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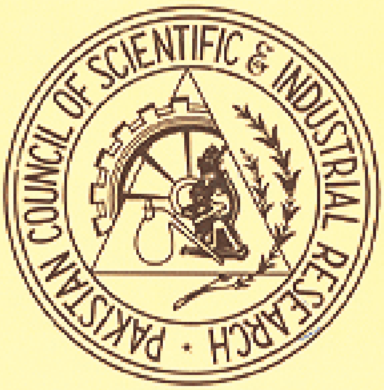

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Editorial

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(Dr Kaniz Fizza Azhar)
Executive Editor

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Vol. 54, No.2, May - June, 2011

Contents

Facile Synthesis and Characterization of Substituted Pyrimidin-2(1*H*)-ones and their Chalcone Precursors
Olayinka Oyewale Ajani, Ruth Itoroabasi Ituen and Ayorinde Falomo 59

Comparative Study of the Characteristics of Seed Oil and Seed Nutrient Content of three Varieties of *Cucumis sativus* L
Md. Abbas Ali, Md. Abu Sayeed, Sandip Kumar Ghosh, Sarmina Yeasmin, Astaq Mohal Khan and Fadzilah Adibah Abdul Majid 68

Removal of Copper from Aqueous Solutions Using Sawdust, Zeolite and Activated Carbon: Equilibrium Time Convergence
Zaman Shamohammadi Heidari, Esmat Jamalia, Milad Ghallehban Tekmedash and Mostafa Khajeh 75

Investigation of Carbon Monoxide at Heavy Traffic Intersections of Karachi (Pakistan) using GIS to Evaluate Potential Risk Areas for Respiratory and Heart Diseases
Akhtar Shareef, Durdana Rais Hashmi, Muhammad Azam and Imran Ahmad Khan 84

Petroleum Hydrocarbon Pollution after the Tasman Spirit Oil Spill of Coastal/Deep Sea Sediment along the Clifton Beach Karachi, Pakistan
Alia Bano Munshi, Fayyaz Ahmed Ansari, Hina Asgar Siddiqi and Mohammad Zeeshan 90

Lead Concentration in Road Side Dust and Selected Vegetables of Lahore City, Pakistan
Naz Imtiaz, Tahir Butt, Muhammad Irfan, Tahira Shafiq and Arshad Chaudhry 98

HPLC Determination of Betamethasone and Prednisolone in Urine Samples Using Monolithic Column
Kamran Abro, Najma Memon, Muhammad Iqbal Bhanger, Shahnaz Perveen and Rehana Jafri 103

Technology for Treatment and Recycling of Wastewater of Automobile Service Stations
Abdur Rahman Khan, Mumtaz Khan, Tehseen Aslam, Naveed Jan, Jehangir Shah and Inayat-ur-Rehman 108

Short Communication

Influence of Expeller Design Parameters on Free Fatty Acid Content and Colour of Palm Kernel *(Elaeis guineensis)* Oil
Rahman Akinoso, Abdulganiy Olayinka Rajib and Joseph Chulwugoziem Igbeka 114

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Facile Synthesis and Characterization of Substituted Pyrimidin-2(1*H*)-ones and their Chalcone Precursors

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Abstract. A new and efficient method has been developed for the quantitative transformation of chalcones to pyrimidine frame work *via* solid support catalysis. Silica supported sulphuric acid (SSA) efficiently catalyzed the reaction of α-β-unsaturated carbonyl, chalcones (**1-10**) with urea to afford substituted pyrimidin-2(1*H*)-ones (**11-20**) in good to excellent yield. The interesting behaviour of SSA lies in the fact that it can be re-used after simple washing with chloroform thereby rendering this procedure more economical. The chemical structures were confirmed by analytical data as well as spectroscopic means.

Keywords: catalyst, spectroscopic means, chalcones, 4-phenylbut-3-en-2-one

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Introduction

The pyrimidine moiety is one of the most widespread heterocycles in biologically occurring compounds, such as nucleic acid components (uracil, thymine and cytosine) and vitamin B_1, and is an important constituent of numerous drug molecules in many therapeutic areas (Kakiya *et al.*, 2002). In the light of the recent findings concerning the role of apoptosis and of tumour cell enzymes in cancer chemotherapy (Rich *et al.*, 2004), the interest in pyrimidine derivatives has greatly increased (Kaufmann and Earnshaw, 2000). Pyrimidine templates have been reported to possess, among others, antimicrobial (Behalo, 2009; Moustafa, *et al.*, 2008; Habib *et al.*, 2007; Vaghasia and Shah, 2007), anticancer (Xie *et al.*, 2009; Singh and Paul, 2006), anticoagulant (Saif, 2008; Ries and Priepke, 2000), antitubercular, (Trivedi *et al.*, 2008; Virsodia *et al.*, 2008; Alksnis *et al.*, 2001), anti-HIV (Al-Masoudi *et al.*, 2008; Murugesh *et al.*, 2008; Balzarini *et al.*, 2007; Miyashita *et al.*, 2003), analgesic (Hafez *et al.*, 2008; Sondhi *et al.*, 2005), anti-inflammatory(Pandas and Chowdary, 2008), anticonvulsant (Paronikyan *et al.*, 2007; Jain *et al.*, 2006), antiplatelet (Husted, 2007; Leoncini *et al.*, 2004), antiviral (Korkach *et al.*, 2007; Holy *et al.*, 2002), antimalarial (Rodenko *et al.*, 2007; Katritzky *et al.*, 2006) antifungal (Youssef *et al.*,2006), antibacterial (Sriharsha *et al.*, 2006), antitumoural (Grigoryan *et al.*, 2005) and antileukemic (Liu *et al.*, 2003) activities.

Although various procedures for the synthesis of pyrimidine derivatives have been developed, it is convenient to synthesize substituted pyrimidines by the reaction of amidine or guanidine derivatives with a variety of 1,3-dielectrophilic three-carbon units such as α,β-unsaturated carbonyl compounds (chalcones). Some series of pyrimido[3,2-a]pyrimidine derivatives have also been designed as targeted structures with modest activity against gram-positive bacterial strains (Al-Thebeiti, 2001).

In a similar manner, many attempts on the synthetic manipulation of chalcones have always been very productive because of biological relevance of this frame work. In fact, all the pyrimidinone derivatives synthesized in the work herein were obtained from the chemical transformation of α,β-unsaturated carbonyl in the presence of urea under acidic condition. Chalcones and pyrimidine derivatives are classes of heterocycles that are of considerable interest because of the diverse range of their biological properties.

