with stirring under cool condition. The reaction mixture was further stirred for another 2 h at room temperature and then refluxed for 4 h. Benzene was removed by distillation, to yield

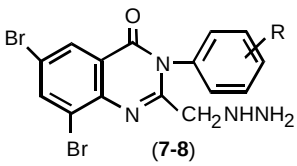
Table Ia. Physical and analytical data of compounds (**3-10**).



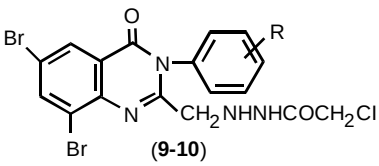
(**3-4**)



(**5-6**)



(**7-8**)



(**9-10**)

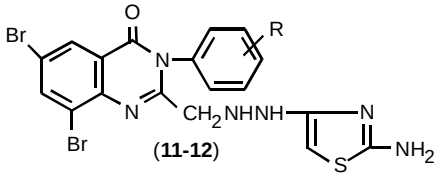
Comp. No.	R	R'	M.P. (ºC)	Yield	Recrystalization solvent	Molecular formula	Elemental analysis					
							%C		% H		%N	
							Calcd.	Found	Calcd.	Found	Calcd.	Found
3	-4OH	-	202	62	Ethanol	$C_{15}H_{10}N_2O_2Br_2$	43.90	43.95	2.43	2.55	6.82	6.94
4	-2Cl	-	196	63	Methanol	$C_{15}H_9N_2OBr_2Cl$	42.00	42.08	2.10	2.01	6.53	6.63
5	-4OH	-	214	70	Ethanol	$C_{15}H_9N_2O_2Br_3$	36.80	36.76	1.84	1.94	5.73	5.48
6	-2Cl	-	210	72	Benzene	$C_{15}H_8N_2OBr_3Cl$	35.46	35.51	1.57	1.50	5.51	5.68
7	-4OH	-	222	64	Methanol	$C_{15}H_{12}N_4O_2Br_2$	40.90	40.84	2.72	2.80	12.72	12.52
8	-2Cl	-	220	62	Acetone	$C_{15}H_{11}N_4OBr_2Cl$	39.25	39.22	2.39	2.46	12.21	12.32
9	-4OH	-	240	70	Methanol	$C_{17}H_{13}N_4O_3Br_2Cl$	39.57	39.64	2.32	2.28	10.86	10.95
10	-2Cl	-	230	71	Ethanol	$C_{17}H_{12}N_4O_2Br_2Cl_2$	38.20	38.28	2.05	2.15	10.34	10.30

the product, which was finally recrystallized from methanol to afford compound **9**, m.p. 242 ºC ; yield 70%; molecular formula $C_{17}H_{13}N_4O_3Br_2Cl$; IR(KBr)ν_{max} cm^{-1}: 610 (C-Br), 760 (C-Cl), 1270 (N-N), 1320 (C-N), 1550 (C=C of aromatic ring), 1715 (C=O of quinazolin ring), 1618 (C=N), 3480 (OH), 3320 (N-H), 2956 (C-H aliphatic), 3055 (C-H aromatic), 3480 (OH); ^{1}H-NMR ($CDCl_3$): δ 2.60 (s, 2H, CH_2), (s, 2H,-CH_2Cl), 7.25-7.80 (m, 6H, Ar-H), 7.86 (brs, 2H, $NHNH_2$), 9.85 (s, 1H, Ar-OH exchangeable). The compound **10** was prepared by employing the afore mentioned method and their physical and analytical data are shown in Table Ia.

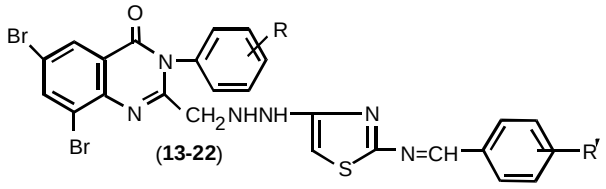
Synthesis of 2–(2'-aminothiazol-4'-yl)hydrazinyl)methyl)-6,8-dibromo-3-(*p*-hydroxy phenyl)quinazolin-4(*3H*)-one (11). A mixture of compound **9** (0.02 mol), thiourea (0.02 mol) and acetone (60 ml) was refluxed for 12 h. The completion of reaction was monitored by TLC. It was then concentrated and cooled, where upon the solid separated out. It was filtered and then recrystallized from methanol. The solid thus obtained was washed with 2% saturated sodium carbonate solution and water to liberate the base, completely dried and recrystallized from ethanol to afford compound **11**, m.p. 250ºC; yield 68%; molecular formula $C_{18}H_{14}N_6O_2SBr_2$; IR(KBr)ν_{max} cm^{-1}: 612 (C-Br), 690 (C-S-C), 1270 (N-N), 1220 (C-N), 1580 (C=C of aromatic ring), 1715 (C=O of quinazolin ring), 1616 (C=N), 3485 (OH) ,3390 (N-H); ^{1}H-NMR ($CDCl_3$): δ 2.55 (s, 2H, CH_2 NH), 6.40 (s, 2H, -NH_2 exchangeable with D_2O), 7.25-7.84 (m, 7H, Ar-H), 7.89 (bs, 2H, $NHNH_2$), 10.00 (s, 1H, Ar-OH exchangeable). The compound **12** was prepared by employing the afore mentioned method and their physical and analytical data are shown in Table Ib.

Synthesis of 2-(2'-(benzylideneamino-thiazol-4'-yl) hydrazinyl)-6,8-dibromo-3-(*p*-hydroxyphenyl) quinazolin-4 (*3H*) -one (13).To the solution of compound **11** (0.01 mole) in methanol (80 ml), benzaldehyde (0.01 mol) with few drops of glacial acetic acid was added and then reaction mixture was refluxed for 10 h; completion of the reaction was monitored by TLC. After distillation of excess of solvent; the reaction mixture was cooled, diluted with cold water and filtered. The solid thus obtained was recrystallized from ethanol to furnish compound **13**, m.p. 260 ºC; yield 58%; molecular formula $C_{25}H_{18}N_6O_2SBr_2$; IR(KBr)ν_{max} cm^{-1}: 608 (C-Br), 690 (C-S-C), 1270 (N-N), 1219 (C-N), 1575 (C=C of aromatic ring), 1717 (C=O of quinazolin ring), 1620 (C=N), 3382 (N-H), 3485 (OH); ^{1}H-NMR ($CDCl_3$): δ 2.56 (s, 2H, -CH_2NH), 7.05-7.90 (m, 12H, Ar-H), 7.85 (bs, 2H, NHNH exchangeable with D_2O), 4.62 (s, 1H, CH, Ar), 10.04 (s, 1H, Ar-OH exchangeable). The compounds **14-22** were prepared by employing the aforementioned method and their physical and analytical data are shown in Table Ib.

Table Ib. Physical and analytical data of compounds (**11-22**).



(**11-12**)



(**13-22**)

Comp. No.	R	R'	M.P. (ºC)	Yield	Recrystalization solvent	Molecular formula	Elemental analysis					
							%C		% H		%N	
							Calcd.	Found	Calcd.	Found	Calcd.	Found
11	-4OH	-	250	68	Ethanol	$C_{18}H_{14}N_6O_2SBr_2$	40.14	40.10	2.60	2.74	15.61	15.69
12	-2Cl	-	240	74	Acetone	$C_{18}H_{13}N_6OSBr_2Cl$	38.81	38.84	2.33	2.35	15.09	15.29
13	-4OH	-H	260	58	Ethanol	$C_{25}H_{18}N_6O_2SBr_2$	47.92	47.84	2.87	2.67	13.41	13.31
14	-4OH	-4OH	270	59	Benzene	$C_{25}H_{18}N_6O_3SBr_2$	46.72	46.76	2.80	3.10	13.08	13.19
15	-4OH	-4OCH_3	265	61	Methanol	$C_{26}H_{20}N_6O_4SBr_2$	47.56	47.64	3.04	3.16	12.80	12.95
16	-4OH	-3OCH_3, -4-OH	240	73	Ethanol	$C_{26}H_{20}N_6O_4SBr_2$	46.42	46.36	2.97	2.90	12.50	12.89
17	-4OH	-4$N(CH_3)_2$	258	71	Acetone	$C_{27}H_{23}N_7O_2SBr_2$	48.43	48.33	3.43	3.54	11.26	11.25
18	-2Cl	-H	250	69	Methanol	$C_{27}H_{17}N_6OSBr_2Cl$	48.46	48.66	2.54	2.45	12.56	12.50
19	-2Cl	-4OH	255	62	Methanol	$C_{25}H_{17}N_6O_2SBr_2Cl$	45.42	45.30	2.57	2.48	12.71	12.61
20	-2Cl	-4OCH_3	272	66	Benzene	$C_{26}H_{19}N_6O_2SBr_2Cl$	46.25	46.35	2.81	2.95	12.45	12.58
21	-2Cl	-3OCH_3, -4-OH	275	63	Acetone	$C_{26}H_{19}N_6O_3SBr_2Cl$	45.18	45.38	2.75	2.87	12.16	12.00
22	-2Cl	-4$N(CH_3)_2$	262	74	Ethanol	$C_{27}H_{22}N_7OSBr_2Cl$	47.12	47.24	3.20	3.15	14.25	14.38

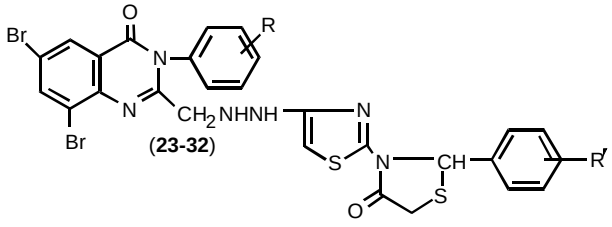
Synthesis of 3-(4-(2-(6,8-dibromo-3-(*p*-hydroxyphenyl)-4-oxo-3,4-dihydro quinazolin-2-yl) methyl) hydrazinyl) thiazol-2-yl)-2-phenylthiazolidin-4-one (23). A mixture of compound **13** (0.01 mol), thioglycolic acid (0.01 mol) and anhydrous $ZnCl_2$ (2 gm) in absolute ethanol was refluxed for 10 h. The progress and completion of reaction was checked by TLC. After refluxing, excess of solvent was distilled off and the residue was poured in cold water, filtered, dried and finally the product was recrystallized from benzene to furnish compound **23**, m.p. 268 ºC; yield 60%; molecular formula $C_{27}H_{20}N_6O_3S_2Br_2$; IR(KBr)$\nu_{max}$ cm^{-1}: 610 (C-Br), 682 (C-S-C), 1217 (C-N), 1574 (C=C of aromatic ring), 1740 (C=O of quinazolin ring), 1622 (C=N), 3370 (N-H), 3515 (OH). ^{1}H-NMR ($CDCl_3$): δ 2.45 (s, 2H,-CH_2NH), 4.05 (s, 2H, -CH_2 of thiazolidinone ring), 7.07-7.10 (m, 12H, Ar-H), 7.94 (bs, 2H, NHNH exchangeable with D_2O), 4.60 (s, 1H, CH-Ar), 10.08 (s, 1H, Ar-OH exchangeable). The compounds **24-32** were prepared by employing the afore mentioned method and their physical and analytical data are shown in Table Ic.

Anticonvulsant activity. The anticonvulsant activity was performed according the method of Toman *et al.* (1946) on Charles foster rats of either sex weighing, between 90-150 g. Rats were divided into groups of ten animals each. The rats were treated with different doses of test drugs or phenytoin sodium 30 mg/kg i.p., After one hour, they were subjected to a shock of 150 m.A by convulsiometer through ear electrodes for 2 sec and the presence or absence of extensor response was noted. Animals in which extensor response was abolished were taken as protected rats. The compounds were also investigated for their acute toxicity ALD_{50} in mice by following the method of Smith (1960).

Results and Discussion

Newly synthesized compounds were evaluated for anticonvulsant activity at a dose of 30 mg/kg i.p., and have shown varying degrees (10% to 90%) of anticonvulsant activity. The characteristic feature of this series is the presence of a five membered thiazole ring at second position of quinazolin moieties which was further substituted with imino arylidenyl or imino substituted arylidenyl group at the second position of five membered thiazole ring. The compounds **3-12** exhibited 10% to 50 % of anticonvulsant activity and compounds **13-22** exhibited 50% to 80% anticonvulsant activity. It was observed that compound **13** and **18** with substituted phenyl group, exhibited 50% and 60% activity, while compounds **16** and **21** with substituted 3-methoxy-4-hydroxyphenyl ring, exhibited 70% and 80% protection against seizures, respectively. Considering the potentiality of the compound **21**, it was studied in detail at three graded doses 7.5, 15, 30 mg/kg i.p., it showed equipotent activity to phenytoin sodium 80%. Compounds **14**, **15**, **17**, **19**, **20** and **22** with substituted 4-hydroxyphenyl ring compounds **14** and **19**; 4-methoxy-phenyl ring compound, **15** and **20** and 4-N, N-dimethyl-phenyl ring compounds **17** and **22** exhibited 60%, 70%, 70%, 70%, 60% and

Table Ic. Physical and analytical data of compounds (**23-32**)



(**23-32**)

Comp. No.	R	R'	M.P. (ºC)	Yield	Recrystalization solvent	Molecular formula	Elemental analysis					
							%C		% H		%N	
							Calcd.	Found	Calcd.	Found	Calcd.	Found
23	-4OH	-H	268	60	Benzene	$C_{27}H_{20}N_6O_3S_2Br_2$	46.28	46.34	2.85	2.80	12.00	12.06
24	-4OH	-4OH	275	54	Acetone	$C_{27}H_{20}N_6O_4S_2Br_2$	45.25	45.20	2.79	2.85	11.73	11.68
25	-4OH	-4OCH_3	272	58	Methanol	$C_{28}H_{22}N_6O_4S_2Br_2$	46.02	46.12	3.01	3.08	11.50	11.55
26	-4OH	-3OCH_3, -4-OH	250	70	Methanol	$C_{28}H_{22}N_6O_5S_2Br_2$	45.04	45.09	2.94	3.05	11.26	11.22
27	-4OH	-4$N(CH_3)_2$	265	69	Ethanol	$C_{29}H_{25}N_7O_3S_2Br_2$	46.83	46.89	3.36	3.30	13.18	13.12
28	-2Cl	-H	242	65	Acetone	$C_{27}H_{19}N_6O_2S_2Br_2$ Cl	45.09	45.12	2.64	2.60	11.69	11.64
29	-2Cl	-4OH	263	60	Methanol	$C_{27}H_{19}N_6O_3S_2Br_2Cl$	44.11	44.28	2.58	2.64	11.43	11.33
30	-2Cl	-4OCH_3	280	63	Methanol	$C_{28}H_{21}N_6O_3S_2Br_2Cl$	4.88	44.66	2.80	2.84	11.22	11.28
31	-2Cl	-3OCH_3, -4-OH	265	60	Ethanol	$C_{28}H_{21}N_6O_4S_2Br_2Cl$	43.95	43.99	2.74	2.70	10.98	10.95
32	-2Cl	-4$N(CH_3)_2$	270	70	Acetone	$C_{29}H_{24}N_7O_2S_2Br_2Cl$	45.69	45.62	3.15	3.10	12.86	12.82

Table 2. Anticonvulsant activity of compound (**1-32**)

Comp. No.	R	R'	Anticonvulsant activity (SMES)[c] Dose (mg/kg i.p.)	No. of animals exhibiting convulsions	Seizure protection (%)	ALD_{50} (mg/kg i.p.)
	P.G.[a]		2 ml	10	0	
	Phenytoin sodium[b]		30	2	80***	
1						
2						
3	-4-OH	-	30	9	10	>1000
4	-2-Cl	-	30	8	20	>1000
5	-4-OH	-	30	8	20	>1000
6	-2-Cl	-	30	8	20	>1000
7	-4-OH	-	30	8	20	>1000
8	-2-Cl	-	30	7	30	>1000
9	-4-OH	-	30	7	30	>1000
10	-2-Cl	-	30	6	40**	>1000
11	-4-OH	-	30	6	40**	>1000
12	-2-Cl	-	30	5	50**	>1000
13	-4-OH	-	30	5	50**	>1000
14	-4-OH	-4-OH	30	4	60**	>1000
15	-4-OH	$-4\text{-}OCH_3$	30	3	70**	>1000
16	-4-OH	$3\text{-}OCH_3$,-4-OH	30	3	70**	>1000
17	-4-OH	$-4N(CH_3)_2$	30	4	60**	>1000
18	-2-Cl	-H	30	4	60**	>1000
19	-2-Cl	-4-OH	30	3	70**	>1000
20	-2-Cl	$-4\text{-}OCH_3$	30	3	70**	>1000
			7.5	9	10	
21	-2-Cl	$3\text{-}OCH_3$,-4-OH	15	6	40**	>1000
			30	2	80***	
22	2-Cl	$-4N(CH_3)_2$	30	3	70**	>1000
23	-4-OH	-H	30	4	60**	>1000
24	-4-OH	-4-OH	30	2	80***	>1000
25	-4-OH	$3\text{-}OCH_3$	30	3	70**	>1000
26	-4-OH	$3\text{-}OCH_3$, 4-OH	30	2	80***	>1000
27	-4-OH	$-4N(CH_3)_2$	30	3	70**	>1000
28	-2-Cl	-H	30	3	70**	>1000
29	-2-Cl	-4-OH	30	2	80***	>1000
30	-2-Cl	$-4\text{-}OCH_3$	30	3	70**	>1000
			7.5	8	20	
31	-2-Cl	$3\text{-}OCH_3$,4-OH	15	5	50**	>2000
			30	1	90***	
32	-2-Cl	$-4N(CH_3)_2$	30	2	80***	>1000

* = P < 0.05, ** = P< 0.01, *** = P < 0.001; [a] = P.G.- propylene glycol standard for control; [b] = phenytoin sodium, reference standard for anticonvu-lsant activity; [c] = supramaximal electroshock seizure pattern test

70% inhibition of seizures, respectively. Furthermore, compounds **23-32** of this series were characterized by the presence of thiazolidinone ring in addition to thiazole ring. The compounds **26** and **31** with substituted 3-methoxy-4-hydroxyphenyl ring have shown 80% and 90% activity, respectively, against seizures. Considering the potentiality of compound **31** i.e. 3-(4-(2-(6,8-dibromo-3- (*p*-chlorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)hydrazinyl) thiazol-2-yl)-2-phenylthiazolidin-4-one, it was further studied in detail at three graded doses 7.5, 15, 30 mg/kg, i.p., for its anticonvulsant activity and showed most potent activity. The results shows better activity than the standard drug. Table 2 show the results of compounds **1-32** and standard drug phenytoin sodium. Compounds **23** and **28** with substituted phenyl ring at the second position of thiazolidinone ring showed 60% and 70% protection, respectively, whereas compounds **24** and **29** having 4-hydroxyphenyl group showed the same degree of protection i.e, 80% against MES test. Compounds having 4-methoxyphenyl ring i.e., compounds **25** and **30** have also shown remarkable protection of 70% each. Compounds **22** and **32** with substituted 4-N, N-dimethylphenyl ring exhibited 70% and 80% inhibition of seizures, respectively. The newly synthesized compounds were also tested for approximate lethal dose ALD_{50} and were found to exhibit a higher value of ALD_{50} i.e., more than 1000 mg/kg, i.p., except compound **31** which exhibited ALD_{50} of more than 2000 (maximum dose tested) thus indicating the safer nature of these compounds. It can be concluded that cyclisation of substituted imino arylidenyl compounds **13-22** into thiazolidinones **23-32** has no remarkable effect on anticonvulsant activity except compound **31** which is the most active compound of this series.

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Synthesis of Blue Pigment from Kaolin

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Abstract: Kaolin of Swat NWFP, Pakistan was analyzed and its suitability was tested for utilizing the raw material for the synthesis of blue pigment. It was successfully utilized for the preparation of ultramarine blue pigment by subsequent reductive and oxidative heating with other ingredients. The pigment was characterized by UV-Vis, IR spectrophotometry and XRD.

Keywords: kaolin, ultramarine blue, pigment, Swat, Pakistan

Introduction

Ultramarines family of pigments are derived from sodalite ($6NaAlSiO_4.2NaCl$) doped with sulphur and are used in the manufacture of textiles, synthetic fibers, detergents, soaps, plastics, toys, ropes and mats, cosmetics etc. The catalytic activity of ultramarine is also reported and is thus used for dehydrogenation, dehydro-sulphurization, cracking and isomerization purposes (Kowalak *et al.*, 2004).

Natural ultramarines i.e., lazurite and lazuli are known. Ultramarine is available in different colours and shades i.e., blue, reddish blue, greenish blue, red, pink and violet. Ultramarine blue consists of alumino-silicate framework, containing sodium cations and poly-sulphide anions (S^{2-}, S^{3-}) (Tarling, *et al.*, 1988) in which the S^{3-} species are dominant over S^{2-}. The S^{3-} is responsible for the intense blue colour of the pigment.