Due to high biological diversity of chalcone reported above, among other things, some of these chalcones have been synthesized earlier by using various approaches (McConville *et al.*, 2009; Kreher *et al.*, 2003; Hayakawa *et al.*, 1984; Lyle and Paradis, 1955). However, chemical transformation of these templates to pyrimidinone derivatives using re-usable silical sulphuric acid (SSA) has not been explored to the best of our knowledge. Thus, it is conceivable to develop a

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*Author for correspondence; E-mail: wajanfresh@yahoo.com series of pyrimidinones using SSA catalyst technique and also compare it with the traditional method of conventional heating in concentrated HCl. Therefore, it was envisaged that the synthetic manipulation of chalcones by incorporating pyrimidine moieties therein might lead to the discovery of more pharmaceutically useful compounds.

Materials and Methods

General condition. Melting points were determined in open capillary tubes on a Stuart melting point apparatus and were uncorrected. Infrared spectra were recorded on a Schimadzu spectrometer. The ultraviolet spectra were run on a Genesys spectrometer using acetone solvent. ^{1}H and ^{13}C NMR were run on JEOL-JNM-GX 300-MHz spectrometer (in δ ppm relative to Me_4Si) using deuteriated chloroform. Mass spectra were run on Finnigan MAT 312 machine. All compounds were routinely checked by TLC on silical gel G plates using $CHCl_3$:CH_3OH (9:1, v/v) solvent system and the developed plates were visualized by UV light. The elemental analysis (C, H, N) of compounds were performed using a Carlo Erba-1108 elemental analyzer.

General procedure for the synthesis of aromatic chalcones (1-7). To a solution of sodium hydroxide (2.5 g) in water (20 mL), was added ethanol (10 mL) with continuous stirring until it cools down to room temperature. To this solution was added a mixture of appropriate ketone (14.15 mmol) and benzaldehyde (14.15 mmol or 28.30 mmol) drop-wise with continuous stirring at room temperature for 30 min. The resulting solution formed coloured precipitate which was filtered by suction, washed and recrystallized from ethanol to afford **1-7.**

4-Phenylbut-3-en-2-one (1). Yield 90.3%; mp. 38-40 °C {Lit. mp. 39-41 °C, McConville *et al.,* 2009}. UV-VIS{λ_{max}(log ε)}: 331 (1.83), 253 (4.18), 232 (3.30), 205 (3.76). IR [ν, cm^{-1}, KBr]: 2928 (CH aliphatic), 1690 (C=O), 1615 (C=C), 1190 (CH aromatic), 1040 (CH_3). ^{1}H NMR (300 Hz, δ ppm, $CDCl_3$): 7.60 (d, 1H, CO-C=CH, *J* = 15 Hz), 7.33-7.60 (m, 3H, Ar-H), 6.69 (d, 1H, CO-CH=C, *J* = 15 Hz), 2.27 (s, 3H, CH_3). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 197.7 (C=O), 142.7, 135.2, 128.6, 128.6, 128.5, 128.5, 127.9, 127.2, 27.4. MS *m/z:* 146[M$^+$, 25%], 131 [M – CH_3, 100%], 69 [M – Ph, 80%]. R_f (TLC): 0.52. Anal. Calcd. for $C_{10}H_{10}O$ (146): C, 82.2; H, 6.8. Found: C, 82.4; H, 6.9.

1-(4-Ethyphenyl)-3-phenylprop-2-en-1-one (2). Yield 95.0%; mp. 58-60 °C {Lit mp. 59-61 °C, (Lyle and Paradis, 1955)}. UV-VIS{λ_{max}(log ε)}: 325 (3.22), 244 (3.15), 226 (3.29), 208 (4.19). IR [ν, cm^{-1}, KBr]: 3010 (CH aliphatic), 1690 (C=O), 1600 (C=C), 1040 (CH_3). ^{1}H NMR (300 Hz, δ ppm, $CDCl_3$): 8.06 (d, 1H, CO-C=CH, *J* = 15 Hz), 7.59 (d, 1H, CO-CH=C, *J* = 15 Hz), 7.01-8.04 (m, 9H, Ar-H), 2.60 (q, 2H, CH_2, *J* = 8Hz), 1.25 (t, 3H, CH_3, *J* = 8 Hz). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 189.7 (C=O), 150.1, 145.1, 135.2, 135.1, 129.8, 129.8, 128.6, 128.6, 128.5, 128.5, 128.2, 128.2, 127.9, 121.3, 28.2 (CH_2), 14.5 (CH_3). R_f (TLC): 0.61. Anal. Calcd. for $C_{17}H_{16}O$ (236): C, 86.4; H, 6.8. Found: C, 86.6; H 6.7.

2-Benzylidenecyclopentanone (3). Yield 73.66%; mp. 55-58 °C {Lit mp. 54-57 °C, (Kreher *et al.,* 2003). UV-VIS {λ_{max}(log ε)}: 346 (4.35), 205 (3.47). IR [ν, cm^1, KBr]: 2928 (CH aliphatic), 1695 (C=O), 1604 (C=C). ^{1}H NMR (300 Hz, δ ppm, $CDCl_3$): 7.60 (d, 2H, Ar-H), 7.33-7.40 (m, 3H, Ar-H), 7.25 (s, 1H, Ar-CH=C), 2.94 (t, 2H, CH_2, *J* = 7.2Hz), 1.96 (t, 2 H, CH_2, *J* = 7.2 Hz), 1.44 (quin., 2H, CH_2, *J* = 7.2 Hz). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 168.4 (C=O), 153.1, 144.9, 140.5, 133.2, 133.2, 124.7, 124.7, 112.4, 35.8, 22.5, 19.7 (CH_2). MS *m/z:* 172 [M$^+$, 50%], 95 [M – Ph, 100%]. R_f (TLC): 0.69. Anal. Calcd. for $C_{12}H_{12}O$ (172): C, 83.7; H, 7.0. Found: C, 83.4; H, 6.9.