Basic raw material for ultramarine is kaolin (Booth *et al.*, 2003; Cork, 1993). Kaolin is largely used in ceramics and as filler in paper, plastics and rubbers. Synthesis of ultramarine is still based on J. B. Guimet method (Kowalak *et al.*, 2004). The aim of the present research was production of ultramarine blue for industrial use utilizing kaolin ($Al_2Si_2O_5(OH)$) of Swat, NWFP, Pakistan. Kaolin deposits of Swat lie 34° 53′ 30″ N, 72′ 53″ 30 E and are among the oldest known kaolin deposits of Pakistan (Siddiqui *et al.*, 2005). The reported deposits of Swat kaolin are 2.5 million tons (Yotoni *et al.*, 1967).

Materials and Methods

Kaolin sample was collected from Shah Dehri, Swat, NWFP, Pakistan. The sample was analyzed gravimetrically (Furman, 1962) and trace metals were determined using atomic absorption spectrophotometer (Hitachi Z8000, Japan) (Table 1). Moisture content, water-soluble content and pH of the sample were also determined (Table 2).

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Steps of pigment production are given in the flow diagramme (Fig. 1). Kaolin sample was activated/dehydrated at 700 °C in a muffle furnace for 2 h and afterwards mixed with sodium carbonate and charcoal in optimized ratio. The mixture was then finely grinded and blended with fine sulphur. All the chemicals were of commercial grade. The blend was packed in ceramic vessel, covered with mud, kept in muffle furnace and heated to 800 °C for 5 h. After cooling the reduced greenish product was grinded and heated in open air in a china dish, till the colour changed to blue. The blue pigment was washed with hot water several times and then with 10% sodium hydroxide solution to remove soluble matter i.e., sulphates. The product was then dried in air.

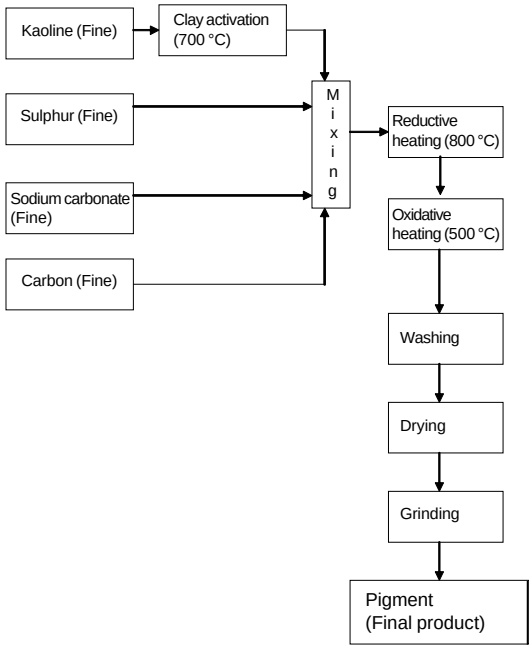

Fig. 1. Flow diagram for the production of blue pigment from kaolin

Density, water-soluble matter and free sulphur of the product were determined (Table 3). The pigment was characterized by double beam UV-visible spectrophotometer (Hitachi, U2000), FTIR (Shimadzu Prestige-21) and XRD (Jeol, JDX3532, Japan).

Results and Discussion

The raw materials used in this synthesis are kaolin, sulphur, coal and Na_2CO_3. The kaolin composition is given in Table 1. The ratio of Si to Al is 1.88. Si and Al form the main framework and their ratio is very important. Variation in Si:Al ratio affects the shade of the pigment (Klinowski *et al.*, 1987). Kaolin having Si:Al, 1.66 has already been successfully used for ultramarine preparation (Landman and De Waal, 2004). Trace metals are not in appreciable quantity to disturb the synthesis of ultramarine. The amount of Fe_2O_3 is 0.8%. The quantity of iron not only affects the synthesis of the pigment but also its quality. Ultramarines having heavy metals are toxic.

Moisture of kaolin is 4.761% (Table 2) and earlier trials with unactivated samples were not successful. The sample was therefore, activated at 700 °C to remove water and hydroxyl ions and also to weaken the structure of kaolin. The pH of the sample was suitable for the synthesis (Table 2).

Table 1. Chemical constituents of kaolin

Compounds	Composition (%)
SiO_2	48.254
Al_2O_3	37.424
CaO	4.479
MgO	1.365
Fe_2O_3	0.805
PbO	0.003
CoO	0.004
CuO	0.033

Table 2. Physical properties of kaolin

Parameters	Value
pH	5.49
Water soluble matter	3.659%
Moisture	4.761%

Table 3. Properties of the synthesized ultramarine blue

Parameters	Value
Moisture	0.08%
Water soluble matter	2.05%
Free sulphur	0.27%
Density	1.429%

The pigment was prepared in two steps i.e., reductive and oxidative heating. In the first step the ingredients were packed in a vessel and tightly covered and then heated at 800 °C in a furnace for 4 h. The green pigment formed was then oxidized in open air, which changed the colour to blue. Overheating and under heating of the reactants in the crucible lead to heterogeneous product.

The nature of these steps is controversial. Some suggests as follows (Gruen *et al.*, 1971).

$$\underset{\text{Yellow}}{S_8} \rightarrow \underset{\text{Yellow}}{S_2} \rightarrow \underset{\text{Blue}}{S_3^-} \quad \text{[1]}$$

While others propose (Cork, 1993):

$$2Na_8[Al_6Si_6O_{24}]S_{2\text{-}3}+SO_2+O_2 \rightarrow 2Na_7[Al_6Si_6O_{24}]S_{2\text{-}3}+Na_2SO_4 \quad \text{[2]}$$

$$2S_{2\text{-}3}^{2-}(s)+SO_2(g)+O_2 \rightarrow 2S_{2\text{-}3}^{-}(s)+SO_4(s) \quad \text{[3]}$$

At 800 °C, alumino silicate framework is formed and the encaged polysulphides act as chromophores. Beta cage consisting of SiO_4 and AlO_4 forms aluminosilicate framework. Sodium cations also enter the cage and those outside the frame act as counter ions to balance the charges of polysulphides. These sodium ions are important and affect the shade of pigment. Ultramarines are similar to zeolites and sodium ions could be exchanged to other cations like K, Li, Ca, Mg, Ag, Rb, Cs, Sr, Ba, Tl, and Pt.

There are two forms of polysulphides i.e., S^{2-} and S^{3-}. In the greenish pigment S^{2-} are present. In the oxidative step S^{2-} is oxidized to S^{3-} form. In the blue pigment both S^{2-} and S^{3-} are present but S^{3-} dominates over S^{2-} species. The UV-visible spectrum of the pigment is shown in Fig. 2. A peak at ~600 nm and ~350 nm conforms the presence of S^{3-}(Lindner *et al.*, 1995). The peak at 350 nm is due to sodalite framework. The absorption at 600 nm indicates blue color of the pigment. Infrared spectroscopy is used for the confirmation of the sodalite structure formation. IR spectrum (Fig. 3) of the pigment also shows the presence of thiosulphatate and sulphate. XRD patterns of

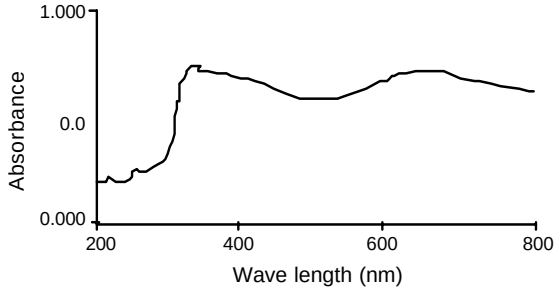

Fig. 2. UV-visible spectrum of blue pigment (suspension in glycerin).

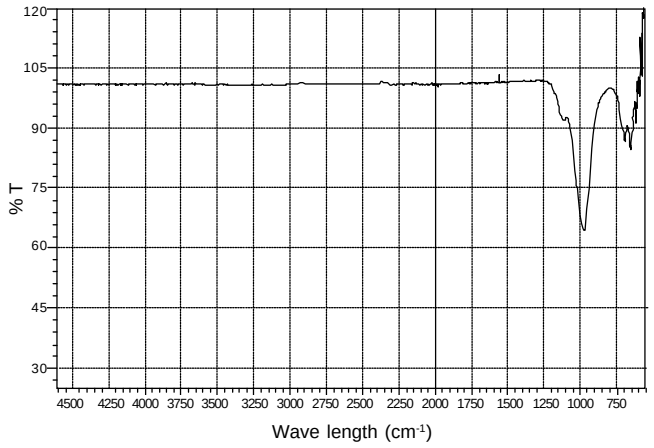

Fig. 3. FTIR spectrum of blue pigment.

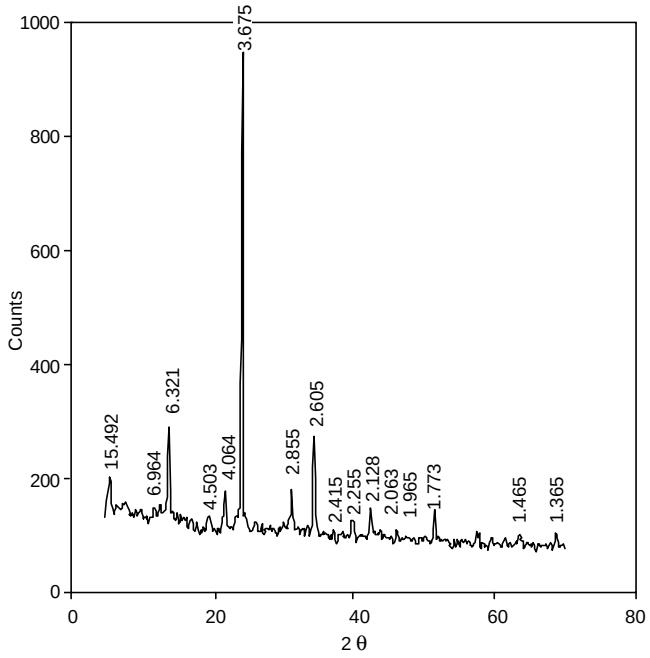

Fig. 4. XRD pattern of blue pigment.

the pigment (Fig. 4) matches well with the XRD pattern of the ultramarine blue (JCPDS-ICCD, 2000). XRD also confirms the presence of sodium sulphate in the pigment.

Conclusion

Composition of Swat kaolin is suitable for the preparation of ultramarine blue. Metals like iron, lead and copper are not in appreciable quantity to affect the synthesis of the pigment. Activated kaolin could be utilized for the preparation of ultramarine blue.

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Biological Sciences

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Evaluation of the Seed Oil of Three *Citrus* species, for the Control of the Bean Beetle, *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae)

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Abstract. On application of the seed oil of ripe and unripe fruits of *Citrus sinensis*, *C. paradisi* and *C. aurantifolia* to the cowpea bruchid, *Callosobruchus maculatus* (F) for three days, a dose of 0.5 ml of *C. sinensis* gave, significantly, high mortality rate upto 85%. In case of *C. aurantifolia*, mortality ranged from 75% to 100%. Same least dosage of seed oil of ripe *C. paradissi* produced 58.8% to 100% mortality, whereas, except the dose of 0.5 ml, all the other treatments of unripe *C. paradissi* resulted in 100% mortality after 24 h.

Keywords: pest control, *Citrus* seed oils, bean beetle, *Callosobruchus maculatus*

Introduction

Insects constitute 85% of all the known animal species (Richards and Davies, 1977), nearly one thousand species of which are associated with stored products (Banks, 1999); majority of them belong to Coleoptera (beetles) and Lepidoptera (moths) (Rees, 2004).

Production of cowpea, *Vigna unguiculata*, the most widely consumed and affordable source of protein in the tropics, is plagued by the field and storage insect pest infestation; some of them include *Aphis cracivora* (K). *Mealurothrips sjostedti* (T), *Maruca virata (testulalis) (Fab), Clavigralla tormentosicolis*, *Riptortus dentipes* and *Ootheca mutabilis* (Singh *et al*., 1990; Booker, 1965).

Dry cowpea seeds are infested by field-to-storage insect pests; damage by one of them i.e., *Callosobruchus maculatus* often leads to deterioration both in the quantity and quality of the produce. Caswell (1981) reported an annual cowpea damage of 24,000 tonnes due to infestation by *C. maculatus.* One hundred percent damage can be recorded within six months of the storage (Seek, 1993).

The most effective means of controlling this notorious pest is the use of synthetic insecticides (Obeng-Ofori and Dankwah, 2004; Anyim, 2003), continuous usage of which has produced some undesirable effects through inhalation of insecticidal dusts and residues. Apart from the high cost, chemicals also have adverse effects on non-target micro- and macro-fauna components of both aquatic and terrestrial ecosystems (Elhag, 2000).

In a bid to curtail the adverse effects of these synthetic insecticides, the activities of some cheap and environmentally friendly botanicals have been evaluated and found effective (Ogunleye, 2004; Tapondjou, 2002; Keita *et al*., 2001; Ogunleye, 2000). Linalool is a natural occurring terpene alcohol found as a major constituent of the essential oils of *Citrus sinensis* (Rutaceae) and *Occimum basilicum* (Lamiaceae) among others. It is also used by professionals as a flea and cockroach insecticide (Wikipedia, 2008a).

Plant-derived-oils have been used as repellant and antifeedant on insect pests by Akou-Edi (1985). According to him, laboratory trials in Togo using red corn treated with neem oil at concentrations of 1, 2, 3, 4 and 5 ml/kg infested with confused flower beetles and corn weevils showed significant difference between the treated and untreated samples. Addition of a little vegetable oil to stored rice or legumes for protection against stored insect pests has been established by researchers. Modes of action, appropriate dosage and duration of efficacy of oils on storage insect pests have been investigated by various workers (Rahman and Talukder, 2006; Singh, 1993). Mwaiko (1992) reported that *Citrus* peel oil extracts was successfully used as mosquito larvae insecticides. Present study was made of the oils of ripe and unripe fruits of three citrus species namely *Citrus sinesis*, *C. paradisi* and *C. aurantifolia* with reference to their effect on cowpea bruchid *Callosobruchus maculatus*.

Materials and Methods

Collection of samples. Seeds of ripe and unripe fruits of *C. sinensis*, *C. paradisi* and *C. aurantifolia* were collected from a farm land in Okitipupa local government area of Ondo State, in western Nigeria. The collected seeds were spread on the laboratory tables under ambient conditions of temperature 37 °C and humidity 51%, for a period of 3 months for complete air drying. They were then grinded with a Kenwood blender to

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powder form. Powders, moisture content of which was 13.00%, were kept separately in glass beakers and labelled appropriately. Temperature of the powders rose sharply to 50 °C during the process of grinding. They were then kept on the laboratory table to assume normal room temperature of 37 °C before the experiment.

Extraction of oil. Two hundred grams (200 g) of each sample of the ground seeds were measured in 500 ml conical flasks separately. To each of these conical flasks were added 450 ml of petroleum ether and thereafter, the mixture was shaken thoroughly at regular intervals for 3 days to extract the oil content of the material. An aluminum foil was used to cover the conical flasks to avoid evaporation of the solvent. After 3 days, the solution from each container was decanted and later filtered into separate 250 ml conical flasks. Conical flasks were left open to allow for escape of the solvents, leaving behind the oil.

Insect culture. Pure culture of *Callosobruchus maculatus* was maintained in the laboratory. Clean uninfested seeds of brown cowpea were kept in kilner-jar like container. Twenty randomly selected species of *C. maculatus* were introduced into the cowpea containers and left on the laboratory table under ambient environmental conditions of 32 °C and 60% relative humidity (RH). The insects multiplied in the containers within six weeks. All the insects used for this experiment were taken from these containers.

Bioassays. Fifty grams (50 g) of cowpea seeds were measured separately into kilner-jars with a Metler balance. The extracted oils were added separately to the jar in the quantities of 0.5 ml, 1.0 ml and 1.5 ml with the aid of hypodermic syringe. The experiment was replicated 3 times. The oil was properly mixed with the cowpea seeds by shaking the containers vigorously to ensure its even distribution on the seeds.

Twenty (20) newly emerged adults of *C. maculatus* (without sexing) were introduced into each kilner-jar with treated and also in untreated (control) cowpea containers. Insect mortality was noted every 24 h for a period of 3 days.

Statistical analysis. All the data were subjected to analysis of variance and means were separated using Fisher's least significant difference (LSD) at 5% level of significance (Wikipedia, 2008b).

Results and Discussion

Results of the effect of different doses of seed oil from the ripe and unripe fruits of *Citrus sinensis* on *C. maculatus*, for 3 days are given in Table 1.

Table 1. Effect of the seed oil of ripe and unripe *C. sinensis* on *C. maculatus*

Treatment	Mean percentage mortality (Days)		
	1	2	3
Ripe *C. sinensis*			
0.5 ml	85±4.1[a]	87.5±8.5[a]	100±0[a]
1.0 ml	93.8±2.5[a]	100±0[a]	100±0[a]
1.5 ml	100±0[a]	100±0[a]	100±0[a]
Unripe *C. sinensis*			
0.5 ml	75±4.1[a]	88.8±8.5[a]	100±00[a]
1.0 ml	81.3±2.5	100±00[a]	100±00[a]
1.5 ml	100±00[a]	100±00[a]	100±00[a]
Control	0±0[b]	1.25±0[b]	1.25±0[b]

Means followed by the same letters are not significantly different at 5% level using Fisher's LSD

At 0.5 ml dose of the seed oil application, mortality rate of *C. maculatus* ranged from 85% to 100% during 3 days. At 1.0 ml dose, mortality was 93.8% on the first day and 100% on the second day. The highest dose of 1.5 ml resulted in 100% mortality within 24 h, post application. Gradual increase in the effectiveness of the oil, with increase in the rate of application, corroborates the findings of Akou-Edi (1985) that the effect of plant-derived oil increased at higher concentration. Mortality rate for the control was 0% on the first day and 1.25% on the second and the third day. In the case of application of oil of unripe fruits, at the doses of 0.5 ml, 75%, 88.8% and 100% mortality was obtained on 3 consecutive days. At 1.0 ml dose, insect mortality reached its peak on the second day and at 1.5 ml dose after 24 h of application.

Statistical analysis revealed that there were significant differences between the treatments and the control at 5% level of probability using Fisher's least significant difference. This indicates effectiveness of the test materials even at the lowest rate.

Ortuno *et al*. (2006) reported that the level of heptamethoxy flavone is high in *C. sinensis* which *in vitro* acted as the defense mechanism of *Citrus* sp., against the fungus, *Penicillium digitatum*.

Table 2 presents the percentage mortality of *C. maculatus* treated with ripe and unripe C*itrus aurantifolia* seed oil. Mortality ranged from 75.0% - 100% for the least dosage of the ripe fruit oil from the 1st to the 3rd day. At higher application rates, mortality increased with increase in dosage. In the present experiment involving unripe *C. aurantifolia*, all the doses of seed oil produced 100% mortality from the 1st day to the 3rd day except the smallest dose which produced insect mortality of 75% and 92.5 on the 1st and the 2nd day and 95% at

Table 2. Effect of the seed oil of ripe and unripe *C. aurantifolia* on *C. maculatus*

Treatment	Mean percentage mortality (Days)		
	1	2	3
Ripe *C. aurantifolia*			
0.5 ml	75±2.9[a]	92.5±6.5[a]	100±0[a]
1.0 ml	92.5±26.5[a]	100±0[a]	100±0[a]
1.5 ml	100±0[a]	100±0[a]	100±0[a]
Unripe *C. aurantifolia*			
0.5 ml	75±2.0[a]	92.5±6.5[a]	100±00[a]
1.0 ml	92.5±6.5[a]	100±00[a]	100±00[a]
1.5 ml	100±00[a]	100±00[a]	100±00[a]
Control	0.0±0[b]	0.0±0[b]	0.0±0

Means followed by the same letters are not significantly different at 5% level using Fisher's LSD

Table 3. Effect of the seed oil of ripe and unripe *C. paradisi* on *C. maculatus*

Treatment	Mean percentage mortality (Days)		
	1	2	3
Ripe *C. paradisi*			
0.5 ml	58.8±8.5[a]	85.0±4.1[a]	100±0[a]
1.0 ml	96.3±4.8[a]	100±0[a]	100±0[a]
1.5 ml	100±0[a]	100±0[a]	100±0[a]
Unripe *C paradisi*			
0.5 ml	75±4.1[a]	96.3±0[a]	100±0[a]
1.0 ml	100±0[a]	100±0[a]	100±0[a]
1.5 ml	100±0[a]	100±0[a]	100±0[a]
Control	0±0[b]	0±0[b]	0±0[b]

Means followed by the same letters are not significantly different at 5% level using Fisher's LSD

1.0 ml dose on the 1st day. The mortality of insects in the control experiment remained at zero level. *C. aurantifolia* oil, at a dosage of 7 ml/kg, caused 100% mortality in adult *C. maculatus* after one hour exposure (Don-Pedro, 1996).

Four coumarines were isolated, purified and identified from *C. aurantifolia*, which are limonene, bergapten, imperatin and isopimpinellin (Tasneem, 1995). The effectiveness of this seed oil might be the result of the presence of these phytochemicals in species of *Citrus*. Isman (2000) reported that plant essential oils were effective in pest and disease management.