2,5-Dibenzylidenecyclopentanone (4). Yield 69.92%; mp.192-194 °C. UV-VIS{λ_{max}(log ε)}: 346 (4.01), 274 (3.30), 253 (3.31), 205 (3.69). IR [, cm^{-1}, KBr]: 3000(CH aliphatic), 1690 (C=O), 1600 (C=C), 1250 (CH aromatic). ^{1}H NMR (300 Hz, δ ppm, $CDCl_3$): 7.60 (m, 4H, Ar-H), 7.37 (s, 2H, 2×Cp=CH), 7.33-7.40 (m, 6H, Ar-H), 3.02 (s, 4H, 2×CH_2, *J* = 7.1 Hz). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 196.6 (C=O), 143.6, 143.6, 135.2, 135.2, 132.8, 132.8, 128.6 (four times), 128.5 (four times), 127.9, 127.9, 29.4 (CH_2), 29.4 (CH_2). R_f (TLC): 0.59. Anal. Calcd. for $C_{19}H_{14}O$ (258): C, 88.4; H, 5.4. Found: C, 88.7; H 5.6.

2,5-Bis(3-methoxybenzylidene)cyclopentanone (5). Yield 71.78%; mp.144-147 °C. UV-VIS{λ_{max}(log ε)}: 358 (3.19), 328 (3.28), 241 (3.06), 208 (3.55). IR [ν, cm^{-1}, KBr]: 2928 (CH aliphatic), 1690 (C=O), 1605 (C=C), 1450 (OCH_3), 1250 (CH aromatic). ^{1}H NMR (300 Hz, δ ppm, $CDCl_3$): 7.37 (s, 2H, 2×Cp=CH), 6.87-7.59 (m, 8H, Ar-H), 3.83 (s, 6H, 2× OCH_3), 3.02 (s, 4H, 2×CH_2, *J* = 7.1 Hz). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 196.6 (C=O), 160.5, 160.5, 143.6, 143.6, 134.8, 134.8, 132.8, 132.8, 129.6, 129.6, 120.8, 120.8, 113.5, 113.5, 113.2, 113.2, 55.8, 55.8, 29.4, 29.4 (CH_2).

R_f (TLC): 0.54. Anal. Calcd. for $C_{21}H_{18}O$ (286): C, 88.1; H, 6.3. Found: C, 88.3; H, 6.5.

2-Benzylidenecyclohexanone (6). Yield 76.43%; mp. 56-57 °C {Lit mp. 53-55 °C, (Kreher *et al.*, 2003)}. UV-VIS{λ_{max}(log ε)}: 348 (3.98), 265 (4.01), 220 (3.87). IR [ν, cm^{-1}, KBr]: 1685 (C=O), 1612 (C=C). ^1H NMR (300 Hz, δ ppm, $CDCl_3$): 7.33-7.60 (m, 5H, Ar-H), 7.25 (s, 1H, Cp=CH), 3.16 (t, 2H, CH_2), 2.81 (t, 2H, CH_2, *J* = 7.1 Hz), 1.67-1.74 (m, 4H, 2×CH_2, *J* = 7.1 Hz). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 202.1 (C=O), 143.8, 135.6, 135.2, 128.6, 128.6, 128.5, 128.5, 127.9, 39.0 (CH_2), 27.2 (CH_2), 26.1 (CH_2), 22.8 (CH_2). R_f (TLC): 0.66. Anal. Calcd. for $C_{13}H_{14}O$ (186): C, 83.9; H, 7.5. Found: C, 83.7; H, 7.4.

2,6-Dibenzylidenecyclohexanone (7). Yield 79.12%; mp. 121-123 °C. UV-VIS{λ_{max}(log ε)}:328 (4.17), 274 (3.15), 247 (3.15), 208 (3.93). IR [ν, cm^{-1}, KBr]: 2980 (CH aliphatic), 1690 (C=O), 1610 (C=C), 1310 (CH aromatic). ^1H NMR (300 Hz, δ ppm, $CDCl_3$): 7.60-7.61 (m, 4H, Ar-H), 7.33-7.40 (m, 6H, Ar-H), 7.37 (s, 2H, 2×Ch=CH), 2.81 (t, 4H, 2×CH_2, *J* = 7.1 Hz), 1.60 (quin., 2H, CH_2, *J* = 7.1 Hz). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 190.4 (C=O), 137.1, 137.1, 135.2, 135.2, 132.2, 132.2, 128.6 (four times), 128.5 (four times), 127.9, 127.9, 26.1 (CH_2), 26.1 (CH_2), 25.1 (CH_2). R_f (TLC): 0.68. Anal. Calcd. for $C_{20}H_{18}O$ (274): C, 87.6; H, 6.6. Found C, 87.7; H, 6.3.

General procedure for the synthesis of heteroaromatic chalcones (8-10). Sodium hydroxide (2.98 g) was dissolved in a mixture of water (20 mL) and methylated spirit (10 mL) in an ice bath with continuous stirring until a clear solution is obtained. To the clear solution, a mixture of furfural (1.95 mL, 23.57 mmol) and appropriate ketone (23.57 mmol) was added with continuous stirring for 2 h under ice bath. A clear solution was obtained. The reaction mixture was neutralized with dilute sulphuric acid and a crystalline product was formed immediately, filtered by suction and recrystallized from aqueous ethanol (1:1) to afford the product **8-10.**

4-(Furan-2-yl)but-3-en-2-one (8). Yield 51.20%; mp. 34-36 °C {Lit. mp. 33-34 °C, (Hayakawa *et al.*, 1984)} VIS{λ_{max}(log ε)}: 348 (3.47), 272 (3.86), 220 (4.11). IR [ν, cm^{-1}, KBr]: 1685 (C=O), 1612 (C=C). ^1H NMR (300 Hz, δ ppm, $CDCl_3$): 8.17 (d, 1H, Fr-H, *J* = 7.5 Hz), 7.65 (d, 1H, Fr-H, *J* = 7.5 Hz), 7.54 (d, 1H, CO-C=CH, *J* = 15 Hz), 6.91 (d, 1H, CO-CH=C, *J* = 15 Hz), 6.87 (m, 1H, Fr-H, *J* = 7.5 Hz), 2.27 (s, 3H, CH_3). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 197.7 (C=O), 151.6, 143.8, 129.1, 123.1, 113.6, 112.7, 26.8 (CH_3). R_f (TLC): 0.70. Anal. Calcd. for $C_8H_8O_2$ (136): C, 70.6; H, 5.9. Found: C, 70.7; H 5.7.

2-(Furan-2-ylmethylene)cyclopentanone (9). Yield 35.90%; mp. 58-61 °C. UV-VIS{λ_{max}(log ε)}: 348 (3.44), 304 (3.77), 216 (4.09). IR [ν, cm^{-1}, KBr]: 2928 (CH aliphatic), 1685 (C=O), 1612 (C=C), 1375 (C-O, epoxy). ^1H NMR (300 Hz, δ ppm, $CDCl_3$): 8.17 (d, 1H, Fr-H, *J* = 7.5 Hz), 7.65 (d, 1H, Fr-H, *J* = 7.5 Hz), 7.27 (s, 1H, Cp=CH), 6.87 (t, 1H, Fr-H, *J* = 7.5 Hz), 2.94 (t, 2H, CH_2, *J* = 7.0 Hz), 1.95 (t, 2H, CH_2, *J* = 7.0 Hz), 1.44 (quin., 2H, CH_2, *J* = 7.0 Hz). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 208.5 (C=O), 151.5, 147.4, 143.7, 119.4, 112.7, 109.6, 38.5 (CH_2), 21.4 (CH_2), 19.8 (CH_2). R_f (TLC): 0.69. Anal. Calcd. for $C_{10}H_{10}O_2$ (162): C, 74.1; H, 6.2 Found: C, 74.4; H, 6.5.

2-(Furan-2-ylmethylene)cyclohexanone (10). Yield 41.20%; mp. 45-47 °C. UV-VIS{λ_{max}(log ε)}: 368 (3.89), 340 (3.78), 220 (4.11). IR [ν, cm^{-1}, KBr]: 1685 (C=O), 1610 (C=C). ^1H NMR (300 Hz, δ ppm, $CDCl_3$): 8.17 (d, 1H, Fr-H, *J* = 7.5 Hz), 7.66 (d, 1H, Fr-H, *J* = 7.5 Hz), 7.27 (s, 1H, Cp=CH), 6.86 (t, 1H, Fr-H, *J* = 7.5 Hz), 3.16 (t, 2H, CH_2, *J* = 7.0 Hz), 2.82 (t, 2H, CH_2, *J* = 7.0 Hz), 1.68-1.75 (m, 4H, 2×CH_2, *J* = 7.0 Hz). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 201.9 (C=O), 151.5, 149.8, 143.7, 119.5, 112.7, 109.4, 38.4 (CH_2), 25.5 (CH_2), 24.8 (CH_2), 22.8 (CH_2). R_f (TLC): 0.57. Anal. Calcd. for $C_{11}H_{12}O_2$ (176): C, 75.0; H, 6.8. Found C, 74.8; H, 6.5.

General procedure for synthesis of pyrimidinone derivatives (11-20). ***Method I.*** A mixture of any of chalcones **1-10** (10 mmol) and urea (1.30 g, 21 mmol) was ground in mortar and quantitatively transferred to a 250 mL quick fit flask containing ethanol (30 mL). Later, concentrated hydrochloric acid (10 mL) was added drop-wise with continuous stirring and the reaction mixture was reflux for appropriate time and reduced by evaporation to half of the original volume. It was then cooled to room temperature and neutralized with 30% sodium hydroxide and left in the freezer chest over night. The solid product obtained was recrystallized from ethanol to afford the corresponding pyrimidinone **11-20** in moderate to good yield.

Method II. To a mixture of any of chalcones **1-10** (10 mmol), urea (1.30 g, 21 mmol) and ethanol (20 mL), a catalytic amount of SSA (100 mg, 0.26 mmol) was added and the reaction mixture was refluxed for appropriate time. The SSA catalyst was extracted with chloroform (20 mL) and removed from the entire solution. The remaining solution was reduced to half of its volume and cooled to room temperature. It was neutralized with 30% sodium hydroxide and left in the freezer chest over night. The solid product obtained was recrystallized from ethanol to afford the corresponding pyrimidinone **11-20** in good to excellent yield.

4-Methyl-6-phenyl-5,6-dihydropyrimidin-2(1H)-one (11). UV-VIS{λ_{max}(log ε)}: 325 (3.96), 274 (3.33), 244 (3.78), 226 (3.44), 202 (3.13). IR [ν, cm^{-1}, KBr]: 3241 (N-H), 2928 (CH aliphatic), 1685 (C=O), 1612 (C=C), 1575 (C=N). ^{1}H NMR (300 Hz, δ ppm, CDCl$_3$): 8.0 (s, 1H, NH, D$_2$O exchangeable), 7.26-7.40 (m, 5H, Ar-H), 4.90 (t, 1H, CH, $J = 7.0$ Hz), 1.94 (s, 3H, CH$_3$), 1.91-1.66 (m, 2H, CH$_2$, $J = 7.0$ Hz). ^{13}C NMR (300 Hz, δ ppm, CDCl$_3$): 180.1 (C=O), 160.2, 143.5, 128.7, 128.5, 128.5, 126.9, 126.9, 126.7, 47.7, 40.0, 22.1 (CH$_3$).

4-(4-Ethylphenyl)-6-phenyl-5,6-dihydropyrimidin-2(1H)-one (12). UV-VIS{λ_{max}(log ε) }: 310 (3.68), 265 (3.86), 230 (3.97), 215(3.77). IR [ν, cm^{-1}, KBr]: 3133 (N-H), 1685 (C=O), 1570 (C=N). ^{1}H NMR (300 Hz, δ ppm, CDCl$_3$): 8.0 (s, 1H, NH, D$_2$O exchangeable), 7.27-7.40 (m, 7H, 2×Ar-H), 7.78 (d, 2H, Ar-H, $J = 7.5$ Hz), 4.90 (t, 1H, CH, $J = 7.0$ Hz), 1.91-1.66 (m, 2H, CH$_2$, $J = 7.0$ Hz). 2.60 (q, 2H, CH$_2$, $J = 8.0$ Hz), 1.25 (t, 3H, CH$_3$, $J = 8.0$ Hz). ^{13}C NMR (300 Hz, δ ppm, CDCl$_3$): 164.6 (C=O), 160.1, 146.7, 143.5, 137.8, 128.5, 128.5, 127.8, 127.8, 127.0, 127.0, 126.9, 126.9, 126.7, 47.3 (CH), 42.7 (CH$_2$), 28.2 (CH$_2$), 14.5 (CH$_3$).