The result of the experiment with *C. paradisi* is presented in Table 3. At 0.5 ml application, mortality was between 58.8%, and 100% during three days. At 1.0 ml application, mortality was 100% after 48 h while the dose of 1.5 ml resulted in 100% mortality even after 24 h.

In the case of unripe *C. paradisi*, except the dose of 0.5 ml, which produced mortality of 75% and 96.3% on the 1st and the 2nd day, all the other treatments resulted in 100% mortality, Whereas the control showed none.

C. paradisi is reported to contain the flavonoid, diglycoside, (Pelt *et al*., 2003). According to Fenaroli (1995), the main constituents of the essential oil obtained by cold expression of the fresh peels of redblush grapefruit (*C. paradisi*) was limonene (90%), while 2 to 3% volatile fraction contained oxygen compound and sesquiterpenes. These findings were corroborated by the research findings of Njoroge *et al*. (2005), according to whom, the volatile constituents of this oil contained limonene (91.1%), terpene (1.3%) and sesquiterpene hydrocarbons (0.4%). This might explain the level of effectiveness.

There were no significant differences in the level of effectiveness of the ripe and unripe seed oils of the three *Citrus* species. It can also be inferred that the mechanism of action of these oils is by contact with the body, through preventing enough oxygen from getting into the internal organs.

Apart from the smallest dosage rate of *C. paradisi*, all other doses of seed oil of ripe and unripe fruits of the three plant species used in the present work were effective against *C. maculatus*. Therefore its use against this notorious pest species is recommended. Furthermore, since the oils are potent at the dose of 0.1 ml per 50 g of cowpea seeds, farmers are encouraged to employ this application rate to reduce costs to the minimum. Oils of either ripe or unripe seeds could be used. The comparative effectiveness of ripe *vis a' vis* unripe seed oils for insecticidal activities has not been reported in literature.

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Growth Measurement of Some Amylolytic *Bacillus* Species in Three Media

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Abstract. Study of the growth pattern of some *Bacillus* species on starchy substrates showed that the metabolic activity affected the enzymatic activity. *B. subtilis* (WBS), *B. licheniformis* (WBL) and *B. coagulans* (MBC) generally had higher growth rate. *B. circulans* (SBC) and *B. coagulans* (WBC) had higher growth on cornstarch medium with corresponding higher ɓ-amylase production as compared to other strains such as *B. polymyxa.* Ten of the 13 *Bacillus* species studied had better performance on cornstarch than on soluble starch except *B. macerans* (MBM), *B. macerans* (SMB2) and *B. subtilis* (WBS). The enzyme production ranged from 0.022 unit/cfu x 10^2 to 0.912 unit/cfu x 10^2 on cornstarch and 0.01 unit/cfu x 10^2 to 0.693 unit/cfu x 10^2 on soluble starch. Relatively higher â-amylase activity was observed in *B. subtilis, B. licheniformis, B. macerans* and *B. circulans* (WBC1).

Keywords: *Bacillus* sp., starch, beta amylase production, enzymatic activity

Introduction

Many environmental factors and culture media components greatly affect the metabolic processes in microorganisms. Ajayi and Fagade (2006), Lin *et al.* (1997) and Amoa-Awua and Jakobsen (1995), in their study demonstrated the metabolic activity of some microbial strains and the corresponding enzymatic productivity. Previous researches have also shown that medium composition affects enzymatic activities as well as sporulation in some microorganisms including *Bacillus* sp., (Ajayi and Fagade, 2006; Ray *et al.*, 1995). Starch induces amylase production but there are reports indicating that starch may not be required for amylase production probably in organisms having constitutive enzymes (Shittu *et el.*, 2005; Srivastava and Baruah, 1986; Burbidge and Collier, 1958). Thus the nature of substrate, including the nitrogen source and mineral element components of culture medium, affects metabolic processes in the microorganisms.

Bacillus species and other forms of microorganisms grow at different rates with specificity to different substrates in culture medium (Tobey and Yosten, 1977). The growth conditions also influence their enzymatic activities (Nortermann, 1992). Generally, media composition, cultural conditions, microbial cell biochemistry and physiology play vital roles in amylase producing mechanisms of *Bacillus* species (Bezbaruah *et al.*, 1994; 1987).

In the present work, study was made of the growth of 13 amylase producing *Bacillus* species on starch and their corresponding amylase production activity, also with reference to carbon source.

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Materials and Methods

The *Bacillus* strains for this study were obtained from wastewater, soil and milk sources in Ibadan, Oyo State, Nigeria. A sporulating chemically defined medium was employed to aid the suitable growth and recovery of *Bacillus* species, as described by Leicth and Collier (1996). Amylolytic *Bacillus* sp., were identified by standard microbiological techniques (Kotzekidou, 1996) and selected for final study by using starch hydrolysis procedure (Cowan and Steel, 1985; Difco, 1984).

Each organism was sub-cultured in nutrient agar medium and incubated for 24 h at 35 °C. Loopful of each sample was transferred to test tube containing sterile distilled water, thoroughly mixed and serially diluted to provide a homogeneous liquid suspension to be used as inoculum containing an estimated 10^6/cfu/ml of broth. Pour plate count technique and microscopy was used for the estimate. Samples were plated out immediately.

The growth pattern of *Bacillus* strains were studied by culturing the samples in different media supplemented with cornstarch, soluble starch and compared with the nutrient broth medium that served as the base medium. One ml of the appropriate dilution with similar range of count was inoculated into nutrient broth base medium supplemented with different carbon sources specified above and the nutrient broth base without supplement. This was cultured for 24 h at 37 °C. Ten fold dilution was made for each sample and analyzed at 6

to 24 h intervals using a spectrophotometer at 610 nm wavelength.

Amylolytic bacterial isolates recovered from sampled sources were cultured in a 50 ml broth medium containing (w/v): peptone (2%), starch (0.5%), K_2HPO_4 (0.3%), and $MgSO_4.7H_2O$ (0.1%) in Erlenmeyer flask of 200 ml capacity for 40 h at 30 °C on a rotatory shaker (Model G24 Environmental Incubator Shaker, N.J., USA) at 150 rpm. The cultivated cells were removed by centrifugation for 15 min at 4000 rpm and the resultant supernatant was used as the enzyme source.

Determination of the saccharifying capability of the enzyme to release the reducing sugar was made by dinitrosalicylic acid (DNSA) method (Bailey, 1988; Murao *et al.*, 1979) as described below.

Soluble starch and white cornstarch substrate 1.0% were dissolved in phosphate buffer (pH 7.0). A measure of 0.1 ml of the crude enzyme was added to 1 ml of the substrate. After incubation for 10 min at 37 °C, the reaction mixture was stopped by adding 2 ml of DNSA reagent. The reaction mixture was heated at 100 °C for 10 min, cooled and then 17 ml of distilled water was added. The reaction mixture was allowed to stand for 15 min at the room temperature. Optical density of each sample was measured using a spectrophotometer (Model Pye Unicam, USA). The spectrophotometer was set up in a regulated environment usually with air conditioner and allowed to warm up for 15 min to enhance accurate reading. The optical meter gauge was standardized with a blank and control sample put into a cuvette that was fixed appropriately into the spectrophotometer. The control sample was buffered substrate solution which was compared with the test enzyme sample to give corresponding values for estimation of reducing sugar released at 530 nm.

Results and Discussion

Bacillus species obtained from various sample sources such as soil, wastewater and food (milk) sources demonstrated relatively higher growth value on the cornstarch, compared with that on the soluble starch while the nutrient broth, which served as control, recorded low growth range as shown in Fig. 1 and Table 1. Ten *Bacillus* strains utilized cornstarch better than soluble starch for enzyme production except three namely *Bacillus macerans* (SBM1), *B. macerans* (MBM) and *B. subtilis* (WBS) (Table 2). The strains *B. subtilis* (WBS), *B. licheniformis* (WBL) and *B. coagulans* (MBC) generally had high growth rate. *B. circulans* (SBC) and *B. coagulans* (WBC) had specific affinity for growth and some enzymatic activity was observed on the cornstarch medium with high growth value of 1.118 and 1.080 units at 48 h. Correspondingly

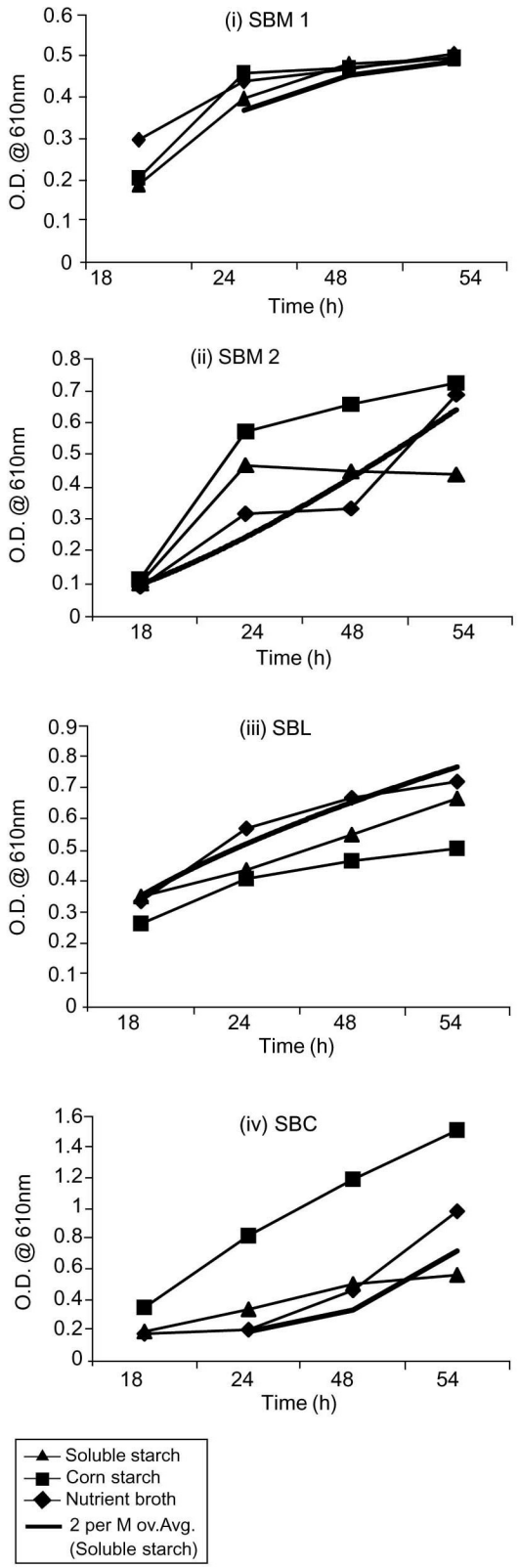

Fig. 1a(i-iv). Comparative (O.D. measurement)growth pattern of the isolated *Bacillus* species in different media. (Soil source: University of Ibadan, Nigeria).

Table 1. Assessment of carbon source utilization by amylolytic *Bacillus* species

Sources	Strain code	*Bacillus* species	Total bacterial count (CFU/ml x10^2)			SE (%)
			Corn starch substrate	Soluble starch substrate	Nutrient broth medium	
Soil, U.I.	SBM	*B. macerans*	8.0	5.0	4.0	12.25
Canned milk, Ibadan	MBM	*B. macerans*	25.0	20.0	9.0	15.16
Wastewater, U.I.	WBC	*B. coagulans*	3.0	3.0	0.4	22.50
Canned milk	MBC	*B. coagulans*	6.0	2.0	1.0	29.40
Soil, U.I.	SBL	*B. licheniformis*	7.0	6.0	0.5	27.80
Wastewater, U.I.	WBL	*B. licheniformis*	20.0	5.0	18.0	18.94
Soil, U.I.	SBC	*B. circulans*	2.0	1.8	2.0	1.98
Wastewater, U.I.	WBC	*B. circulans*	16.0	34.0	2.7	29.79
Soil, U.I.	SBG	*B. megaterium*	12.0	11.0	12.0	1.66
Wastewater, U.I.	WBP	*B. polymyxa*	8.0	7.4	15.0	13.90
Wastewater, U.I.	WBS	*B. subtilis*	9.0	7.0	19.0	18.37
ATCC, (USA)	ATCC 11778	*B. cereus*	2.5	1.9	1.7	6.32
	Mean		9.87	8.67	7.11	10.73

SE = standard error; U.I. = University of Ibadan, Nigeria

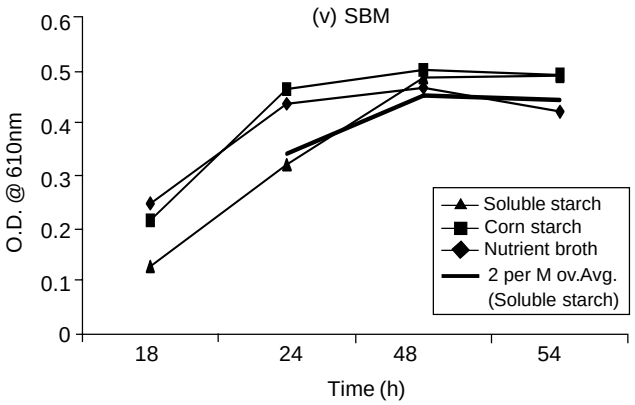

Fig. 1a(v). Comparative (O.D. measurement)growth pattern of the isolated *Bacillus*s pecies in different media. (Soil Source: University of Ibadan, Nigeria)

ь-amylase production was higher with values of 0.606 unit/O.D. and 0.667 unit/O.D., respectively, as compared to the other lower growth indices observed in *B. polymyxa* (WBP) of 0.425 unit but higher enzyme production value of 1.129 unit/O.D (Fig. 1).

The *Bacillus* species demonstrated different patterns of growth rates with relatively higher values in starchy medium (Fig. 1a-c; Table 1). The enzyme production values ranged from 0.022 unit/cfu x 10^2 by *B. circulans* (WBC) to 0.912 unit/cfu x 10^2 by *B. licheniformis* (WBL) for cornstarch and 0.01 unit/cfu x 10^2 by both *B. megaterium* (SBG) and *B. licheniformis* (SBL) to 0.693 unit/cfu x 10^2 by *B. subtilis* (WBS) for soluble starch (Table 2). These results agree with those of Hensley *et al.* (1980) who reported good yields of ь-amylase on corn steep liquor among various complex media by selected strains of *Bacillus* species, like *B. circulans.* Srivastava and Baruah (1986) also found corn steep liquor to be the best. The disadvantage of the corn steep liquor was that it contains many chemical ingredients, and it was difficult to ascertain which of them induced amylase production. Therefore, the use of chemically defined medium as used in this study is required for enzyme production activities (Lederberg, 1992; Srivastava and Baruah, 1986). Some amylolytic enzymes of *B. macerans* were active in starch-containing media, and the enzyme accumulated as the concentration of the carbon source declines (Priest, 1977). During the study, *B. macerans* was encountered among the amylolytic *Bacillus* species. In this study the organisms used have capabilities to produce amylase and this was influenced by the effect of the regulated conditions especially in the utilization of cornstarch substrates compared with other carbon sources. This greatly affected the quality or characteristics of the enzymes produced and it conformed with the studies of Montgomery *et al*. (1990) and Srivastava and Baruah (1986); they stated that the nature and characteristics of enzymes produced by different species of bacteria, depends on the strains of bacteria involved, moreover an optimal growth condition may be determined for each strain.

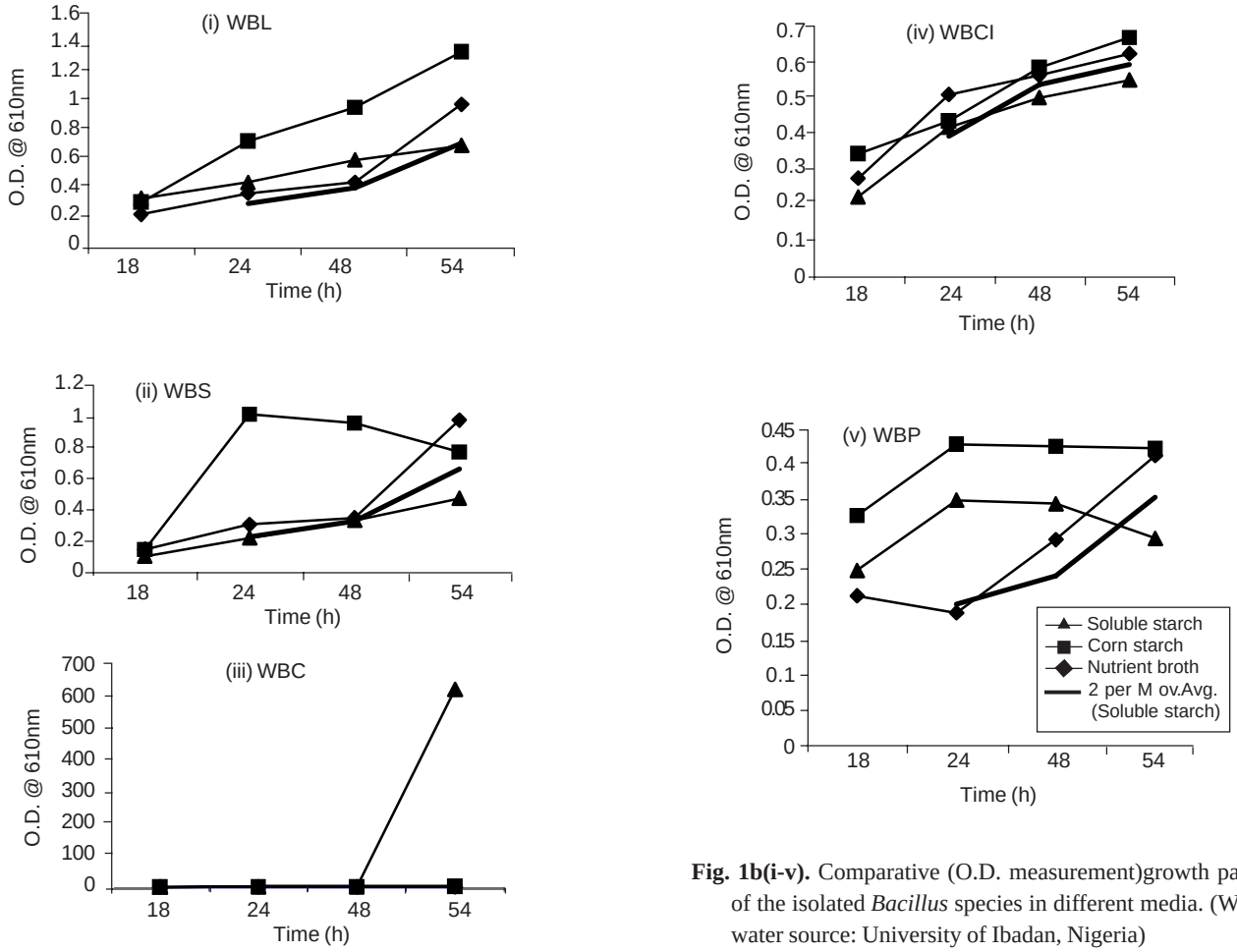

Fig. 1b(i-v). Comparative (O.D. measurement)growth pattern of the isolated *Bacillus* species in different media. (Waste-water source: University of Ibadan, Nigeria)

Table 2. Comparative enzyme production in soluble starch and cornstarch carbon sources

		Corn starch medium			Soluble starch medium		
Strain code	*Bacillus* species	Amylase (unit/ml)	*Bacillus* population (cfu x 10^2)	Amylase (unit/cfu x 10^2)	Amylase (unit/ml)	*Bacillus* population (cfu x 10^2)	Amylase (unit/cfu x 10^2)
SBM	*B. macerans*	1.32	8.0	0.165	0.72	5.0	0.144
MBM	*B. macerans*	1.80	25.0	0.072	3.0	20.0	0.15
SBM1	*B. macerans*	1.80	16.0	0.112	1.56	15.0	0.104
SBM2	*B. macerans*	0.72	17.4	0.41	1.80	1.5	1.2
WBC	*B. coagulans*	0.72	3.0	0.24	0.36	3.0	0.12
MBC	*B. coagulans*	1.32	3.0	0.44	0.84	2.0	0.42
SBL	*B. licheniformis*	0.72	7.0	0.102	0.12	6.0	0.02
WBL	*B. licheniformis*	4.56	6.0	0.912	4.2	5.0	0.84
SBC	*B. circulans*	0.72	2.0	0.36	0.12	1.8	0.06
WBCI	*B. circulans*	0.36	16.0	0.022	0.72	34.0	0.02
SBG	*B. megaterium*	0.72	12.0	0.06	0.12	11.0	0.01
WBP	*B. polymyxa*	0.48	8	0.06	0.12	7.4	0.016
WBS	*B. subtilis*	0.72	9.0	0.08	6.24	7.0	0.89

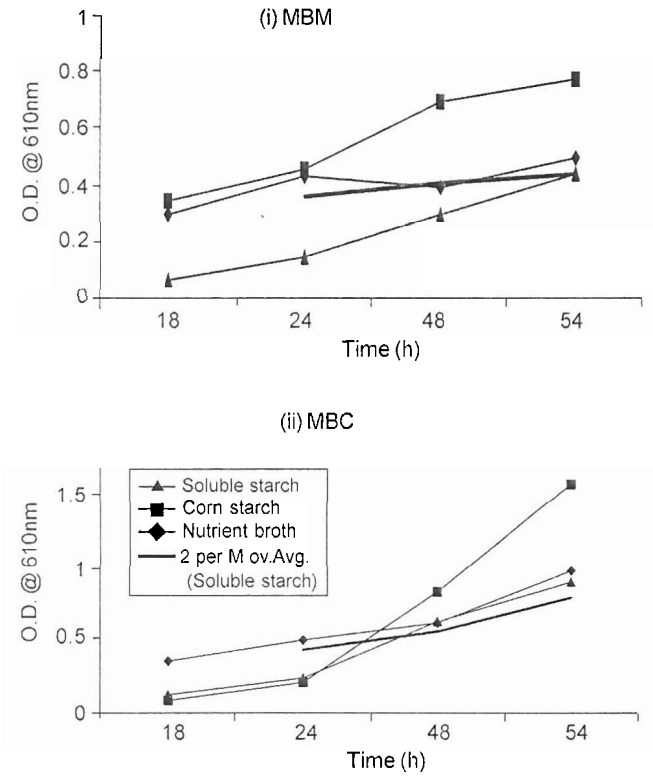

Fig. 1c. Comparative (O.D. measurement) growth pattern of the isolated *Bacillus* species in different media (canned milk).