4-Phenyl-3,4,4a,5,6,7-hexahydro-2H-cyclopenta [d] pyrimidin-2-one (13). UV-VIS{λ_{max}(log ε)}: 328 (4.12), 274 (3.39), 247 (3.41), 208 (4.02). [IR ν, cm^{-1}, KBr]: 3295 (NH), 2928 (CH aliphatic), 1690 (C=O), 1600 (C=C), 1565 (C=N). ^{1}H NMR (300 Hz, δ ppm, CDCl$_3$): 8.01 (s, 1H, NH, D$_2$O exchangeable), 7.25-7.41 (m, 5H, Ar-H), 4.92 (d, 1H, CH), 2.67-2.84 (m, 5H, Cp-H), 1.22-1.41 (m, 4H, 2×CH$_2$, $J = 7.1$ Hz). ^{13}C NMR (300 Hz, δ ppm, CDCl$_3$): 208.4 (C=O), 150.0, 146.1, 142.9, 135.0, 135.0, 128.1, 128.1, 115.0, 115.0, 39.1 (CH$_2$), 23.8 (CH$_2$), 20.4 (CH$_2$). MS *m/z:* 214 [M$^+$, 12.5%],137 [M$^+$–Ph, 75%], 109 [M$^+$–Ph–CO, 100%].

7-Benzylidene-4-phenyl-3,4,4a,5,6,7-hexahydro-2H-cyclopenta[d]pyrimidin-2-one (14). UV-VIS{λ_{max} (log ε)}: 330 (3.98), 208 (4.14). IR [ν, cm^{-1}, KBr]: 3387 (NH), 1685 (C=O), 1612 (C=C), 1575 (C=N). ^{1}H NMR (300 Hz, δ ppm, CDCl$_3$): 8.0 (s, 1H, NH, D$_2$O exchangeable), 7.27-7.60 (m, 10H, 2×Ar-H), 6.34 (s, 1H, CH), 4.91 (d, 1H, CH, $J = 7.0$ Hz), 2.69 (t, 1H, CH, $J = 7.0$ Hz), 1.22-2.02 (m, 4H, 2×CH$_2$, $J = 7.1$ Hz). ^{13}C NMR (300 Hz, δ ppm, CDCl$_3$): 163.0 (C=O), 160.1, 141.5, 137.1, 135.2, 130.8, 128.6, 128.6, 128.5 (four times), 128.1, 128.1, 127.9, 125.9, 49.9, 45.3, 33.6 (CH$_2$), 31.3 (CH$_2$).

7-(3-Methoxybenzylidene)-4-(3-methoxyphenyl)-3,4,4a,5,6,7-hexahydro-2H-cyclopenta[d]pyrimidin-2-one (15). UV-VIS{λ_{max} (log ε)}: 366 (3.98), 345 (3.77), 210 (4.14). IR [ν, cm^{-1}, KBr]: 3387 (NH), 1685 (C=O), 1612 (C=C), 1575 (C=N). ^{1}H NMR (300 Hz, δ ppm, CDCl$_3$): 8.0 (s, 1H, NH, D$_2$O exchangeable), 6.82-7.59 (m, 8H, 2×Ar-H), 6.35 (s, 1H, CH), 4.90 (d, 1H, CH, $J = 7.0$ Hz), 3.84 (s, 6H, 2×CH$_3$, $J = 7.0$ Hz), 1.81-2.32 (m, 5H, Cp-H, $J = 7.1$ Hz). ^{13}C NMR (300 Hz, δ ppm, CDCl$_3$): 163.1 (C=O), 160.5, 160.4, 160.1, 141.5, 141.5, 134.8, 133.2, 130.9, 129.6, 129.5, 120.8, 120.3, 113.5, 113.2, 111.5, 55.8 (2 × OCH$_3$), 50.3, 45.3, 33.4, 31.2.

4-Phenyl-4,4a,5,6,7,8-hexahydroquinazolin-2(3H)-one (16). UV-VIS{λ_{max}(log ε)}: 375 (3.69), 344 (3.87), 210 (4.02). IR [ν, cm^{-1}, KBr]: 3387 (NH), 1685 (C=O), 1600 (C=C), 1573 (C=N). ^{1}H NMR (300 Hz, δ ppm, CDCl$_3$): 8.0 (s, 1H, NH, D$_2$O exchangeable), 7.27-7.41 (m, 5H, Ar-H), 4.91 (d, 1H, CH, $J = 7.0$ Hz), 2.19 (q, 1H, CH, $J = 7.0$ Hz), 1.19-1.41 (m, 8H, 4×CH$_2$, $J = 7.1$ Hz). ^{13}C NMR (300 Hz, δ ppm, CDCl$_3$): 164.7 (C=O), 160.1, 137.1, 128.5, 128.5, 128.1, 128.1, 125.9, 49.8, 41.9, 33.8, 27.0, 24.8, 24.2.

8-Benzylidene-4-phenyl-4,4a,5,6,7,8-hexahydroquinazolin-2(3H)-one (17). UV-VIS {λ_{max} (log ε)}: 378 (3.84), 362 (4.01), 220 (3.91). IR [ν, cm^{-1}, KBr]: 3385 (NH), 1684 (C=O), 1612 (C=C), 1573 (C=N). ^{1}H NMR (300 Hz, δ ppm, CDCl$_3$): 8.0 (s, 1H, NH, D$_2$O exchangeable), 7.60 (d, 2H,Ar-H), 7.28-7.40 (m, 8H, Ar-H), 6.35 (s, 1H, Ph-CH=C), 4.90 (d, 1H, CH, $J = 7.0$ Hz), 2.19 (q, 1H, CH, $J = 7.0$ Hz), 1.97 (t, 2H, CH$_2$, $J = 7.1$ Hz), 1.20-1.39 (m, 4H, 2 × CH$_2$, $J = 7.1$ Hz). ^{13}C NMR (300 Hz, δ ppm, CDCl$_3$): 164.6 (C=O), 160.1, 137.0, 135.2, 130.1, 128.6, 128.6, 128.5(five times), 128.1, 128.1, 127.8, 125.9, 50.2, 38.2, 27.4, 24.6, 24.6.

6-(Furan-2-yl)-4-methyl-5,6-dihydropyrimidin-2(1H)-one (18). UV-VIS{λ_{max}(log ε)}: 365 (3.66), 335 (3.59), 210 (3.72). IR [ν, cm^{-1}, KBr]: 3365 (NH), 1675 (C=O), 1610 (C=C), 1575 (C=N). ^{1}H NMR (300 Hz, δ ppm, CDCl$_3$): 8.0 (s, 1H, NH, D$_2$O exchangeable), 6.30-6.61 (m, 3H, Fr-H), 5.11(t, 1H, CH, $J = 7.0$ Hz), 1.94 (s, 3H, CH_3, $J = 7.0$ Hz), 1.65-1.91 (d, 2H, CH_2). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 180.1 (C=O), 160.2, 151.0, 141.5, 110.0, 109.3, 48.7, 38.2, 21.5(CH_3).

4-(Furan-2-yl)-3,4,4a,5,6,7-hexahydro-2H-cyclopenta [d] pyrimidin-2-one (19). UV-VIS {λ_{max} (log ε)}: 368 (4.03), 345 (3.87), 210 (4.14). IR [ν, cm^{-1}, KBr]: 3371 (NH), 1690 (C=O), 1612 (C=C), 1572 (C=N). ^{1}H NMR (300 Hz, δ ppm, $CDCl_3$): 8.00 (s, 1H, NH, D_2O exchangeable), 7.66 (d, 1H, Fr-H), 6.43-6.47 (t, 2H, Fr-H), 5.10 (d, 1H, CH, $J = 7.0$ Hz), 2.67-2.82 (m, 3H, Cp-H), 1.20-1.41 (m, 4H, 2 × CH_2, $J = 7.1$ Hz). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 164.6 (C=O), 160.1, 150.1, 141.6, 110.0, 108.9, 50.8, 45.7, 37.4, 24.7, 22.8.

4-(Furan-2-yl)-4,4a,5,6,7,8-hexahydroquinazolin-2(3H)-one (20). UV-VIS {λ_{max} (log ε)}: 379 (3.92), 365 (3.75), 210 (4.01). IR [ν, cm^{-1}, KBr]: 3272(N-H), 1673(C=O), 1605(C=C), 1575(C=N). ^{1}H NMR (300 Hz, δ ppm, $CDCl_3$): 8.00 (s, 1H, NH, D_2O exchangeable), 7.65 (d, 1H, Fr-H), 6.43-6.47 (t, 2H, Fr-H), 5.10 (d, 1H, CH, $J = 7.0$ Hz), 2.19 (q, 1H, CH, $J = 7.0$ Hz), 1.18-1.39 (m, 8H, 4 × CH_2, $J = 7.1$ Hz). ^{13}C NMR (300 Hz, δ ppm, $CDCl_3$): 164.5 (C=O), 160.1, 150.0, 141.5, 110.0, 108.8, 51.0, 40.0, 33.3, 27.0, 24.1, 21.8.

Results and Discussion

In the first part of this study, α,β-unsaturated carbonyls **(1-7)** were synthesized *via* condensation of benzaldehyde with ketones in basic medium while replacing of benzaldehyde with heteroaromatic aldehyde, furfural, resulted in the formation of α,β-unsaturated carbonyls **8-10** (Scheme 1). Although, compounds **1-7** were formed in good yields *via* a continuous stirring at room temperature, **8-10** violated this reaction protocol at room temperature but were obtained in improved yields *via* continuous stirring in ice bath at a controlled temperature of 0 °C. Later, compounds **(1-10)** were subsequently reacted with urea under two different conditions to afford pyrimidinone derivatives **(11-20)**. The difference in the condition lied in the nature of the catalyst. Hence, the synthesis of pyrimidinone in the presence of concentrated HCl (Method I) was compared with one using solid support catalyst, silica sulfuric acid (SSA) (Method II). The products of the reactions were monitored through thin layer chromatography (TLC) spotting using chloroform: methanol (9:1, v/v) solvent system. Each of the reactions gave one spot with R_f values varying from 0.40 to 0.85. The main method used to construct the pyrimidine skeleton is the [3+3]cyclocondensation of N-C-N and C-C-C units.

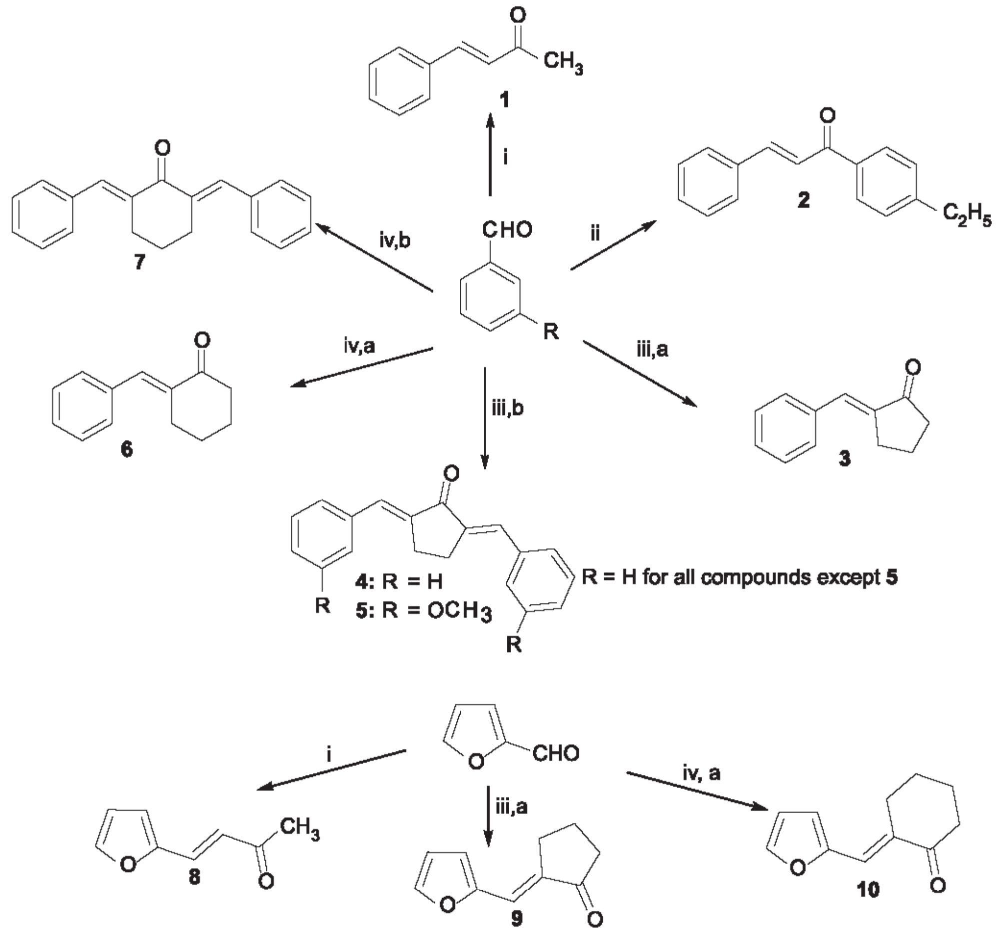
As a case study, condensation of an equimolar mixture of benzaldehyde with cyclopentanone affords 2-benzylidenecyclopentanone **(3)**. The chalcone **3** was subsequently treated with urea in ethanol in the presence of either concentrated hydrochloric acid (Method I) or silica sulphuric acid (Method II) under reflux at 140 °C to afford 4-phenyl-3,4,4a,5,6,7-hexahydro-2*H*-cyclopenta[*d*] pyrimidin-2-one, **(13)**, Scheme 2. This procedure was repeated for the chemical transformation of other chalcones to their corresponding pyrimidinone derivatives. In Method I, upon completion (TLC), the reaction was worked up to afford **13** in moderate yield 51% after refluxing for 9 h. However, in Method II, where conc. HCl was replaced with solid support catalyst SSA, the reaction time did not only reduced drastically to 3 h but also led to the formation of the product **(13)** at a higher yield, 91% (Table 1). The SSA catalyst was recovered with chloroform (20 mL). The resulting filtrate was reduced to half its volume and cooled. It was neutralized with ammonium hydroxide and filtered by suction to afford 4-phenyl-3,4,4a,5,6,7-hexahydro-2*H*-cyclopenta[*d*]pyrimidin-2-one, **(13)**. In a nutshell, it was observed that SSA did not only emerge as an efficient catalyst in this study but also afforded the pyrimidinone products in higher yields (75-93%) within smaller reaction time (3-4 h) compared with concentrated hydrochloric acid which gave smaller yields (40-71%) at higher reaction time of 8-9 h (Table 1).