The carbon sources used in the study were soluble starch, cornstarch, glucose and sucrose. They all influenced the activity of amylases (Table 3). Soluble starch carbon source

Table 3. Effect of carbon sources on amylase production (unit/ml) on soluble starch buffered substrate

Carbon substrate	SBM	MBM	MBC	WBL	WBS
Soluble starch	0.72	3.0	0.84	4.2	6.24
Corn starch	0.84	0.84	1.32	3.72	0.12
Glucose	0.84	0.84	0.6	0.72	1.20
Sucrose	0.24	0.72	–	0.48	0.96

favoured high enzymatic activity which ranged between 0.12 unit/ml for *B. licheniformis* (SBL strain), *B. megaterium* (SBG) and *B. polymyxa* (WBP) to 6.24 unit/ml by *B. subtilis* (WBS). Cornstarch substrate in the culture medium also recorded high yield of amylase ranging from 0.12 unit/ml for *B. macerans* (SBM2), *B. coagulans* (WBC), *B. licheniformis* (SBL), *B. megaterium* (SBG), *B. subtilis* (WBS) to 3.72 unit/ml by *B. licheniformis* (WBL). The enzymatic activity of the *Bacillus* strains with use of sucrose as carbon source was very low. This ranged from 0.24 unit/ml to 0.72 unit/ml among the three strains that showed some activity. Nevertheless, results with reference to the cornstarch buffered substrate varied (Fig. 1a-c).

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Endemicity of Urinary Schistosomasis in Ogbese-Ekiti Community of Ise-Orun Local Government Area of Ekiti State, Nigeria

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Abstract. In random examination of 191 students of Ogbese-Ekiti community of Nigeria for urinary schistosomasis, 170 (89%) were found positive for *Schistosoma haematobium* eggs in their urine. The prevalence in the secondary school was 97.4%, while the prevalence in the primary school was 87.5%. The overall mean intensity of *S. haematobium* eggs/10 ml of urine in this community was 339.4. Also, 5.9% of the infected pupils excreted above 1000 eggs/10 ml of urine, while 59.8% had moderate intensity (50-499 eggs/10 ml of urine). The perentage macrohaematuria was 84. Among five aquatic snails *Bulinus* (*B*) *forskalii, Bulinus* (*B*) *globosus*, *Pila ovata*, *Potadoma moerchi* and *Melanoides tuberculata* of river Ogbese, only *B.* (*P*) *globosus* shed the characteristics cercariae of *S. haematobium.* A monthly mean of *B. globosus* in river Ogbese was 53.2 and an increase in the population density of the snail occurred between November and May, 2004. The highest infection rate of *B.* (*P*) *globosus* with *S. haematobium* occurred in the month of March.

Keywords: schistosomiasis, aquatic snail, *S. haematobium*, Ogbese-Ekiti

Introduction

Schistosomiasis is widespread in tropical Africa and considerable amount of work has been done on African schistosomiasis (Cowper and Woodword, 1961). The prevalence of the disease and the distribution of the snail intermediate hosts differ in different parts of the continent (WHO, 1980). Schistosomiasis is endemic in Nigeria (Adewumi *et al.*, 1991; Ozumba *et al.*, 1989; Edungbola, *et al*., 1988). Investigation carried out in Nigeria indicates a widespread and intensive transmission of schistosomiasis, with exceptionally high prevalence of the disease among children living where water based activities are very common (Akogun and Okin, 1993; Betterton, 1984). The status of urinary schistosomiasis in southwestern Nigeria has been the subject of many publications (Okoli and Odaibo, 1999; Mafiana and Adesanya, 1994; Cowper and Wood Ward, 1961). The status of urinary schistosomiasis in each of the Local Government Areas of Ekiti State has already been documented (Ologunde, 2004). Ogbese-Ekiti is located in Ise-Orun Local Government Area of Ekiti State and no published data is available on the status of urinary schistosomiasis in this community. This paper describes the status of urinary schistosomiasis in Ogbese community of Ise-Orun Local Government Area of Ekiti State, Nigeria.

The study area – Ogbese-Ekiti. The study area, Ekiti-State of Nigeria is situated between latitudes 7°.15′ N to 8°,10′ N and longitudes 4°.45′ E to 5°,45′ E. Osun, Kwara, Kogi and Ondo States bound Ekiti-State. Ekiti-State lies in the southern climatic belt, which is characterized by the rainy season of about eight months (March-October) and the dry season of about four months (November-February) (Barbour *et al*., 1982). Ogbese river which is the major source of water to all inhabitants of Ogbese-Ekiti is a stretch of several kilometers that runs through several communities in Ekiti, Ondo and Edo States. The river is rich in aquatic vegetation, particularly *Nymphaea lotus, Pistia stratiotes, Ludwigia octovalvi*, which alter in density from season to season. During the dry season, many pockets of water are found along the river course. Human activities in river Ogbese include swimming, fishing, washing of legs, clothes and vehicles, wading and using water for domestic purposes.

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Materials and Methods

Collection and examination of urine. Three schools (two primary and one post primary) in Ogbese-Ekiti were surveyed for urinary schistosomiasis (Table 1). Total student population of these three schools was 602. School children were randomly selected from the names in class registers to participate in the survey. Urine samples were collected from 191 school children aided by their class teachers between 10.00 and 13.00 h and the samples were labelled appropriately. In the laboratory, each of the sample was thoroughly mixed to ensure even distribution of contents. An aliquot of 10 ml of each sample was centrifuged at 2000 rpm for 5 min. The supernatant (9 ml) was decanted and the sediment was pipetted on to microscopic slides and the number of eggs were counted using hand counter. The number of eggs in 10 ml of each urine sample was calculated from the mean of results of two counts

by proportionality and recorded as number of eggs/10 ml of urine.

Snail sampling. A survey was carried out in Ogbese river to determine the types of aquatic snails present between January 2005 to August 2006. Sampling was done using a long handled sieve net, 1 mm mesh size. Snails were sampled twice a month. At each visit, the population of *B.* (P) *globosus* was determined by carefully searching for 15 minutes as described by Fashuyi (1976) and Ologunde (2004). The number of snails was recorded as average number of snails/man/15 min and the population was recorded as a mean of two collections. Only snails of a shell diameter of over 4 mm were examined for infections (Ologunde, 2004; Fashuyi, 1976; Shiff, 1964).

Water contact activities. Water contact activities were determined from questionnaire data (Okoli and Odaibo, 1999; Udonsi, 1990; Chandiwana, 1986). Each student aided by the class teacher and/or member of the survey team was asked to complete a questionnaire, for providing information concerning his/her water contact and water usage. Water contact was classed according to the extent of body immersion. Complete long term contact (swimming) was given a weightage of 5. Partial and medium term contact (clothes washing, fetching of water, fishing, washing of legs/hands and washing of plates) was given a weightage of 3 and limited and short term contacts (wading across) was given a weightage of 1. These gave the exposure index of the children to each water contact activity (Okoli and Odaibo, 1999). A total of 80 students filled the questionnaire on water contact activities in the study area.

Results and Discussion

The data on the prevalence of urinary schistosomiasis among the school children in Ogbese community are shown in Table 1. 170 (89%) of the students consisting of 107 (62%) males and 63 (38.0%) females were found to be infected with *S. haematobium*. As shown in Table 2, infection occurred evenly among different age groups with peak among the 17-20 years age group (100.0%). Chi square analysis shows that there is significant difference in the prevalence of infection between sex ($P<0.5$). The distribution of egg counts by sex (Table 3) shows a similar pattern for both sexes. Over one hundred and forty seven (86.5%) of the 170 infected school children excreted 50 or more eggs per 10 ml urine. One hundred and forty three (84.5%) infected school children had haematuria. The snail fauna of the area are predominantly *B.* (*B*) *globosus*, *B.* (*B*) *forskalii, Potadoma moerchi* and *Pila ovata*. The population and infection dynamics of *B.* (*P*) *globosus* is presented in Fig. 1. The highest number of *B.* (*P*) *globosus* was recovered in February. The water contact activities of the primary and post primary school children is presented in Table 4.

Table 1. Prevalence and mean intensity of *Schistosoma haematobium* infection among primary and secondary school pupils in Ogbese community of Ise Orun Local Government Area of Ekiti-State

Name of School	No. of pupils examined	No. of pupils infected	Prevalence (%)	Mean intensity
St. Andrew's Anglican Primary School	99	81	81.8	291.9
Ogbese Community Primary School	16	15	93.8	339.82
Ogbese Comprehensive High School	76	74	97.4	362.5
Total	191	170	89.0	339.4

Table 2. Intensity of *Schistosoma haematobium* infection by age of primary and secondary school pupils in Ogbese Community of Ise-Orun Local Government Area of Ekiti-State

Age group (years)	No. of pupils examined	No. of pupils infected	Prevalence (%)	Mean intensity
5-10	53	42	79.2	267.4
11-16	128	118	92.2	362.1
17-20	10	10	100.0	373.3
Total	191	170	89.0	339.4

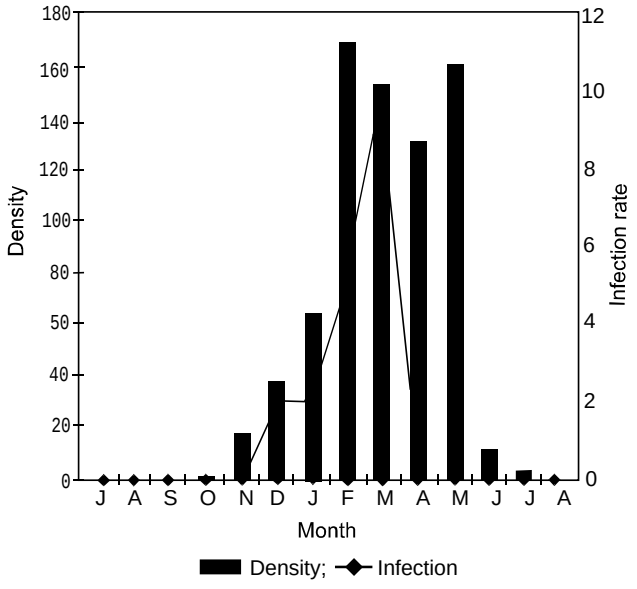

Fig. 1. Density and infection rate of *B.* (*P*) *globosus* in Ogbese river.

Table 3. Intensity of *Schistosoma haematobium* infection by sex in the primary and secondary school pupils in Ogbese Community of Ise-Orun Local Government area of Ekiti State

Sex	No. of infected pupils	Eggs intensity				<50 eggs/10 ml	Overall mean intensity
		Low intensity (1-49/10 ml)	Moderate intensity (50-499/10 ml)	High intensity (500-999/10 ml)	Above <1000/10 ml		
Male	107(62%)	13(12.1%)	57(53.3%)	29(27.1%)	8(7.4%)	94(87.9%)	388.00
Female	63(38%)	10(15.9%)	43(68.3%)	8(12.7%)	2(3.2%)	53(84.1%)	290.0
Total	170	23(13.5%)	100(59.8%)	37(21.8%)	10(5.9%)	147(86.5%)	339.4

Table 4. Water contact activities of primary and post-primary school children in Ogbese-Ekiti Community

Water contact activity	Frequency of exposure	Exposure total index	Exposure (%)	Frequency of exposure	Male exposure index	Exposure (%)	Frequency of exposure	Female exposure index	Exposure (%)
Complete and long term exposure									
Swimming	49	245	29.6	29	145	30.7	20	100	28.1
Partial and medium term exposure									
Clothes washing	45	135	16.4	23	69	14.6	22	66	18.5
Water fetching	51	153	18.5	29	57	18.4	22	66	18.5
Fishing	9	27	3.3	7	21	4.5	2	6	1.7
Washing of legs/hands	59	177	21.4	37	111	23.5	22	66	18.5
Washing plates	17	51	6.2	7	21	4.5	10	30	8.4
Short term contact									
Wading	40	40	4.8	18	18	3.8	22	22	6.2
Total exposure	270	828	100	150	472	57.0	142	359	43.0

With an overall infection rate of 89% among primary and secondary school pupils, prevalence of schistosomiasis is very high in Ogbese-Ekiti community. Extensive work had earlier been carried out by Ologunde (2004) on the status of urinary schistomiasis in each Local Government Area of Ekiti State. The prevalence rate according to that study ranged between 1.9-36.3%. Ogbese community is perhaps the only community within Nigeria especially in southwestern Nigeria with such high prevalence rate of disease in Ekiti State.

The age and sex related pattern of distribution of urinary schistosomiasis infection in man has been widely reported by various authors (Ugbomoiko, 2000; Okoli and Odaibo, 1999; Fajewonyomi and Afolabi, 1994). This study shows that prevalence and intensity of infection varied with sex and age of school children. Urinary schistosomiasis was most prevalent between the ages of 17 and 20 years (100%). Analysis of the egg output of the infected children in this study shows that all the different age groups (Table 2) were important in relation to the transmission of the disease. An overall mean intensity of 339.4 eggs/10 ml of urine is very high and accounts for the high prevalence rate. Mass chemotherapy and health education are therefore recommended as measurers of preventing and controlling the disease especially among the affected school children. It will reduce potential of transmission, reduce morbidity and minimize the risk of developing complications later in life.

Ogbese River is the only source of water available for Ogbese community. The water serves both domestic and recreational purposes. Water contact activities of the community with reference to the river is high (Table 4). And since the appropriate intermediate host and egg excretion is very high, prevalence rate will definitely be high. Only five aquatic snails were recovered from river Ogbese; among these only *B.* (*P*) *globosus* shed the characteristic cercariae of *S. haematobium.* The population and infection dynamics of *B.* (*P*) *globosus* over a period of fourteen months is presented in Fig. 1. The chance of acquiring infection is high in the month of November onwards, at the onset of the dry season and

continue to increase steadily up to the month of March at the onset of the rain. Any strategy adopted for control of the snail (intermediate host) should, therefore, be affected between November and March. Again, in the month of February the water level had greatly reduced with only few pockets of water along the river course. Focal molluscide during this month will, therefore, be one of the effective methods of controlling the snail intermediate host. Efforts should be made to reduce water contact activities through provision of alternative water supply that reduces chances of acquiring infection. The high prevalence of urinary schistosomiasis in Ogbese-Ekiti can be used to assess the success or failure rate of the Federal and State Governments primary health care programme. A more integrated approach is required to effectively control schistosomiasis in Nigeria.

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Dynamics of Clay Mineralogy With Profile Depth in Relation to Long Term Potassium Fertilizer Application to Sugar Cane Crop

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Abstract. The experiment consisted of treatment of sugar cane crop with N, NP, NPK and farmyard manure and determination of its effect on soil mica, vermiculite and montmorillonite over a period of 18 years. The NPK treatment had greater mica in coarse clay, but less in fine clay than NP and control treatments. Vermiculite in coarse clay fraction, in NPK treatment, increased with the depth as compared to other treatments. The fertilizer treatment effect on smectite content was obvious only in AP horizon in fine clay fraction.

Keywords: clay mineralogy, potassium fertilizer, sugar cane

Introduction

Potassium requirements of plants are mostly met from soil K resources. With respect to availability to plants, soil K exists as structural, exchangeable and soluble potassium. Mineral K occurs as mica and feldspars and amounts to about 98% of all the soil K, while readily available form of K is only 1-2% and occurs as exchangeable and soluble potassium. Sugar cane takes up K from solution that is buffered with exchangeable and structural potassium of soil system. Therefore, solution K depletion due to plant growth enhances weathering of mica and K feldspars. Among the two types of mica, biotite weathers at a rate faster than muscovite. Hence, biotite in soil system maintains greatest solution K than muscovite. Potassium feldspars in fine silt and clay fraction serves as an important source of K, though usually less significant than mica. Bajwa (1989) and Al-Ravi and Al-Mohammadi (1979) inferred that amongst the feldspars, only orthoclase is important in releasing potassium.

Mica on weathering is transformed to vermiculite with concurrent release of interlayer K (Fanning *et al.*, 1989). Mineral vermiculite entraps added K and renders it unavailable to plants. On K fixation, the expensive minerals, beidellite and vermiculite contract and revert to mica-like-structure (Alexiades and Jackson, 1965).

Mittal *et al.* (1989) observed that increasing cropping intensity resulted in depletion of soil K, yet the intensity of K depletion was associated with cropping and fertilizer scheme. Akhtar and Ali (1993) also reported K depletion with intensive cultivation of rice-wheat without fertilizer K in an alluvial camborthid soil. Tributh *et al.* (1987) observed that removal of K by plants results in depletion of interlayer K in illite followed by the degration of clay minerals. Cropping without K application enhanced the depletion of structure K from mica minerals leading to the transformation of mica to vermiculite and smectite. The removal of K by plants resulted in depletion of interlayer K from illite and an increase in smectite minerals. These phenomena induce changes in clay mineralogy in soil profile with depth.

Singh and Goulding (1997) observed no changes in mica and no K depletion in 153 years experiment on soil that was put under winter wheat cultivation at Rothamsted Experiment Station. However,contrary to this, Srivastava *et al.*(2002) observed depltion of non-exchangeable K in 27 years in NP treatment compared to NPK+FYM in alluvial mixed mineralogy in typic ustochrept soil under maize-wheat-cowpea cropping system.

Shaikh *et al.* (2007), in a five year study of mineral composition of Ustic Haplocamborthid soils of Sindh, under cotton-wheat system, observed that NPK treatment has more mica in coarse and fine clay fractions in AP (0-14 cm) horizon than control and NP treatments, indicating greater weathering of mica in NP than NPK treatment in surface horizon wherein K-less treatment increased weathering of sand and silt size mica. Dhaliwal *et al.* (2006) observed that soils containing sufficient quantity of K fix lower quantity of K.

Fertilizer application to sugar cane in Pakistan is primarily skewed towards nitrogen, followed by phosphorus and only nominal quantities of potassium are applied. The sugar cane crop of 125-Mg/ha removes about 168 kg K/ha per year.

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Potassium application in K deficient soils causes an increase in sugar cane yield and also tolerance against environmental stresses including moisture stress as well.

Ahmad (2000) reported that soils in alluvial plains of Pakistan are intensively cultivated without application of K fertilizer that causes a negative K balance in soil. Continuous crop production with K application may result in mica weathering particularly that of biotite into vermiculite.Hence the study was conducted with the objective to investigate the dynamics of clay mineralogy with profile depth in relation to long term potassium fertilizer application to sugar cane crop.

Materials and Methods

Soil samples were collected from the site under sugar cane crops from 1978 to 1996 at Soil Chemistry Section, Ayub Agricultural Research Institute, Faisalabad. The study site was typical camborthid (Soil Survey Staff, 1970). The soil was loam variant of Hafizabad soil series. It was medium textured calcareous, well drained, developed in late Pleistocene, mixed mineral alluvium. The annual precipitation was 350-400 mm; rainfall occurred mostly during summer and average annual temperature was 24 °C. Fertilizer treatments are presented in Table 1.