From the spectroscopic studies, using **13** as a typical representative of the pyrimidones, the UV-visible absorption spectrum in chloroform gave rise to wavelength ranging from 208 nm to 328 nm. The peak at $\lambda_{max} = 208$ (log ε = 4.02) was as a result of $\pi \rightarrow \pi^*$ of benzene nucleus, while the highest one at $\lambda_{max} = 328$ (log ε = 4.12) was as a result of $n \rightarrow \pi^*$ transition due to presence of iminone and additional conjugation. Two shoulders were noticed at 247 nm and 274 nm. The infrared spectrum of **13** showed absorption bands due to the stretching vibrations of N-H and C-H aliphatic at 3295 cm^{-1} and 2928 cm^{-1}, respectively, while the band at 1690 cm^{-1} depicted the presence of conjugated C=O. The infrared band of C=C aromatic and C=N of pyrimidine were confirmed at 1600 cm^{-1} and 1565 cm^{-1} respectively. The chemical shifts and multiplicity patterns of ^{1}H and ^{13}C NMR correlated well with that of the proposed structures. For instance, the ^{1}H NMR spectrum of **13** in deuteriated chloroform showed NH signal, which was exchangeable with D_2O, as a singlet down

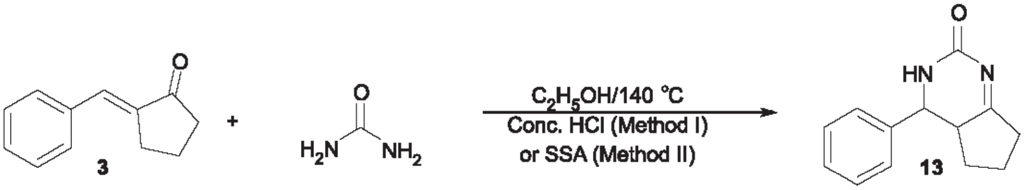
Table 1: Physicochemical properties of synthesized pyrimidinone **(11-20)**

Comp. code	Molecular formula	Mol. Wt.	M.P. (°C)	R_f*	Colour	Method I		Method II	
						Time** (h)	Yield (%)	Time** (h)	Yield (%)
11	$C_{11}H_{12}N_2O$	188	124-127	0.77	Yellow	8	45	3	77
12	$C_{18}H_{18}N_2O$	278	211-213	0.56	White	9	58	3	82
13	$C_{13}H_{14}N_2O$	214	184-186	0.85	Green	9	51	3	94
14	$C_{20}H_{17}N_2O$	301	227-229	0.49	Green	8	63	3	89
15	$C_{22}H_{21}N_2O_3$	355	240-242	0.69	Green	9	60	4	91
16	$C_{14}H_{16}N_2O$	228	198-200	0.76	Orange	7	48	3	75
17	$C_{21}H_{20}N_2O$	316	>320	0.63	Black	8	71	3	95
18	$C_9H_{10}N_2O_2$	178	106-108	0.55	Yellow	7	68	3	90
19	$C_{11}H_{12}N_2O_2$	204	133-135	0.68	Black	8	71	3	92
20	$C_{12}H_{14}N_2O_2$	218	144-147	0.40	Orange	7	40	3	75

* = solvent system: $CHCl_3$:CH_3OH (9:1, v/v); ** = reaction under reflux at 140 °C.



Scheme 1. (i) acetone (ii) 4-ethyl acetophenone (iii) cyclopentanone, a=1 eq, b=0.5 eq (iv) cyclohexanone, a=1 eq. b=0.5 eq. Reaction conditions for **1-7** = NaOH/EtOH/ H_2O/RT while conditions for **8-10** = NaOH/methylated spirit/H_2O/ice bath at 0 °C.



Scheme 2. Synthesis of 4-phenyl-3, 4, 4a, 5, 6, 7-hexahydro-2*H*-cyclopenta[d]pyrimidin-2-one (**13**).

field at δ 8.01 while five aromatic protons were observed as a multiplet at δ 7.25-7.41. The only proton on carbon adjacent to NH resonated as a doublet δ 4.92. All the seven protons from cyclopentyl (Cp) group were noticed up field; three of them as a mutiplet at δ 2.67-2.84 while the remaining four (2 x CH_2, Cp) were observed as a multiplet at δ 1.22-1.41. In addition, ^{13}C NMR spectrum of **13** showed the presence of twelve carbon atoms with the signals ranging from 208.4(C=O) to 20.4 (CH_2) ppm. In the mass spectral data of **13**, the molecular ion peak observed at *m/z* 214 corresponded with its molecular mass while the base peak found at *m/z* 109 was as a result of the loss of a phenyl radical and a stable ethylene molecule. Loss of a phenyl radical from the molecular ion peak accounted for the daughter fragment with *m/z* of 137.