Table 1. Treatments of the experiment

Treatments	Fertilizer applied (kg/ha)		
	N	P_2O_5	K_2O
Control	0	0	0
N	170	0	0
NP	170	110	0
NPK	170	110	110
FYM	85 kg N from fertilizer and 85 kg N from FYM.*		

* = 85 kg N from FYM amounts to 12.5 tons of FYM per hectare, as analysis depicted 0.68% N in FYM (farm yard manure)

Fertilizer treatments were applied each year to sugar cane crop and was incorporated in AP horizon.All the fertilizer treatments and control had three replications. All the processes including cultural and fertilizer applications were uniform for the experiment.The designe of the experiment was completely randomized block designe. Soil samples were collected after harvesting sugar cane crop of 1996.The profile site was taken at random and was representative one. The composit, representative soil samples were collected from genetic horizon; AP: 0-15 cm, BW: 15-40 cm and BWK: 40-50 cm depth. Soil samples were air dried, crushed by wooden roller, passed through 2mm sieve and analyzed for electrical conductivity (Ec_e), saturation percentage, soil reaction (pH), organic matter, total nitrogen and available phosphorus, according to the methods described by Page *et al.* (1982). Physical and chemical characteristics of the soil are given in Table 2.

Table 2. Physical and chemical characteristics of soil

Soil characteristics	Horizon depth (cm)		
	AP(0-15)	BW(15-40)	BWK(40-56)
Sand (%)	47.1	46.4	45.2
Silt (%)	43.0	43.6	42.8
Clay (%)	9.9	10.0	12.0
Textural class	Loam	Loam	Loam
Ec_e (dsm^{-1})	1.30	1.32	1.70
PH	7.52	7.92	7.86
Organic matter (%)	0.65	0.50	0.50
Total nitrogen (%)	0.033	0.025	0.025
Available phosphorus (ppm)	3.5	3.0	4.5

Mineralogical determination of soil samples was conducted at National Agricultural Research Centre, Islamabad and soil samples were prepared for mineral analysis. For removal of cementing agent, 15 g soil sample was taken. Carbonates were removed with 1N NaO AC buffered at pH 5.0, organic matter was removed with 30% H_2O_2 and iron oxide was removed with citrate bicarbonate, dithionite bufferd at pH 7.3 (Jackson, 1979). For separation of soil into various fractions the treated samples were dispersed in Na_2CO_3 (pH 10) solution by 15 sec sonification using macro tip from the dispersed suspension. Sand was separated by wet sieving and silt by five repeated centrifugation washes which each time dispersed the suspension. The clay was further separated into coarse and fine clay fractions by similar five-repeated centrifugation treatments. Coarse and fine clay fractions were made salt-free by dialysis and were freeze dried. Mica, vermiculite and smectite in coarse and fine clay fractions were determined according to the methods described by Jackson (1979) (Table 3).

Results and Discussion

Changes in mica. In coarse clay fraction, NPK treatment at all the three depths had greater mica as compared to other fertilizer treatments at respective depths (Table 4, Fig. 1). In NPK treatment, mica in AP was 34 g/l00 g which increased with depth and at the lowest depth, maximum of 41g/l00 g was recorded. The increase in mica from AP to BW was less as compared to the increase from BW to BWK. The lowest amounts of mica at the upper two depths were recorded in

Table 3. Mica, vermiculite and smectite in clay*

Horizon	Depth (cm)	Fraction of profile		
		Mica	Vermiculite	Smectite
		(g /l00g)		
Coarse clay fraction				
AP	0-15	39.0	38.0	14.6
BW	15-40	40.0	14.0	19.2
BWK	40-56	20.0	19.0	17.9
Fine clay fraction				
AP	0-15	19.0	40.0	12.5
BW	15-40	41.0	32.0	17.9
BWK	40-56	35.0	48.0	20.1

* = the data is average of three replications

Table. 4. Effect of fertilizer treatments on mica, vermiculite and smectite in clay fractions*

Horizon	Depth (cm)	Fertilizer treatment				
		Control	N	NP	NPK	FYM
		Mica (g/100 g)				
Coarse clay fraction						
AP	0-15	27.0	27.0	26.0	34.0	25.0
BW	15-40	36.0	27.0	24.0	37.0	23.0
BWK	40-56	23.0	24.0	21.0	41.0	26.0
Fine clay fraction						
AP	0-15	22.0	33.0	17.0	21.0	36.0
BW	15-40	19.0	21.0	38.0	20.0	21.0
BWK	40-56	38.0	55.0	30.0	19.0	25.0
		Vermiculite (g/100 g)				
Coarse clay fraction						
AP	0-15	21	20	19	10	17
BW	15-40	26	28	25	16	21
BWK	40-56	20	16	14	25	13
Fine clay fraction						
AP	0-15	42	40	40	46	25
BW	15-40	46	35	35	42	25
BWK	40-56	32	3	32	48	37
		Smectite (g/100 g)				
Coarse clay fraction						
AP	0-15	20.3	17.2	16.2	12.6	18.5
BW	15-40	21.3	16.3	17.6	20.9	17.6
BWK	40-56	19.4	20.6	19.6	33.0	20.9
Fine clay fraction						
AP	0-15	29.8	23.9	16.1	29.8	27.5
BW	15-40	32.9	26.1	22.5	19.2	28.7
BWK	40-56	26.8	17.4	31.5	27.5	27.5

* = the data is average of three replications

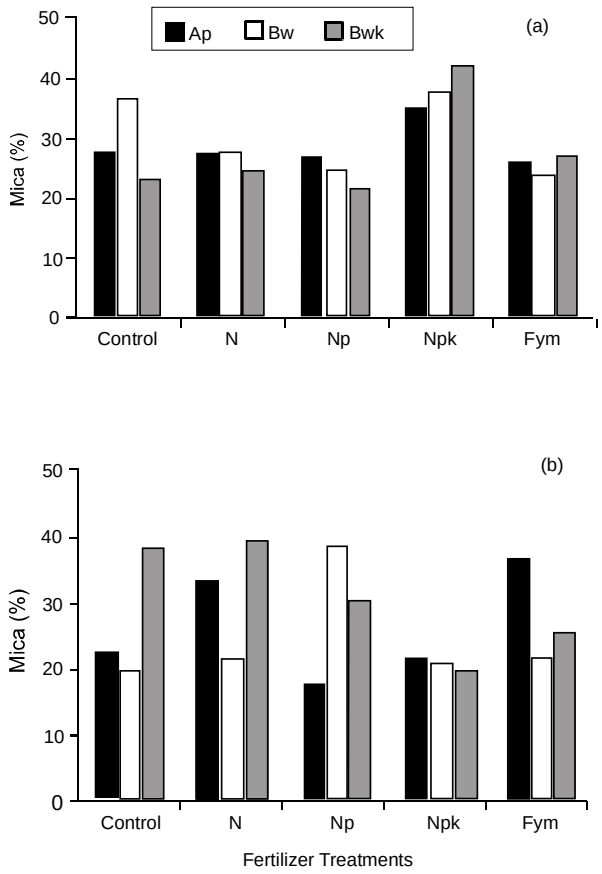

Fig. 1. Fertilizer treatment effect on mica: (a) coarse clay (2-0.2 µm) and (b) fine clay (<0.2 µm) based on total K contents assuming 10% K_2O in crystal (Jackson, 1979).

FYM and NP treatments as compared to other fertilizer treatment. As against NPK, in NP treatments, mica decreased as the profile depth increased. In case of the control treatment, mica was 27 g/l00 g soil. In AP, it increased in BW and decreased in BWK horizon. In treatment with N, mica was identical in the upper two depths and decreased in the lowest profile depth. Mica in FYM treatment was 25, 23 and 26 g/ l00 g in 0-15, 15-40 and 40-56 cm depths, respectively.

In fine clay fraction, FYM treatment had the highest mica in Ap horizon and the lowest at this depth was recorded in NP treatment. Mica in BW horizon decreased in all fertilizer treatments except in NP treatment, wherein it increased considerably. Maximum amount of mica (55 g/100 g) was observed in N treatment, that was substantially higher as compared to all other fertilizer treatments at all depths of the soil profile. On the basis of total mica of the three horizons in fine clay fraction, the highest mica was recorded in N treatment followed by NP, FYM control and NPK treatments. In NP

treatment, mica in fine clay increased from Ap to Bw horizon, suggesting greater weathering at surface than subsurface soil. The result of the study is in line with those reported by Akhtar and Ali (1993), who reported K depletion in intensive rice-wheat cropping system without K fertilizer application in an alluvial camborthid soil.

Changes in vermiculite. Vermiculite in coarse clay fraction was greater in the control, N and NP than in FYM and NPK treatments in the upper two profile depths (Table 4, Fig. 2). In the lowest profile depth, vermiculite was the highest in NPK as compared to all other fertilizer treatments. In NPK treatment, vermiculite in coarse clay fraction increased from AP to BWK horizon and was 1.6 and 2.5 times in BW and BWK horizon, respectively, as compared to AP horizon. In the control, N, NP and FYM treatments, vermiculite increased from AP to BW horizon and, thereafter, it decreased in Bwk horizon. In fine clay fraction in the three profile depths, cumulative vermiculite followed the order NPK > Control > N> NP> FYM treatments. Vermiculite in fine clay fraction in the control increased in Bw as compared to AP and then decreased substantially in BWK as compared to BW horizon. In the case of N treatment, vermiculite decreased in BW in comparison to AP and was identical in both BW and BWK profile depths. In NP treatment, vermiculite decreased with increase in the soil depth. In fine clay fraction, in NPK treatment vermiculite decreased in BW and then increased in BWK depth. In case of FYM treatment, vermiculite was identical in AP and BW depths and it was 1.48 times more in Bwk depth. On overall basis, vermiculite in fine clay fraction was almost double than that in the coarse clay fraction in all the fertilizer treatments and it was also, in general, more than mica in the fine clay fraction. This evidently indicates greater transformation of mica to vermiculite. These results are in line with the finding of Fanning *et al.* (1989) who reported transformation of mica to vermiculite on weathering.

Changes in smectite. Smectite, in coarse clay fraction, in AP horizon followed the order of control >FYM>N>NP>NPK treatments (Table 4, Fig. 3). In NPK and NP treatments, smectite increased substantially with the depth, with comparatively meagre increase in case of NP. In N and FYM treatments, smectite decreased in Bw depth and increased in BWK depth, and *vice versa* in the control treatment. In fine clay

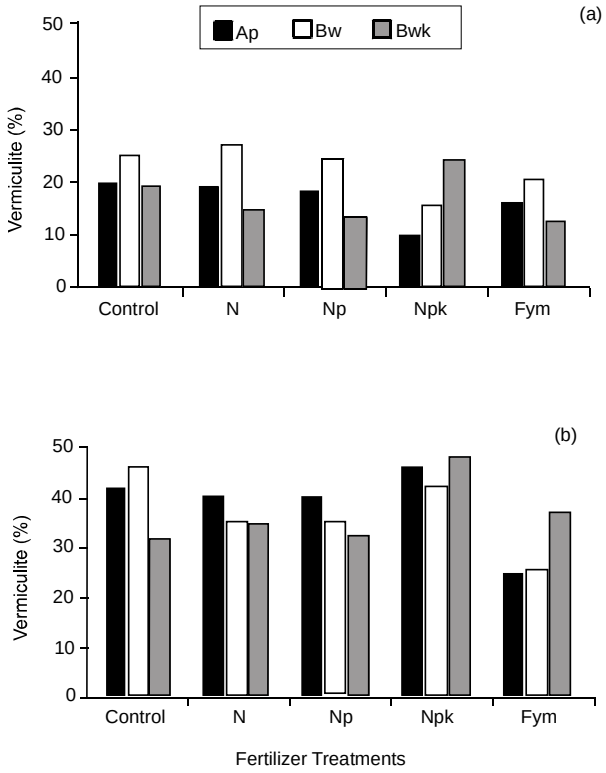

Fig. 2. Fertilizer treatment effect on vermiculite: (a) coarse clay (2-0.2 μm) and (b) fine clay (<0.2) μm) based on Ca/Mg and K/NH_4 CEC (Jackson, 1979).

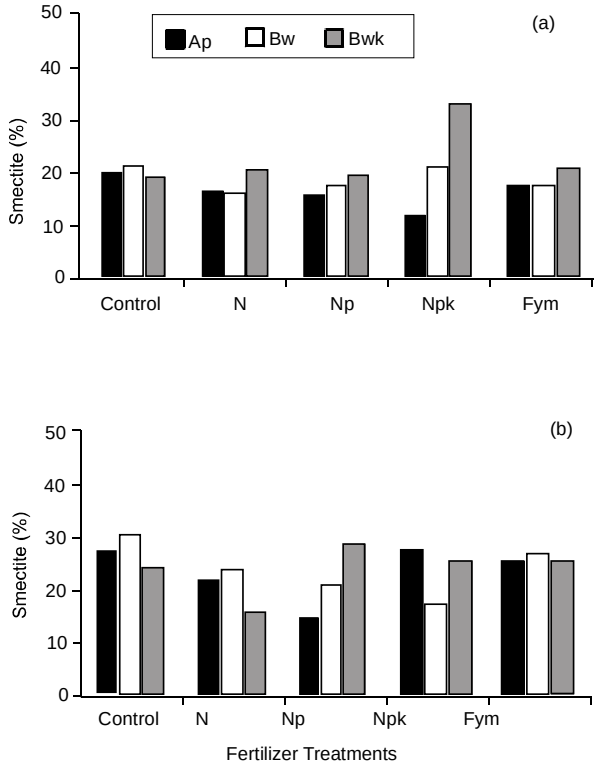

Fig. 3. Fertilizer treatment effect on smectite: (a) coarse clay (2-0.2 μm) and (b) fine clay (<0.2) μm) based on Ca/Mg and K/NH_4 CEC (Jackson, 1979).

fraction, maximum amount of smectite (29.8 g/l00 g) was observed in AP depth in control and NPK treatments followed by FYM treatment. In these three fertilizer treatments, smectite was quite higher than N and NP treatments that had 23.9 and 16.l g/l00 g smectite, respectively. In BW depth maximum smectite was recorded in the control. In BWK depth, smectite was in the order of NP > NPK = FYM > Control > N treatment. This reveals that fertilizer treatments invariably affect smectite content in soil. Tributh *et al.* (1987) observed that cropping without K application enhanced depletion of structural K in mica minerals leading to transformation of mica into vermiculite and smectite.

It can be concluded from the study that changes in clay mineral with profile depth of soil do occur through long term K-fertilizer application to sugar cane crop. Thus potassium application to sugar cane crop may be based on mineralogy of the soil at various depth.

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The Effects of Industrial Soil Pollution on *Prosopis juliflora* Swartz Growth Around Karachi

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Abstract. Study of the effect of soils of towel, garment, rubber and ply-wood factories of Korangi and Landhi industrial estates of Karachi and that of the University of Karachi on the growth of *Prosopis juliflora* Swartz plants growing in these areas demonstrated detrimental effect of industrial soils on the growth of plants of all the areas particularly on the plants growing at the University site.

Keywords: plant growth, *Prosopis juliflora*, soil pollutants, industrial pollution

Introduction

Rapid industrialization and phenomenal growth in population have created environmental pollution problem in Karachi city (Iqbal and Shafiq, 1999a). Major contributors to the environmental degradation include the industrial sector. Naqvi and Khattak (1995) reported increased amount of heavy metals, chromium, nickel, copper and lead in the waste effluents of Landhi Industrial Trading Estate of Karachi, Pakistan. Kullberg (1974) has described damages to vegetation caused by industrial effluents particularly to water plants. Iqbal and Qadir (1973) observed higher reduction in seed germination, root and shoot length in seeds collected from the industrial polluted areas as compared to other areas. Various kinds of industrial pollutants have adverse effects on *Triticum aestivum* var. UP-262 (Habib and Iqbal, 1996). Physical properties of soil, such as soil strength, bulk density, texture and structure, influences greatly the root penetration, growth and yield of various crops (Gerard *et al*., 1982).

Prosopis juliflora (Family, Mimosaceae) is a perennial deciduous thorny shrub/small tree, used as forage for cattle. It provides fuel wood; its timber stabilizes sand dunes and it is used as shade plant and wind breaker also (Khoshoo and Subramanyam, 1985). *P. juliflora*, *Abutilon indicum* and *S. holosericea* are distributed world-wide (Atiq-ur-Rehman and Iqbal, 2008). It is found in South Africa, India, West Indies and Mexico and has been recorded in Pakistan as well. *P. juliflora* is the most dominant species of plants growing in the Karachi University and is among the eight leading species of plants growing in the vicinity of Korangi and Landhi industrial areas (Atiq-ur-Rehman, 2007). In soil of Malir river, some heavy metals such as lead, copper and zinc were detected in large amounts, which influenced the composition of plant communities at this locality (Qamar-uz-Zaman and Iqbal, 1994).

In the present study, an effort has been made to study the destructive and hazardous role of towel, garment, rubber and ply board industries etc. in the proximity of Korangi and Landhi industrial places of Karachi, with reference to their effect on growth of *P. juliflora* in comparison to that of the Karachi University soil and plants.

Materials and Methods

The experiment was conducted in greenhouse at the Department of Botany, University of Karachi under uniform natural environmental conditions. Healthy and uniform-sized seeds of *Prosopis juliflora* Swartz, were chosen from Korangi and Landhi industrial areas of Karachi and Karachi University Campus. Due to hard seed coat, the seeds were slightly cut at one end and sown in garden soil (loam soil) at 1 cm depth in large pots, and were irrigated daily. After 21 days, uniform-sized seedlings were transplanted to pots of 19.8 cm dia and 9.6 cm depth, in soils collected from a towel, a garment, a tech rubber and a tech ply board factories of the Korangi and Landhi industrial areas of Karachi at 0.30 cm depth. The soil of Karachi University was used as control. 50% Soil of the respective areas (including control) was mixed with 50% garden soil (one part manure + two parts fine sand), since, in the preliminary studies, pure soils of the industrial area hardly showed any response to seed germination and seedling growth. There were six replicates for each treatment and the experiment was completely randomized. Only one seedling was grown in each pot and plants were irrigated daily. Every week, pots were reshuffled to avoid light/shade or any other greenhouse effect. Seedling height, number of leaves and plant cover were recorded after every week for eight weeks.

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After eight weeks, number of leaflets and leaf area of each plant were recorded and all the plants of *P. juliflora* were carefully removed from the pots and washed thoroughly to measure root, shoot and seedling length. Root, shoot and leaves were separated for drying in an oven at 80 °C for 24 h. Oven-dried weight of root, shoot and leaves and total plant dry weight were determined. Root/shoot ratio, leaf weight ratio, specific leaf area and leaf area ratio were also determined as follows:

Determination of growth variables

$$\text{Root/shoot ratio} = \frac{\text{root dry weight}}{\text{shoot dry weight}}$$

$$\text{Leaf weight ratio} = \frac{\text{leaf dry weight}}{\text{total plant dry weight}}$$

$$\text{Specific leaf area (cm}^2\text{/g)} = \frac{\text{leaf area}}{\text{leaf dry weight}}$$

$$\text{Leaf area ratio (cm}^2\text{/g)} = \frac{\text{leaf area}}{\text{total plant dry weight}}$$

For soil analysis, two soil samples of each site were air-dried, lightly crushed and passed through a 2 mm sieve and kept in the laboratory. For mechanical analysis of soil, coarse sand was selected using 0.05 mm sieve (USDA, 1951). Maximum water holding capacity (W.H.C.) was measured by the method of Keen (1931). Soil organic matter was determined according to the methods of Jackson (1958). Calcium carbonate concentration was determined by acid neutralization as described by Qadir *et al.* (1966). Bower and Wilcox (1965) methodology was used to determine total soluble salts whereas**,** soil pH was recorded by a direct MP 220 pH Meter (Mettler, Toledo). Available sulphate in soil was determined by the turbidity method as described by Iqbal (1988), using a colorimeter (Photoelectric Colorimeter AE-11M). Soil analysis was also conducted for heavy metals. In this regard, one g dried soil sample was taken in 50 ml beaker and digested with 5 ml concentrated nitric acid (HNO_3) + 5 ml concentrated perchloric acid ($HClO_4$), through heating at 90 °C for two and a half hours. Thereafter, little amount of distilled water was added in the digested residue and filtered through Whatman filter paper No. 42. Solution was made up to 50 ml using distilled water and was diluted 10 times for copper, zinc and chromium analyses by atomic absorption spectrophotometer (Perkin Elmer Model No. 3100).

All data was statistically analyzed by ANOVA (Steel and Torrie, 1984) and DMRT (Duncan, 1955) ($p<0.05$) using personal computer software packages, Costat version 3.0 and SPSS version 10.0.

Reduction in percentage of growth was determined in treated soils of the factories relative to control soil using the following formula:

$$\text{Reduction in growth (\%)} = \frac{\text{growth in control soil - growth in treated soil}}{\text{growth in control soil}} \times 100$$

Results and Discussion

Plant height (28.00 cm) (Fig. 1a), number of leaves (39.33) (Fig. 1b) of *P. juliflora* of the university population were considerably retrogressed by the towel factory soil while the garment factory soil noticeably hampered the plant cover (33.17 cm) (Fig. 1c) comparative to the plant height (34.75 cm), number of leaves (42.50) and plant cover (36.58 cm) in the university soil. On the other hand, whole industrial zone soils augmented plant height, predominantly that of the tech ply board factory soil (37.50 cm) (Fig. 2a) as correlated to the plant height (27.67 cm) in university soil. Number of leaves declined only in the towel factory soil (44.17) (Fig. 2b) than in the university soil (47.83) while plant cover (40.17 cm) (Fig. 2c) was apparently amplified in the tech ply board factory soil relative to plant cover (35.08 cm) in the university soil. Atiq-ur-Rehman (2007) also reported earlier increase in growth in *Leucaena leucocephala, T. populnea, Peltophorum pterocarpum* and *Azadirachta indica* in a tech ply board factory soil comparative to other factories soils.

Almost aggregate growth assortments of *P. juliflora* from University area were poor except shoot dry weight in the towel factory in comparison to the garment, tech ply board and tech rubber factory soils. Drastically lesser growth was examined in shoot, seedling length, leaflets numbers, leaf area, leaf dry weight, root/shoot ratio and leaf weight/leaf area ratio of *P. juliflora* of university population in the towel factory soil correlatively to the university soil (Table 1 and 2). *P. juliflora* plants from industrial locations, had enhanced growth in the garment, tech rubber and tech ply board factories soils whereas the towel factory soil relatively more conversely affected the industrial plants. Number of leaflets, roots, shoots, leaves and total plant dry weight were conspicuously minimized in the plants of industrial estates growing on towel factory soil as compared to that of the university soil (Table 1 and 2). Total soluble salts were in appreciable quantity in the entire industrial soils particularly the towel factory soil had the highest amount of total soluble salts than the university soil (Table 3) which produced restricted growth in both the populations of *P. juliflora.* These results tally with those of Shereen *et al.* (2005) who reported that early seedling stage of rice plants showed that salinity caused a significant repression in seedling growth very soon after transplanting in a saline solution.

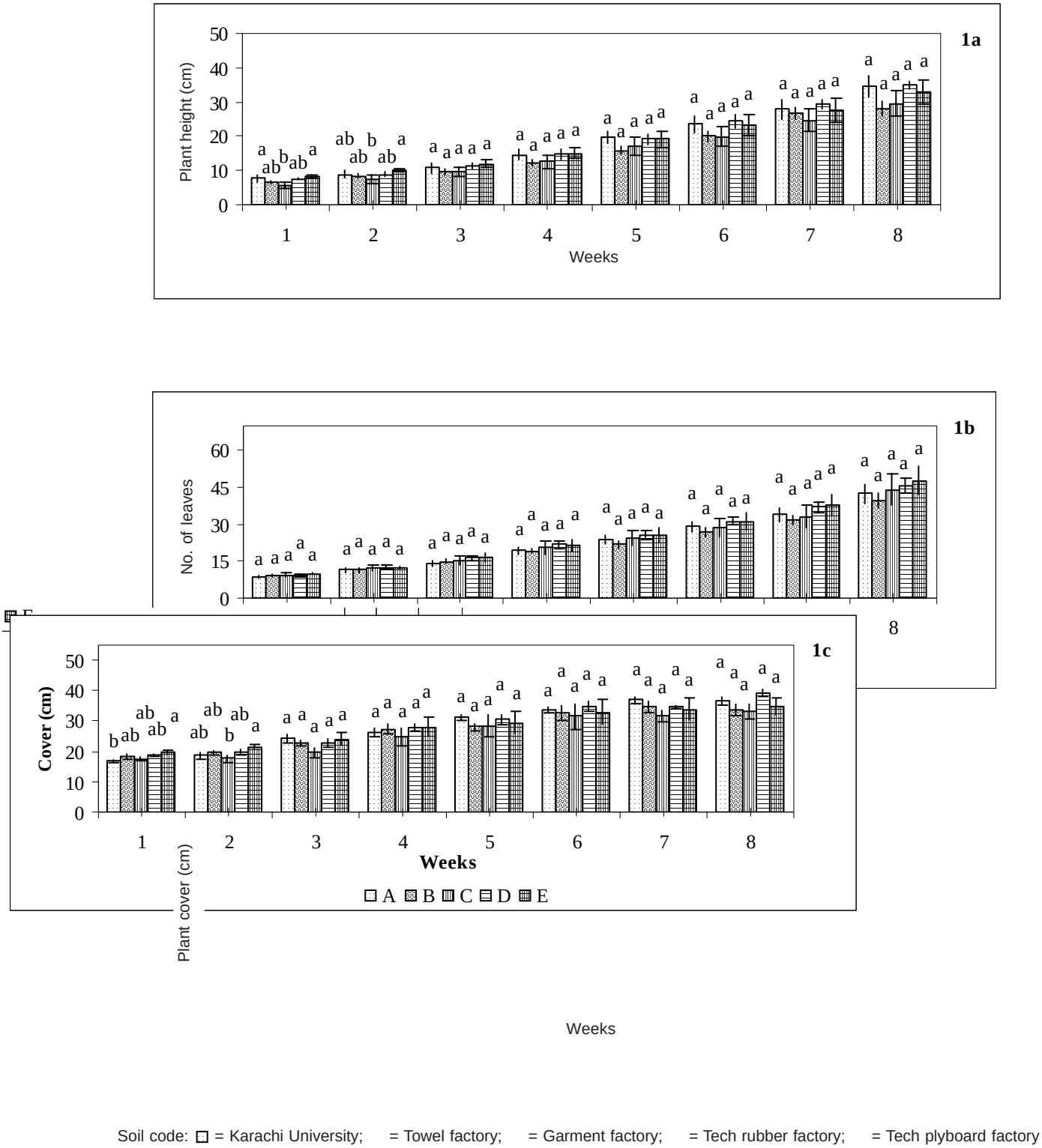
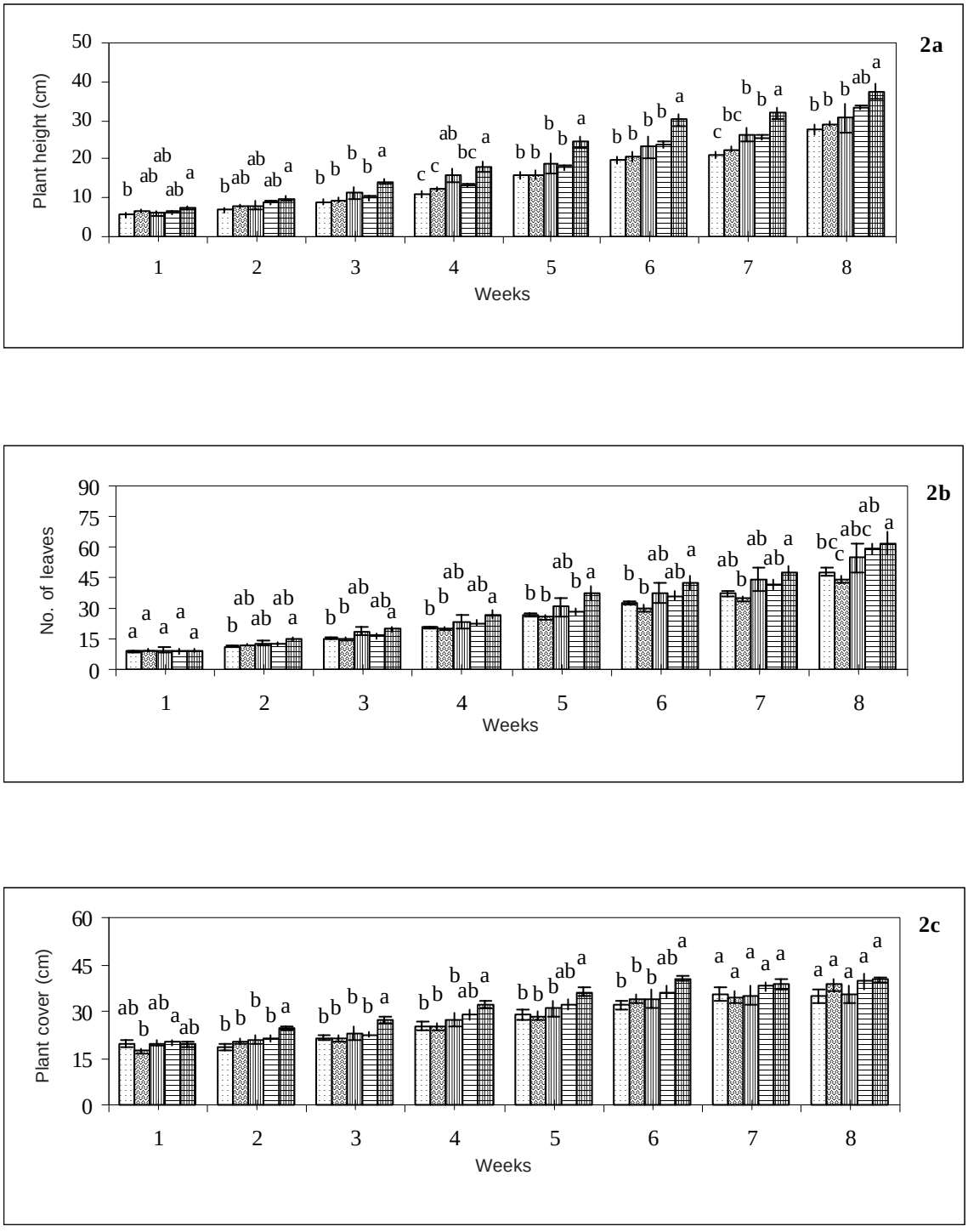


Fig. 1. Plants from seeds of *Prosopis juliflora*, collected from Karachi University, in soils of different areas. (50% soil was mixed with 50% garden soil). Statistical significance was determined by analysis of variance; same letters in a row are not significantly different ($p<0.05$) according to Duncan's Multiple Range Test.



Figs. 2. Plants of *P. juliflora* from seeds, collected from Korangi and Landhi industrial areas, in soils of different areas. (50% soil was mixed with 50% garden soil). Statistical significance was determined by analysis of variance; same letters in a row are not significantly different ($p<0.05$) according to Duncan's Multiple Range Test.

Table 1. Growth of two populations of *Prosopis juliflora* in soils of different areas

Plants	Treatment[a]	Root length (cm)	Shoot length (cm)	Seedling length (cm)	No. of leaflets	Leaf area (cm^2)	Root dry weight (g)	Shoot dry weight (g)	Leaf dry weight (g)	Total plant dry weight (g)	Root/ shoot ratio	Leaf/ weight ratio	Specific leaf area (cm^2/g)	Leaf area ratio (cm^2/g)
PJ[1]	A	22.07[b] ±2.19	36.00[a] ±2.66	58.07[ab] ±2.17	1102.56[a] ±104.52	127.20[a] ±13.70	0.17[a] ±0.01	0.28[a] ±0.04	0.44[a] ±0.05	0.89[a] ±0.10	0.65[a] ±0.11	0.49[a] ±0.01	295.54[ab] ±17.66	146.31[ab] ±9.15
	B	20.55[b] ±2.16	29.45[a] ±2.18	50.00[b] ±1.04	982.00[a] ±101.15	93.62[a] ±9.66	0.15[a] ±0.02	0.29[a] ±0.04	0.33[a] ±0.03	0.77[a] ±0.06	0.57[a] ±0.12	0.44[a] ±0.04	284.73[b] ±10.84	123.70[b] ±11.06
	C	21.77[b] ±0.88	31.01[a] ±3.78	52.78[b] ±4.13	1079.33[a] ±199.99	94.16[a] ±20.28	0.14[a] ±0.03	0.25[a] ±0.07	0.37[a] ±0.08	0.76[a] ±0.18	0.75[a] ±0.16	0.50[a] ±0.02	259.52[b] ±19.22	130.47[ab] ±11.81
	D	27.05[a] ±1.30	36.10[a] ±1.21	63.15[a] ±1.43	1131.11[a] ±90.01	117.87[a] ±8.07	0.19[a] ±0.03	0.30[a] ±0.03	0.43[a] ±0.02	0.92[a] ±0.07	0.61[a] ±0.04	0.47[a] ±0.01	274.64[b] ±14.65	130.16[ab] ±8.19
	E	20.13[b] ±1.19	34.27[a] ±3.52	54.40[b] ±3.13	1249.11[a] ±209.16	133.32[a] ±28.04	0.17[a] ±0.03	0.25[a] ±0.06	0.40[a] ±0.09	0.82[a] ±0.17	0.76[a] ±0.16	0.48[a] ±0.03	342.32[a] ±25.04	163.86[a] ±13.47
	LSD	4.76	8.23	7.68	437.86	51.18	0.07	0.15	0.17	0.37	0.36	0.07	52.76	31.75
PJ[2]	A	21.43[a] ±1.49	28.80[b] ±1.48	50.23[b] ±2.13	1287.78[b] ±107.48	125.45[b] ±13.39	0.22[bc] ±0.02	0.32[bc] ±0.03	0.46[b] ±0.03	1.00[bc] ±0.06	0.71[a] ±0.05	0.46[a] ±0.02	275.92[a] ±25.63	127.56[a] ±12.98
	B	22.90[a] ±1.73	30.57[b] ±1.26	53.47[ab] ±2.59	1166.56[b] ±63.83	143.46[ab] ±11.55	0.20[c] ±0.02	0.27[c] ±0.03	0.44[b] ±0.03	0.91[c] ±0.05	0.77[a] ±0.07	0.49[a] ±0.02	333.32[a] ±40.27	162.36[a] ±21.17
	C	25.30[a] ±3.09	31.68[b] ±3.87	56.98[ab] ±3.21	1468.78[ab] ±231.25	153.43[ab] ±23.57	0.30[ab] ±0.05	0.35[abc] ±0.08	0.52[ab] ±0.09	1.17[b] ±0.14	1.52[a] ±0.72	0.43[a] ±0.06	302.96[a] ±30.53	129.53[a] ±18.07
	D	22.17[a] ±0.93	34.13[ab] ±0.62	56.30[ab] ±1.24	1574.78[ab] ±101.74	156.23[ab] ±9.28	0.26[abc] ±0.02	0.44[ab] ±0.06	0.56[ab] ±0.04	1.26[ab] ±0.09	0.63[a] ±0.06	0.46[a] ±0.03	282.31[a] ±15.49	125.81[a] ±7.70
	E	21.30[a] ±2.66	38.70[a] ±1.72	60.00[a] ±2.29	1784.67[a] ±209.90	193.91[a] ±22.48	0.32[a] ±0.03	0.49[a] ±0.04	0.65[a] ±0.04	1.45[a] ±0.08	0.65[a] ±0.04	0.46[a] ±0.03	299.29[a] ±25.56	135.03[a] ±16.30
	LSD	6.21	6.12	6.93	457.77	49.76	0.08	0.14	0.15	0.26	0.95	0.10	83.44	46.39

Soil code: A = Karachi University; B = towel factory; C = garment factory; D = rubber factory; E = tech plyboard factory (50% sample soil + 50% garden soil); PJ[1] = seeds collected from Karachi University; PJ[2] = seeds collected from Korangi industrial areas; statistical significance was determined by analysis of variance; numbers followed by the same letters in the same column are not significantly different, according to Duncan's Multiple Range Test; LSD = least significance difference, values at $p<0.05$ level ± standard error.

Root, total plant dry weights and specific leaf area of University population were virtually subdued while only leaf weight ratio of industrial population was impeded by the garment factory soil. Atiq-ur-Rehman and Iqbal (2007a) also reported distinct repression in *L. leucocephala* plant in garment factory soil correlative to the Karachi university soil. Organic matter was deficient, whereas zinc concentration was greater in this soil relative to the university soil which might have caused decline in the growth of plants particularly in the plants of University site. This result is in agreement with the report of Iqbal and Shafiq (1999b) about adverse effects of Cu and Zn on seed germination and seedling growth of wheat of *Triticum aestivum* var. J 78 and var. P 85.

Leaf area, leaf dry weight, root/shoot and leaf weight/leaf area ratios and specific leaf area of the university population were restricted in the rubber factory soil. The tech rubber factory soil had elevated magnitude of coarse sand, literal concentrations of calcium carbonate and chromium and lowest quantity of water holding capacity as related to the university soil. Soil texture and water holding capacity presented factual correlation and influenced the growth in the rubber factory soil. Iqbal and Atiq-ur-Rehman (2002) reported that increase in concentration of Cr reduced the dry weight of *L. leucocephala*.

Root length and shoot dry weight of the university population was minimized by the tech ply board factory soil while root length and root/shoot ratio were simply deteriorated in industrial region population by the tech ply board factory soil as compared to the university soil. Available sulphate and copper concentration were adequately increased in the tech ply board factory soil in comparison to the university soil which might be considered decrease in growth of plants.

Table 2. Percentage reduction in growth of two populations of *Prosopis juliflora* in soils of different factories versus control

Plants	Soil	Plant height	No. of leaves	Plant cover	Root length	Shoot length	Seedling length	No. of leaflets	Leaf area	Root dry weight	Shoot dry weight	Leaf dry weight	Total plant dry weight	Root/ Shoot ratio	Leaf weight ratio	Specific leaf area	Leaf area ratio
PJ[1]	A	19.4	7.5	8	6.9	18.2	13.9	10.9	26.4	11.8	3.6^{+}	25.0	13.5	12.3	10.2	3.7	15.5
	B	15.3	3.5^{+}	9.3	1.4	13.9	9.1	2.1	26.0	17.6	10.7	15.9	14.6	15.4^{+}	2.0^{+}	12.2	10.8
	C	0.7^{+}	7.1^{+}	7.5^{+}	22.6^{+}	0.3^{+}	8.7^{+}	2.6^{+}	7.3	11.8^{+}	7.1^{+}	2.3	3.4^{+}	6.2	4.1	7.1	11.0
	D	5.0	12.2^{+}	5	8.8	4.8	6.3	13.3^{+}	4.8^{+}	0.0	10.7	9.1	7.9	16.9^{+}	2.0	15.8^{+}	12.0^{+}
PJ[2]	A	5.1^{+}	7.7	9.7^{+}	6.9^{+}	6.1^{+}	6.5^{+}	9.4	14.4^{+}	9.1	15.6	4.3	9	8.5^{+}	6.5	20.8^{+}	27.3^{+}
	B	10.2^{+}	14.3^{+}	0.7^{+}	18.1^{+}	10.0^{+}	13.4^{+}	14.1^{+}	22.3^{+}	36.4^{+}	9.4^{+}	13.0^{+}	17.0^{+}	114.1^{+}	6.5^{+}	9.8^{+}	1.5^{+}
	C	21.1^{+}	24.0^{+}	13.0^{+}	3.5^{+}	18.5^{+}	12.1^{+}	22.3^{+}	24.5^{+}	18.2^{+}	37.5^{+}	21.7^{+}	26.0^{+}	11.3	0.0	2.3^{+}	1.4
	D	35.5^{+}	29.3^{+}	14.5^{+}	0.6	34.4^{+}	19.5^{+}	38.6^{+}	54.6^{+}	45.5^{+}	53.1^{+}	41.3^{+}	45.0^{+}	8.5	0.0	8.5^{+}	5.9^{+}

A = towel factory soil; B = garment factory soil; C = tech rubber factory soil; D = tech ply board factory soil; PJ[1] = seeds were collected from Karachi University site; PJ[2] = seeds were collected from Korangi industrial areas; + = percentage increase.

Table 3. Soil characteristics of Karachi University and industrial areas soils

Sites	Coarse sand (%)	W.H.C. (%)	Organic matter (%)	$CaCO_3$ (%)	Total soluble salts (%)	pH	Available sulphate	Cu (µg/g)	Zn (µg/g)	Cr (µg/g)
A	58±0^{b}	27±0^{b}	2.0±0.3^{b}	17.8±0.3^{c}	5.9±0.7^{c}	8.4±0.0^{a}	8±0d	0.002±0.002^{c}	0.029±0.017^{bc}	6.066±0.046^{a}
B	24±2^{d}	29±3^{b}	2.1±0.2^{b}	29.5±1.5^{b}	14.0±2.0^{a}	8.0±0.1^{ab}	575±13^{a}	0.023±0.012^{b}	0.033±0.001^{b}	4.139±0.093^{b}
C	47±0^{c}	31±2^{b}	0.9±0.0^{c}	24.5±0.5^{b}	8.0±0.0^{c}	8.3±0.1^{a}	108±23^{c}	0.008±0.002^{bc}	0.090±0.002^{a}	4.229±0.111^{b}
D	88±1^{a}	17±3^{c}	1.1±0.1^{c}	36.5±2.5^{a}	12.0±0.0^{ab}	8.2±0.1^{ab}	401±11^{b}	0.002±0.002^{c}	0.019±0.002^{bc}	6.899±0.978^{a}
E	26±2^{d}	40±0^{a}	3.3±0.4^{a}	17.5±1.5^{c}	9.0±1.0^{bc}	7.8±0.2^{b}	608±45^{a}	0.074±0.002^{a}	0.003±0.002^{c}	1.404±0.406^{c}
LSD	5	8	0.8	5.4	3.8	0.4	86	0.02	0.027	1.738

A = Karachi university soil; B = towel factory soil; C = garment factory soil; D = tech rubber factory soil; E = tech ply board factory soil; W.H.C. = water holding capacity; numbers followed by the same letters in the same column are not significantly different; LSD = least significance difference; values at $p<0.05$ level; ± = standard error.

According to Bell *et al*. (1979), constant SO_2 concentration causes significant reductions in the dry matter accumulation and yield of *Lolium perenne* L. cv. S23. Additionally, most of the industrial sites soils had lower range of coarse sand and chromium concentrations and sufficient water holding capacity which may cause sufficient growth in industrial plant population.

Conclusion

It could be concluded that *Prosopis juliflora* plant population of the university campus was sensitive to industrial estates soils whereas the plant native to Korangi and Landhi industrial regions, remarkably tolerated minimum growth hindrance in industrial zone soils. Atiq-ur-Rehman (2007) as well reported that *Albizia lebbeck* growth was intensely retrograded while *T. populnea* growth was definitely greater in the soils of Korangi industrial sites. He also elucidated that *A. lebbeck* and *L. leucocephala* growth was severely restrained by dissimilar industrial soil ratios especially at enhanced concentration of 75% towel factory soil; *A. indica* seedlings were also harshly impaired in the towel factory soil. Atiq-ur-Rehman and Iqbal (2007b) also observed that *P. juliflora* from the university site was evidently obstructed by the industrial area soil extracts over the *P. juliflora* of industrial locations. So, towel factory soil was more deleterious for both of the populations especially for the University plant population and is excessively noxious for most of the plants.

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Short Communication

Investigation of Starch Modification Potential of 'Kanwa' – an Alkaline Salt

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Abstract. Cassava-starch-modification potential of 'Kanwa' at different concentrations was studied. Kanwa modified cassava-starches showed better swelling power, paste clarity, viscosity, peak viscosity, freeze-thaw stability and reduced gelatinization time over native starch. However, native starch had better water solubility and set back viscosity.

Keywords: kanwa, modified starches, starch modifiying property

Native starches are unsuitable for many industrial applications due to poor characteristics exhibited under processing conditions such as extreme temperature, high shear pressure and diverse pH (Wang *et al.*, 1993). Modified starches in comparison have generally better paste clarity, gel stability, increased resistance to retrogradation, increased solubility and improved freeze-thaw stability which increase their application as stabilizer, filler, binder and adhesive. Starches are modified by physical, enzymatic, biological and chemical methods in order to reduce their limitations in industrial uses. Chemical modification of cassava and other plant starches with improved qualities and applications have been reported by several workers (Ahmed *et al.*, 2005; Iyothi *et al.*, 2005; Nurulislam and Azemi, 1997). Use of naturally occurring food products as starch modifying agents has been advocated. Some natural products such as alum and ginger have been reported to improve the functional properties of cassava and rice starches (Daramola and Osanyinlusi, 2006; Lee *et al.*, 1995). 'Kanwa' also known as 'trona' or sodium sesquicarbonate is a naturally occurring alkaline rock salt with trace amounts of Ca, Mg, Fe, Zn, S, Cl, Si, P and K (Makanjuola and Beetlestone, 1975). It is used not only as a tenderizer but also as flavouring agent, food preservative and as a prophylactic (Uzogara *et al.*, 1988). It is relatively inexpensive, less hazardous and requires less safety precautions in use. Although its food uses have been established, information on its starch modification potential is not available in literature.

The present work aims at modifying cassava starch with low 'kanwa' concentrations and determines the pasting properties of the modified starches. For this prupose starch was extracted from an improved cassava variety 82/00058, aged 12 months at harvest, obtained from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. Subsequently, cassava roots were grated, water was added and the slurry was sieved. The filtrate was allowed to settle for 24 h before decantation. The starch was drained, dried and milled into powder. 'Kanwa' was purchased from a retail market in Ibadan and used without further treatment while other chemicals used were of analytical grade.

The slurry method described by Agboola *et al.* (1991) was employed for the preparation of the modified cassava starches. Whereas unmodified (native) starch was used as control.

The yield of the substituted starch on a dry matter basis, solubility at 60 ^{o}C, swelling power, ash and moisture content of native and substituted starches were determined (AOAC, 1980; Smith, 1967; Leach *et al.*, 1959).

Moreover, pH of the samples, paste clarity, pasting properties and freeze-thaw stability over four cycles were also determined. (Craig *et al.*, 1989; Knight, 1974; Smith, 1967). Analysis was carried out in triplicate except pasting properties which were determined only twice.

The values obtained for some of the physical and functional properties of 'kanwa'-modified starches are shown in Table 1. Yield of the modified starches was high and varied with kanwa concentrations ranging between 90.5-95.7%. This shows that leaching of the starch constituents is reduced at alkaline conditions unlike acidic conditions which reduced yield through thinning of the starch (Ahmed *et al.*, 2005; Lawal *et al.*, 2004). Increase in 'kanwa' concentration from 0.1 to 1.0% increased the yield. The swelling power of the modified starches (4.9 - 5.3) was higher than that of the native (unmodified) starch (3.5). This could be attributed to weakening of the starch

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Table 1. Physical and functional characteristics of native and 'kanwa' modified starches*

Sample	Yield (%)	Swelling power (%)	Solubility (%)	M.C. (%)	Paste clarity (%T)
Cassava starch + 0% kanwa	NA	3.5±0.01	7.7±0.01	11.0±0.03	78.3±0.09
Cassava starch + 0.1% kanwa	90.5±0.12	5.2±0.02	4.2±0.01	10.3±0.04	80.6±0.06
Cassava starch + 0.5% kanwa	95.7±0.16	5.3±0.01	5.0±0.02	11.3±0.01	80.6±0.07
Cassava starch + 1.0% kanwa	94.2±0.09	4.9±0.03	7.0±0.02	11.6±0.04	82.4±0.09

*= values are means of triplicate determinations; NA = not applicable

Table 2. Brabender amylograph reading for native and 'kanwa' modified cassava starches*

Sample	Pasting temperature (°C)	Peak viscosity (BU)	Viscosity at 95 °C (BU)	Viscosity at 50 °C (BU)	Setback viscosity (BU)	Time (min)
Cassava starch + 0% kanwa	68	195	165	110	85	24
Cassava starch + 0.1% kanwa	67	370	220	200	170	23
Cassava starch + 0.5% kanwa	68	395	160	290	105	22
Cassava starch + 1.0% kanwa	68	335	180	200	135	23

*= values are means of duplicate determinations

granules by the substituent ions which allow penetration of water molecules into the granules. The solubility of the modified starch samples was lower (4.2 -7.0%) compared to the native starch (7.7%). The lower solubility recorded for 'kanwa'-modified starches may be attributed to the non-thinning effect of the alkaline salt used. Acids and acidic materials have been reported to create thinning effect which results in a greater fluidity and introduction of the hydrophilic substituent group which allows water molecule retention because of their ability to form hydrogen bonds which increase solubility (Lawal *et al.*, 2004; Betancur *et al.*, 1997; Khalil *et al.*, 1995). The paste clarity of the modified starches was higher (80.6 - 82.4%T) than the native starch (78.3%T). This is a reflection of higher swelling power exhibited by the modified starches. The paste clarity increased as the 'kanwa' concentration was increased from 0.1-1.0%. The pH of the modified starches (10.1 -10.3) was higher than that of the native starch (8.0), which is an indication of the alkaline nature of the modifying agent.

The pasting properties of the modified starches and the native starch are shown in Table 2. Pasting temperature range of 67-68°C was obtained for the modified starches while the native starch had pasting temperature of 68 °C. Addition of kanwa had no effect on the pasting temperature of the starch. The gelatinization time was 22-23 min and 24 min for modified starches and native starch, respectively. Cooking time has energy-cost implication during starch application in the industry. The setback viscosity of modified starches (105-170 BU) was higher as compared to 85 BU recorded for native starch with cassava starch, treated with 1.0% kanwa, having the highest (170 BU). This reflects the instability of the cooked paste of the modified starches against retrogradation. The modified starches exhibited increase in viscosity when cooled from 95 °C to 50 °C and higher peak viscosity compared to the native starch. This indicates that the modified starches could be used as pie filling where thickening and stability are required.

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Technology

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Bactericidal Efficacy of Silver Impregnated Activated Carbon for Disinfection of Water

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Abstract. When highly contaminated water was passed through two types of silver coated activated carbon and their mixtures with sand, the former was found to be far better medium for disinfection of water, with bactericidal efficacy of about 2.5 times that of the latter.

Keywords: bactericidal efficacy, activated carbon, water disinfection, silver impregnation

Introduction

For provision of safe drinking water, the bacteriological quality of water is of paramount importance in addition to monitoring of indicator bacteria such as coliform and faecal coliforms, effective gadgetries and means and devices for providing safe drinking water, as per WHO standards (WHO, 1984).

The germicidal effect of silver was first explored by Carlon in 1893; since then several devices containing silver as water disinfectant have been proposed and their bactericidal efficacy has been investigated (Kim *et al.,* 2008; Bell, 1991; Pierce *et al.,* 1978; Berger *et al.,* 1976; Barranco *et al.,* 1974; Spadaro *et al.,* 1974). In addition to being bactericidal, silver also increases the lability of viruses when used for disinfection of water (Mahnel and Schmidt, 1986). Some bacteria can develop resistance to silver (Russell *et al.,* 1994) but other evidence suggests that no resistant strains have been encountered clinically (Lansdown, 2002). Activated carbon/coal-based sorbents have been observed to remove enteric viruses satisfactorily (Chaudhri and Sattar, 1989; 1986). Activated carbon is unique and versatile adsorbent because of its extensive surface area, microporous structure and high adsorption capacity; hence, it is widely used as filter medium in drinking water purification devices (Jayadev and Chaudhri, 1990; Osman and Chaudhuri, 1990; Prasad and Chaudhuri, 1989; Protheroe *et al.,* 1989; Tikhonova *et al.,* 1989). Activated carbon is also assuming increasing importance in the control of air pollution, in purifying and controlling the general chemical environment, in certain biomedical applications and for removal of organic matter from water and wastewater (Usmani *et al.,* 1994).

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In the present work, samples of granular activated carbon, prepared locally from an indigenous raw material, and imported granular carbon were coated with silver and used for disinfecting water. The objective of the study is to assess and compare the disinfection efficacy and capability of the two types of silver coated products, separately as well as mixed with untreated graded sand.

Materials and Methods

The imported activated carbon used in this study was granular carbon of M/s. Norit, Holland. The locally produced granular activated carbon was prepared from coconut shells. The shells were first disintegrated in a pilot disintegrator followed by carbonization in an inert atmosphere of nitrogen void of air and then physically activated with a mixture of superheated steam and air in a fluidized bed reactor (Usmani *et al.,* 1999). Both activated carbon samples i.e. imported (I) and locally produced (L), were broken in a rod mill, classified to a particle size of 1.00-2.00 mm on a sieve shaker and then utilized for impregnation of silver. The samples were initially washed thoroughly with water to get rid of any foreign material etc., and then dried at 120 °C to constant weight, prior to silver coating.

Silver was impregnated on both carbon samples by the method described by Beg *et al.* (1986) for the treatment of sand. Briefly, 500 g of graded, washed and dried sample of each activated carbon was treated with 0.1% A.R. grade silver nitrate solution, allowed a maturing time of one h and then treated with 10% sodium hydroxide solution. Carbons samples were then initially treated with 10 ml 1:1 NH_4OH solution and afterwards with 15 ml of reducing sugar solution washed with distilled water to pH 7 and then finally dried at 120 °C.

The respective activated carbon sample (1000 g), either alone or a mixture with sieved, graded, washed and dried sand in the ratio of 1:1, was placed into a cylindrical plastic container 4″x 12″. The cycling have adjustable inlet and outlet PVC valves at the top and bottom, respectively, and a polyethylene screen of suitable mesh size at the bottom for supporting the carbon or carbon-sand mixture in the container. In column '1', in which there was only silver coated carbon, the mass of carbon was 1.34 kg and that of silver was 2.68 g. In column '2', in which there was mixed bed of 1:1 silver coated carbon and untreated sand, the masses of carbon, sand and silver were 1kg, 1kg and 2 g, respectively. Mass transfer limits have influenced the results as may be seen in Table 5-6. The whole study was performed at a flow rate of one litre/min of water passing through the columns and empty bed, whereas, contact time in both the cases was five minutes.

Experiments were carried out, using artificially contaminated feed water having chemical and microbiological analysis as shown in Table 1, to asses the disinfection efficiency of silver impregnated activated carbon samples and carbon-sand mixtures. The water was artificially contaminated with common indicator organisms *E. coli* in the range of 10^3-10^4/ 100 ml. A sample was drawn from the bulk of well mixed contaminated feed water and termed as "Control". The contaminated water was passed through plastic containers packed with silver coated locally produced and imported activated carbon samples and also through mixtures of graded sand and silver coated activated carbon and graded sand at a flow rate of 1 litre/min. Samples of water after passing through the plastic containers were collected in sterile flasks and alongwith the control, subjected to microbiological and, silver analyses to compare the disinfection efficacy of the activated carbons alone and carbon-sand mixtures and also to determine the amount of released silver in treated water.

Microbiological tests were performed according to the methods described in American Public Health Association Standard Methods (APHA, 1998) and WHO standards (WHO, 1984). For presumptive coli tests two sets, each consisting of three tubes containing 10 ml, 1 ml and 0.1 ml of the sample in MacConkey broth, were used; one set was incubated at 37 °C for 24 h and the other at 44 °C for 48 h. Most Probable Numbers (MPN/dl) were ascertained from McGrady's table.

Analysis of eluted silver in treated water samples was performed on Z-8000 Hitachi atomic absorption spectrophotometer with Zeeman effect background corrections equipped with a graphite furnace, a microprocessor and a built-in printer. Determination of silver was carried out by flameless (ETAAS) atomic absorption spectrometry employing standard addition technique (APHA, 1998).

Results and Discussion

In an earlier study of the bactericidal efficacy of silver coated sand mixtures, Mahmood *et al.* (1993) obtained upto 1800 litre, microbiologically safe water (coliforms and faecal coliforms <3 MPN/dl), however, the efficacy of silver coated sand mixture dropped significantly, after passage of 2000 litre of contaminated feed water. The present study has been carried out to assess the efficacy of silver coated granular carbon samples (L and I), either alone or as mixture with untreated, washed and graded sand.

All the chemical parameters of feed water used in the present study were within permissible limits (Table 1). Locally produced granular activated carbon sample (L), prepared by fluidized bed activation of coconut shells, had better physical and chemical characteristics as compared with the imported sample (I) of NORIT, Holland (Table 2).

The data recorded in Table 3-4 relate to disinfection of contaminated feed water, after passing through the containers packed with silver coated activated carbon and their 1:1 sand mixtures. The holding time of contaminated water in both the cases was almost zero and samples were drawn immediately after feeding of contaminated water. It was observed that feed water was highly contaminated having coliform and faecal coliform 1100^+ MPN/dl, before treatment with silver coated activated carbon, whereas, the number of coliforms on the water, after treatment with both the carbon samples, reduced

Table 1. Chemical and microbiological analysis of feed water

Parameters	Measured value ± SD
Chemical analysis	
pH	7.1 ± 0.1
Electrical conductivity	588 µg/cm ± 8
Calcium	35 mg/l ± 0.8
Magnesium	11 mg/l ± 0.8
Sodium	37 mg/l ± 0.5
Potassium	06 mg/l ± 0.2
Chloride	49 mg/l ± 1
Sulfate	54 mg/l ± 4
Bicarbonate	130 mg/l ± 4
Alkalinity	107 mg/l ± 3
Calcium hardness	87 mg/l ± 1
Magnesium hardness	45 mg/l ± 1
Total dissolved solids	338 mg/l ± 5
Microbiological tests	
Coliforms (MPN/dl)	>1100
Faecal coliforms (MPN/dl)	>1100

significantly to <3, which is of desired WHO standard (WHO, 1984). The data further reveals, that safe water, as per WHO standard, was obtained for the first 4400 litre of feed water in both the samples 'L' and 'I'. Afterwards, in both the cases, this number gradually increased. However, treated water samples obtained after passing 4600 and 4800 litre of contaminated water through carbon sample 'L' had comparatively less coliforms (9 and 23 MPN/dl, respectively) than that of carbon sample "I" (23 and 43 MPN/dl) (Table-3). This comparatively better bactericidal efficacy of the local carbon sample (L) may be due to its better adsorptive properties, higher surface area and lower bulk density (Table 2).

A critical review of the data obtained in case of 1:1 carbon-sand mixtures ('LS' and 'IS') (Table 4), clearly indicates that bactericidal efficacy of both silver coated carbon samples is drastically affected and disinfection capacity is reduced upto almost 50% (2200 litre), when they were mixed with sand in equal proportions ('LS' and 'IS').

Several studies have been reported in literature indicating that silver impregnated activated carbon filters used in disinfection of water are suspectible to colonization and after some time shed bacteria into the water (Taylor *et al.,* 1979; Burkhead *et al.,* 1978; Hanes, 1978; Wallis *et al.,* 1974; Fiore and Babineau, 1971). However, contrary to the observations of Pierce *et al.* (1978), the present results (Table 3), clearly suggest that silver coated carbon effectively and significantly disinfect contaminated water and that activated carbon is a promising and potential medium for impregnation of silver for disinfection of water. Further, its disinfection efficacy up to 4400 litre of highly contaminated water, is far better than the other silver coated media like sand (Mahmood *et al.,* 1993). Disagreement between the results obtained in this study with those of other workers, with reference to bactericidal efficiency of the two silver coated carbon samples, may be due to the difference in the method of treatment adopted for coating of silver on activated carbon samples. This method may have resulted in a more effective and long lasting impregnation of silver on carbon which is thus released gradually in contaminated water in just sufficient quantity required for its oligodynamic bactericidal action for disinfection of water. This point has further been substantiated by the systematic study carried out on the quantitative estimation of the amount of silver eluted with the passage of a certain amount of water *viz* 50, 100, 200, 400, 600 litre, through silver impregnated activated carbon samples at a flow rate of 1 liter/min, established after a series of experiments. Level of silver up to 0.7 mg/litre (0.1 ppm) can be tolerated without any risk to health (WHO, 2003).The results (Table 5), clearly show that the amount of silver being released is gradual and regular and that the silver coated carbon samples 'L' and 'I' are effective for the disinfection of highly contaminated water in the silver elution range of 11-56 ppb.

Table 2. Main physical and chemical characteristics of granular activated carbon samples

Characteristics determined	Locally produced sample ('L') (Measured value ±SD)	Imported sample ('I') (Measured value ±SD)
Bulk density	0.5028 g/cc ± 0.005	0.5892 g/cc ± 0.006
Ash content	1.35% ± 0.01	2.03% ± 0.02
Iodine	1036 mg/g ± 10.4	818 mg/g ± 8.2
Methylene blue	247 mg/g ± 4.8	190 mg/g ± 3.8
Carbon tetra Chloride adsorption	60% ± 3	54% ± 2
BET surface area	950 m^2/g ± 9.20	810 m^2/g ± 7.9

Table 3. Disinfection of contaminated feed water after passing through silver coated activated carbon samples

Water passed (litre)	L(MPN/dl)	I(MPN/dl)	Control
50-4400	<3	<3	1100^+
4600	9	23	1100^+
4800	23	43	1100^+

Table 4. Disinfection of contaminated feed water after passing through 1:1 mixture of graded sand with locally produced (LS) and imported (IS) activated carbon samples

Water passed (litre)	LS(MPN/dl)	IS(MPN/dl)	Control
50-2200	<3	<3	1100^+
2400	4	4	1100^+
2600	21	21	1100^+
2800	43	43	1100^+
3000	43	43	1100^+
3200	93	93	1100^+
3400	150	150	1100^+
3600	1100^+	1100^+	1100^+
3800	1100^+	1100^+	1100^+
4000	1100^+	1100^+	1100^+
4200	1100^+	1100^+	1100^+
4400	1100^+	1100^+	1100^+
4600	1100^+	1100^+	1100^+
4800	1100^+	1100^+	1100^+

It may be further noted that the amount of silver, released from the coated activated carbons gradually and steadily decreases with the increasing volume of contaminated water passing through carbon. Moreover, there is a definite correlation (Table 5 and 6) between the amount of silver released (ppb) by the silver coated carbon samples in the treated water and their bactericidal efficiency (Masaru, 1991; Yoshinari, 1989). In either of the cases, safe water of MPN/dl<3 was obtained upto minimum silver elution limit of 11 ppb. Afterwards, when the release silver is less than 11 ppb, the bactericidal property of silver is markedly inhibited, as may be seen by the higher MPN/dl of 23 and 43 obtained in product water samples of 'L' and 'I', respectively, which is definitely higher than the desired WHO standard. A somewhat identical pattern of the systematic and gradual release of oligodynamic element, although in smaller quantity, logically due to comparatively lesser amount of impregnated silver available in the mixture of carbon and sand samples 'LS' and 'IS' than carbon samples 'L' and 'I', was found (Table 6). This data further confirms the threshold limit of 11 ppb silver, just the minimum quantity required for bactericidal action/disinfection of water. Data (Table 5 and 6) further confirms that silver impregnated carbon is a better medium for providing safe drinking water, as its silver elution is within permissible limits. 'Safe Drinking Water Act' in USA. requires that siwer content in drinking water should be below 50 ppb (Oya *et al.,* 1994). In the present study, the amount of silver ions eluted in samples 'L' and 'I' is slightly >50 ppb in the first 50 litres of water (Table 5); however this excess silver may be initially removed by washing with water while using coated activated carbon for obtaining disinfected potable water.

Table 5. Estimation of the amount of silver in treated water after passing through silver coated activated carbon samples

Water passed (litre)	Silver concentration in Water(ppb) (L)	Silver concentration in Water(ppb) (I)
50	56	56
100	49	48
200	48.1	46.1
400	46.2	42.4
600	38.3	39.8
800	34.2	32.2
1000	33.6	30.7
1200	32.1	28.8
1400	29.9	26.2
1600	28.7	25.4
1800	26.4	24.0
2000	25.1	22.7
2200	23.9	21.9
2400	22.7	21.1
2600	21.4	20.5
2800	20.1	19.3
3000	19.0	18.1
3200	18.8	17.5
3400	17.7	16.8
3600	16.5	15.7
3800	15.8	14.6
4000	14.9	13.8
4200	13.0	12.9
4400	11.2	11.0
4600	10.1	10.2
4800	9.2	9.4

Table 6. Estimation of the amount of silver in treated water after passing through silver coated carbons and mixtures

Water passed (litre)	Silver concentration in Water(ppb) (LS)	Silver concentration in Water(ppb) (IS)
50	26.7	26.1
100	25.2	25.0
200	24.1	24.2
400	23.2	22.8
600	21.1	20.9
800	21.5	19.5
1000	19.2	18.0
1200	18.0	16.9
1400	17.0	15.4
1600	16.1	14.2
1800	14.9	13.1
2000	12.2	12.0
2200	11.3	11.3
2400	10.1	10.1
2600	9.4	8.7
2800	8.2	7.6
3000	7.1	6.2
3200	6.2	5.4
3400	5.0	4.8
3600	4.3	3.7
3800	3.2	2.3
4000	2.3	1.2
4200	1.0	0.29
4400	0.18	-
4600	-	-
4800	-	-

Conclusion

It may be inferred from the study that though activated carbon is comparatively more expensive than the graded sand, but it is a better medium than graded sand for impregnation of silver to be used for water disinfection /decontamination. The bactericidal efficacy of silver coated activated carbon, obtained by the specific coating technique developed by Beg *et al.,* (1986) is about 2.5 times higher (4400 litre) than that of coated graded sand mixture (1800 litre), for disinfection of highly contaminated water. The technology for the preparation of granular activated carbon from an indigenous raw material, used for coating of silver is locally available. However, this is a preliminary study; further investigations to assess the effects of various variables such as exposure time, pH, temperature, chemical quality of water etc., are required so that this effective and inexpensive technology may be used for water sanitization.

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A ^{15}N Tracer Study to Evaluate the Effects of Nitrogen and Copper Fertilization on Fertilizer Nitrogen Efficiency in Rice Production

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Abstract. In the study of the effects of nitrogen and copper fertilization on rice yield when four rates of N (0, 60, 120 and 180 kg N/ha) as ^{15}N labelled urea and three rates of Cu (0, 5 and 10 kg Cu/ha) were applied, grain yield increased significantly with increasing N rates upto 120 kg N/ha. The recovery of fertilizer N was around 40% irrespective of N and Cu rates. Copper application at 10 kg/ha increased grain yield by 0.53 t/ha insignificantly. Cu content in the straw was below the critical deficiency level of 6 mg/kg. Thus higher rate of Cu fertilizer (above 10 kg/ha) in soil increase rice yield and fertilizer N efficiency if Cu is applied as basal. Alternately, Cu may be applied as foliar spray on standing crop to avoid Cu adsorption in the soil.

Keywords: ^{15}N tracer study, copper, rice, fertilizer nitrogen efficiency

Introduction

Rice is the main food crop of an estimated 40 percent of the world's population (Buresh and De Datta, 1990). The rice crop removes from 16 to 17 kg N and 8 g of Cu for the production of one ton of rough rice including straw (Choudhury and Kennedy, 2005; Sahrawat, 2000; Ponnamperuma and Deturck, 1993; De Datta, 1981). Most of the rice soils of the world are deficient in N and biological nitrogen fixation by cyanobacteria and diazotrophic bacteria meets only fraction of the N requirement (Sattar *et al*., 2008; Hashem, 2001; Baldani *et al*., 2000; Tran Van *et al*., 2000). Fertilizer N applications are thus necessary to meet the crops demand. In wetland rice soils, the availability of water soluble Cu decreases due to decrease in redox potential (Ponnamperuma, 1985, 1972). Cu deficiency in soil increases sterility in rice grain resulting in a decrease in the yield (Ambak and Tadano, 1991). This problem can be solved by applying proper amount of Cu fertilizer.

The largest rice growing area of Malaysia is located in the Muda Irrigation Scheme, Kedah that covers an area of about 95,000 ha. Recent investigations showed that there is a tendency of yield decline in many sites of this area due to Cu deficiency (Samy *et al*., 1992a). Investigations showed that soils of some locations of this irrigation scheme are deficient in Cu (Choudhury and Khanif, 2000a). Farmers are applying a single fertilizer dose of 80 kg N/ha, 30 kg P_2O_5/ha and 20 kg K_2O/ha in rice fields throughout the Irrigation Scheme (Samy *et al*., 1992b). Indiscriminate application of fertilizers throughout the irrigation scheme caused low yield in many locations due to Cu deficiency. Cu fertilization may result in increased rice yield as well as fertilizer N efficiency. The ^{15}N tracer technique is used as the precise method to estimate fertilizer N use efficiency (Cong *et al*., 2008; Fan *et al*., 2007; Kongchum *et al*., 2007; Nishida *et al*., 2007).

The present study was undertaken to evaluate the effects of N and Cu fertilization on rice yield and fertilizer N efficiency using the ^{15}N tracer technique.

Materials and Methods

A greenhouse experiment was conducted at Universiti Putra Malaysia to evaluate the effects of N and Cu fertilization on rice yield and fertilizer N efficiency. The study was conducted in two soils (Idris and Tebengau series). In this paper the findings on one soil (Idris series) are discussed. The taxonomy of the soil is typic plinthaquept, very fine clayey, mixed, isohyperthermic, pallid (Paramananthan, 1998). The soil was collected from rice growing areas of the Muda Irrigation Scheme, Kedah, about 500 km north of Kuala Lumpur, Malaysia. Soil samples were collected from 0-15 cm depth, air dried, ground and sieved through 2 mm sieve. Soil was analysed for organic matter, pH, cation exchange capacity (CEC), total N and available Cu. Organic matter was analysed by potassium dichromate and sulphuric acid digestion method (Walkley and Black, 1934). Soil pH was measured by glass electrode (Peech, 1965). Total N was determined by sulphuric-salicylic acid digestion method (Bremner and Mulvaney, 1982). Cation exchange capacity was determined by ammonium acetate extraction method (Schollenberger and Simon, 1945). Available Cu was analysed by 0.05 N HCl extraction method (Ponnamperuma *et al*., 1981). The soil had a pH of 3.9 with

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CEC 22.78 cmol/kg. It contained 3.65% organic matter, 0.14% total N and 0.09 mg/kg available Cu.

Four N rates (0, 60, 120 and 180 kg N/ha) in the form of ^{15}N-labelled urea (8.378% atom excess) and three Cu rates (0, 5 and 10 kg Cu/ha) as copper sulphate ($CuSO_4$. $5H_2O$) were used in the experiment. The experiment was laid out in randomised complete block design with four replications. The soil used for the study was collected from the plough layer of the field and was filled into plastic pots of 15 litre capacity to 10 cm below the brim of the container. The height and diameter of the pots were 29 cm and 28 cm, respectively. The soil was flooded and preincubated for three weeks to stabilize the physicochemical properties before seed sowing. Sprouted rice seeds of variety MR185 were sown. The number of seeds needed per pot was calculated on the basis of surface area of the pot and a sowing rate of 40 kg/ha. Ten sprouted seeds were equally spaced in the puddle soil of each pot.

Phosphorus (30 kg P_2O_5/ha) and K (20 kg K_2O/ha) fertilizers as triple superphosphate (TSP) and muriate of potash (MOP), respectively, were applied as basal dressings to all pots. Nitrogen was applied in three splits (half as basal and one fourth each at active tillering stage and at panicle initiation stage). Copper application was done all as basal. Nitrogen and copper were applied in solution form. Amount of fertilizers was calculated on the basis of soil surface area of each pot. Rice crop was harvested at maturity. Grain and straw weights (g/pot) were recorded. Grain and straw yields were calculated as t/ha considering the surface area of each pot. About 10 g of representative grain and straw samples were ground to pass through 1 mm sieve and were kept in plastic containers for chemical analyses. Total N of the plant tissue was analysed by H_2SO_4 digestion followed by steam distillation method (Bremner and Mulvaney, 1982), and subsequently the ^{15}N content of the plant samples was analysed by using emission spectrometry (Hauck, 1982). Total N uptake by grain and straw were calculated from yield and N content data. The fertilizer N uptake and recovery percentage of added N by rice plant were calculated following the procedure of Panda *et al*. (1995). Copper content of the plant samples was analysed following 1 N HCl extraction method (Yoshida *et al*., 1976). Total Cu uptake by grain and straw was calculated from yield and Cu content data.

All the data were analysed through analysis of variance (ANOVA) and the means were compared using the Duncan's multiple range test (DMRT) where the F-test was significant. All the analyses were done following Statistical Analysis System (SAS Institute Inc., 1987).

Results and Discussion

Grain and straw yields. Grain yield ranged from 2.11 to 4.93 t/ha while straw yield ranged from 3.95 to 6.90 t/ha (Table 1). Effect of N on both grain and straw yields was significant, whereas the effect of Cu was not significant on either of the parameters. Grain and straw yields increased significantly due to N fertilization up to 120 kg N/ha which was expected as the soil was deficient in N and is in agreement with the previous findings (Choudhury and Bhuiyan, 1994, 1991). Copper application did not increase grain and straw yields although the soil was deficient in Cu. The non-significant effect of Cu was attributed to higher Cu adsorption in this soil (Choudhury and Khanif, 2000b). Plant analysis indicated that the Cu content in the straw was below the critical deficiency level of 6 mg/kg (Yoshida *et al*., 1976). This indicates that application of 5 or 10 kg Cu/ha was not sufficient to increase grain yield in this soil; higher rates of Cu over these rates might be useful. Further research is needed to get optimum Cu rate for this soil.

Table 1. Effect of N and Cu on grain and straw yields of rice

N rate (kg/ha)		Cu rate (kg/ha) 0	5	10	Mean
		Grain yield (t/ha)			
0		2.11	2.97	3.23	2.77[c]
60		3.70	4.04	3.73	3.82[b]
120		4.48	4.34	4.93	4.58[a]
180		3.75	4.11	4.26	4.04[b]
	Mean	3.51	3.87	4.04	
		Straw yield (t/ha)			
0		4.34	3.95	4.36	4.22[c]
60		5.12	5.95	5.08	5.38[b]
120		6.19	6.08	6.90	6.39[a]
180		6.28	6.15	6.86	6.43[a]
	Mean	5.48	5.53	5.80	

Effect of Cu was not significant on either grain or straw yield; values followed by different letters within a parameter in a column are significantly different at 5% level by Duncan's Multiple Range Test (DMRT)

Nitrogen content and uptake. Nitrogen content ranged from 0.97 to 1.49% in the grain, and from 0.34 to 0.78% in the straw (Table 2). Nitrogen content in the grain increased significantly due to N fertilization up to 180 kg N/ha whereas Cu effect was not significant. Interactive effect of N and Cu was significant on N content in straw (Table 2). While application of Cu (at 5 kg Cu/ha) increased N content in straw significantly at 0 and 120 kg N/ha, its effect was not significant at 60 and 180 kg N/ha. Increase in N content in the straw due to Cu application was attributed to the decreases in straw yields at these levels

of Cu application (Table 1) as Cu was less diluted in straw due to less straw yields. At 5 kg Cu/ha, N content in straw significantly increased over control due to N fertilization at 120 kg N/ha while the significant effect of N fertilization on this parameter was noticed at 180 kg N/ha at other Cu rates (0 and 10 kg Cu/ha). Total N uptake by the whole plant (grain and straw) ranged from 35.35 to 122.45 kg/ha (Table 2). Total N uptake increased significantly due to N fertilization up to 180 kg N/ha. Copper fertilization increased the total uptake significantly over the control at 5 kg Cu/ha; beyond this rate there was no further increase. The increase in total N uptake by the whole plant (grain and straw) due to N fertilization was attributed to the increase in grain and straw yield due to N fertilization as well as due to the increase in N content in grain and straw at higher N rates. Similar results were obtained in previous investigations (Cong *et al*., 2009; Choudhury *et al*., 1997; Panda and Mohanty, 1995). Significant increase in total N uptake due to Cu fertilization was attributed to the significant increase in N content in straw at higher Cu rates.

Table 2. Effect of N and Cu on N content in straw and grain and total N uptake by rice plant

N rate (kg/ha)		Cu rate (kg/ha)			Mean
		0	5	10	
		N content in straw (%)			
0		0.34^{bB}	0.47^{cA}	0.39^{bAB}	-
60		0.50^{bA}	0.55^{bcA}	0.49^{bA}	-
120		0.50^{bB}	0.68^{abA}	0.51^{bB}	-
180		0.68^{aA}	0.78^{aA}	0.75^{aA}	-
		N content in grain (%)			
0		0.97	1.06	1.06	1.03^{c}
60		1.11	1.08	1.07	1.09^{c}
120		1.16	1.32	1.16	1.21^{b}
180		1.48	1.49	1.41	1.46^{a}
	Mean	1.18	1.24	1.18	-
		Total N uptake by whole rice plant			
0		35.35	49.51	50.79	45.22^{d}
60		65.57	73.83	64.05	67.82^{c}
120		81.97	97.48	91.67	90.37^{b}
180		105.62	122.45	122.19	116.75^{a}
	Mean	72.13^{B}	85.82^{A}	82.18^{A}	-

^{1}N and Cu interaction was significant on N content (%) in straw; Cu effect on N content (%) in grain was not significant; values followed by different small letters within a parameter in a column, and capital letters in a row are significantly different at 5% level by DMRT

^{15}N Atom excess. ^{15}N atom excess in the grain ranged from 2.588 to 5.244% and in straw, from 3.105 to 5.598% (Table 3). ^{15}N atom excess in grain and straw increased significantly at higher N rates, whereas Cu effect was not significant. Increase in ^{15}N atom excess in grain and straw with increasing N rates indicates that fertilizer contributed more in total N uptake at higher N rates. This finding is in agreement with the previous findings of Choudhury and Khanif (2001).

Table 3. Effect of N and Cu on ^{15}N atom excess in rice grain and straw

N rate (kg/ha)		Cu rate (kg/ha)			Mean
		0	5	10	
		^{15}N atom excess in grain (%)			
60		2.872	2.588	2.666	2.709^{c}
120		4.596	4.309	4.322	4.409^{b}
180		5.244	4.947	5.219	5.137^{a}
	Mean	4.237	3.948	4.069	-
		^{15}N atom excess in straw (%)			
60		3.241	3.495	3.105	3.280^{c}
120		4.967	4.544	4.524	4.678^{b}
180		5.598	5.403	5.555	5.519^{a}
	Mean	4.602	4.481	4.395	-

Effect of Cu on ^{15}N atom excess (%) in either grain or straw was not significant; values followed by different letters in a column within a parameter are significantly different at 5% level by DMRT

Fertilizer N uptake and recovery. Fertilizer N uptake by the whole plant (grain and straw) ranged from 21.73 to 78.92 kg/ha (Table 4) and increased significantly at higher N rates whereas the Cu effect was not significant. This was attributed to higher ^{15}N atom excess at higher N rates (Table 3) as well as due to higher total N uptake at higher N rates (Table 2); this is in agreement with the previous findings (Bandyopadhyay and Sarkar, 2005; Guindo *et al*., 1994b). Fertilizer N recovery (quan-

Table 4. Effect of N and Cu on fertilizer N uptake and recovery by rice plant

N rate (kg/ha)		Cu rate (kg/ha)			Mean
		0	5	10	
		Fertilizer N uptake by whole rice plant (kg/ha)			
60		23.60	25.89	21.73	23.74^{c}
120		46.28	51.35	48.22	48.62^{b}
180		68.44	75.96	78.92	74.44^{a}
	Mean	46.11	51.07	49.62	-
		Fertilizer N recovery by rice plant (%)			
60		39.32	43.15	36.22	39.56
120		38.56	42.79	40.18	40.51
180		38.02	42.20	43.85	41.36
	Mean	38.63	42.71	40.08	-

Effect of N was not significant on fertilizer N recovery (%); effect of Cu was not significant on both fertilizer N uptake and recovery (%); values followed by different letters in a column are significantly different at 5% level by DMRT

tified by ^{15}N atom excess) by rice plant ranged from 36.22 to 43.85% (Table 4). Effects of N and Cu were not significant on fertilizer N recovery. In general, the recovery of fertilizer N by rice plant was around 40%. It is in agreement with the previous findings (Craswell and Vlek, 1979; Guindo *et al*., 1994a). Copper application did not increase fertilizer N recovery by rice plant significantly. This was due to the non-significant response of rice crop to added Cu. The available Cu content in the soil was below the critical deficiency level of 0.1 mg/kg (Ponnamperuma *et al*., 1981), and it was expected that Cu application might increase rice yield and thereby increase fertilizer N uptake. But due to higher adsorption of Cu in this soil the effect of Cu was not significant. Higher doses of Cu (above 10 kg Cu/ha) might be useful to increase grain yield and N uptake. Further research using various levels of Cu is needed to draw the inference.

Copper content and uptake. Copper content in grain ranged from 3.63 to 7.22 mg/kg while in straw, ranged from 4.58 to 5.99 mg/kg (Table 5). Copper content in grain and straw increased significantly due to both N and Cu fertilization. Nitrogen fertilization enhanced plant growth. This might contribute in increase in Cu content of both grain and straw due to higher Cu absorption capacity of the rice plants. Although Cu content in straw increased significantly due to Cu fertilization, it was below the critical deficiency level of 6 mg/kg (Yoshida *et al*., 1976). This indicates that the applied Cu rates were not enough in this soil to meet the demand of the rice plants. As a consequence, grain yield did not increase due to Cu fertilization. A follow-up laboratory experiment indicated that Cu adsorption capacity of this soil was high (Choudhury and Khanif, 2000b). Maximum Cu adsorption capacity (calculated from the Langmuir equation) in this soil was 588 mg/kg (Choudhury and Khanif, 2000b). So, higher rate of Cu (more than 10 kg Cu/ha) is needed to get response in this soil if Cu is applied as basal. Alternately, Cu may be applied as foliar spray on standing crop to avoid Cu adsorption in the soil. Copper uptake by the whole rice plant (grain + straw) ranged from 27.39 to 71.88 g/ha (Table 5). Copper uptake by rice plant increased significantly due to both N and Cu fertilization which was attributed to the increases in Cu content in grain and straw due to N and Cu fertilization. The increase in Cu content in grain and straw due to Cu fertilization is in agreement with some other findings (Ambak and Tadano, 1991; Choudhury and Khanif, 2002).

Table 5. Effect of N and Cu on Cu content in grain and straw, and total Cu uptake by rice plant

N rate (kg/ha)		Cu rate (kg/ha)			Mean
		0	5	10	
		Cu content in grain (mg/kg)			
0		3.63	3.85	4.73	4.07^{c}
60		4.51	5.23	5.57	5.10^{b}
120		5.45	6.76	6.54	6.25^{a}
180		5.87	6.85	7.22	6.65^{a}
	Mean	4.87^{C}	5.67^{B}	6.02^{A}	
		Cu content in straw (mg/kg)			
0		4.58	5.44	5.52	5.18b
60		5.74	5.74	5.99	5.82a
120		5.19	5.57	5.72	5.49a
180		5.64	5.79	5.97	5.80a
	Mean	5.29^{C}	5.64^{B}	5.80^{A}	
		Total Cu uptake by whole rice plant			
0		27.39	33.09	39.44	33.31^{c}
60		46.29	55.18	51.35	50.94^{b}
120		56.76	62.99	71.55	63.77^{a}
180		56.86	64.48	71.88	64.41^{a}
	Mean	46.83^{B}	53.94^{A}	58.56^{A}	

Values followed by different letters within a parameter in a column and different capital letters in a row are significantly different at 5% level by DMRT

Conclusion

The present study indicates that copper application did not increase grain yield and fertilizer N efficiency significantly although the soil was deficient in Cu. This was attributed to higher Cu adsorption in this soil. It indicates that application of 5 or 10 kg Cu/ha was not enough to increase grain yield and fertilizer N efficiency in this soil. Higher rates of Cu over these doses may increase grain yield and fertilizer N efficiency if Cu is applied as basal. Alternately Cu may be applied as foliar spray on standing crop to avoid Cu adsorption in the soil. Further research is needed to find out optimum Cu rate and method of application for this soil.

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