Conclusion

Silica sulphuric acid (SSA) was found to be a mild, efficient and reusable solid catalyst for the reaction of α,β-unsaturated carbonyl with urea to furnish the corresponding pyrimidinone derivatives in good to excellent yield. The interesting behaviour of SSA lies in the fact that it can be re-used after simple washing with chloroform thereby rendering this procedure more economical compared with concentrated HCl method. In addition, SSA gave better yields in a reduced reaction time. Thus, the pyrimidinone library synthesized herein could be very useful candidates for further studies in terms of toxicity effect and structural activity relationship (SAR) in order to improve their biological and pharmacological activities.

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Comparative Study of the Characteristics of Seed Oil and Seed Nutrient Content of three Varieties of *Cucumis sativus* L.

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Abstract. The physicochemical characteristics of oils and nutrient contents of the seeds of three varieties of *Cucumis sativus* namely, Shahi-50, Naogaon-5 and Naogaon-Green, have been reported. Profile of fatty acid composition was not wholly similar in all varieties and unsaturated fatty acids were more than 77%, of which linoleic acid was 61.9-62.2%. High degree of unsaturation was indicated with lower peroxide value (3.7-4.2) and FFA (1.1-1.6%). Triacylglycerols and neutral lipids were the most abounded components recorded as 82.1-83.7% and 92.1-94.0%, respectively. The seeds contained potentially useful amounts of lipid (28.0-31.1%) and protein (14.8-15.9%) and other nutrients.

Keywords: seed oil, fatty acid, nutrient contents, *Cucumis sativus*

Introduction

For developing a healthy population the relation between food, nutrition and health has to be reinforced. Developing countries try to achieve this purpose through the exploitation of available local resources. Knowledge of the nutritive value of local dishes and local foodstuff is necessary for increased cultivation and consumption of highly nutritive crops, and supplementing the nutrients of the staple carbohydrate foods of those who cannot afford adequate proteinaceous foods of animal origin.

The Cucurbitaceae is a medium-size plant family, primarily found in the warmer regions of the world, consisting of economically important species, whose fruits are used for nutrition and medicinal purposes. Many Cucurbitaceae seeds are rich in oil and protein, and although none of these oils has been utilized on an industrial scale, many of them are used as cooking oil in some countries of Africa and the Middle East (Mariod *et al.*, 2009). *Cucumis sativus*, locally known as Sassa, is one of the species which is cultivated largely in Bangladesh. Its three popular local varieties which vary morphologically from each other, are Shahi-50 (Type-1: Fruit long, dark green, covered with long triangular ovate leaves; seed narrow, straw in colour), Naogaon-5 (Type-2: Fruit medium, light green, covered with medium triangular ovate leaves; seed medium, straw in colour) and Naogaon-Green (Type-3: Fruit small, deep green, covered with small triangular ovate leaves; seed straw in colour). *C. sativus* seeds, besides possessing medicinal qualities, are also a rich source of proteins (28.68%) and lipids (53.76%) (Achu *et al.*, 2005).

Extensive researches have been carried out on oils in many countries where vegetable oils are used as house hold and commercial purposes. Peris-Vicente *et al.* (2005) studied drying oils by analysis of fatty acids obtained after acidic hydrolysis of the oils, using HPLC with fluorescence detection for obtaining best resolution of peaks and detector selectivity than with GC-FID methods, and better sensitivity than that achieved with HPLC-UV-VIS detection. Peris-Vicente *et al.* (2007) developed an analytical method for the study of drying oils by analyzing the released fatty acids, using direct infusion mass spectrometry with negative ion electrospray ionization (ESI), avoiding derivatization and separation. Peris-Vicente *et al.* (2006) developed a chromatographic method for characterizing natural waxes by means of their characteristic chemical

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*Author for correspondence; E-mail: radwiya44@yahoo.com composition. For characterizing the waxes, fatty acids and hydrocarbons were taken as characteristic compounds. Peris-Vicente *et al.* (2004) presented an analytical study of drying oils by analysis of the fatty acids, obtained by acid hydrolysis of the oils, using HPLC by UV-VIS detection for obtaining best resolution of peaks and detector selectivity (FID) than with gas chromatography methods.

The proximate and fatty acids composition of seeds or seed kernels of *C. sativus* from different origins and varieties have been reported widely (Fokou *et al.*, 2009; Mariod *et al.*, 2009; 2004; Achu *et al.*, 2005). Three of the prominent local varieties of *C. sativus*, as mentioned above, have gained large acceptance in the northern part of Bangladesh. The aim of this work was to determine physicochemical characteristics, acylglycerol class, lipid class and fatty acid composition of oils and nutrient contents of the seeds of the three varieties (Type-1, Type-2 and Type-3) of *C. sativus* as a basis of comparison.

Materials and Methods

Plant materials and chemicals. Ripe fruits of the three varieties of *C. sativus* were collected in the year 2006 from an experimental plot located in Rajshahi city, Bangladesh. The seeds were separated from the fruits manually and washed several times with water to remove foreign materials. Afterwards, the seeds were dried in sunlight for four consecutive days and then in an electric oven at 40 °C until constant weight was reached. The seeds were ground to a fine powder, packaged and stored at 4 °C prior to analysis. Solvents such as petroleum ether, diethyl ether, benzene, chloroform, acetone, methanol etc. were obtained from Merck (Darmstadt, Germany) and BDH (Poole, England). Silica gel (60-120 mesh) and silica gel (HF_{254}) were products of Merck (Darmstadt, Germany). Esters of fatty acids and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade unless otherwise specified and results were expressed on dry weight basis.

Analysis of *C. sativus* seed oil. The oil from the powdered seeds was extracted with light petroleum ether (40-60 °C) in a soxhlet apparatus for about 24 h and the solvent was removed by rotary vacuum evaporator (Buchi Labortechnik AG, Postfach, Switzerland) and the percentage of oil content was computed.

Physical and chemical characteristics. Specific gravity of the oil was determined at 23 °C with the help of a specific gravity bottle. Refractive index of clear oil was determined at 28 °C using Abbe Refractometer (ATAGO T-series, Model-3T, Texas, USA) following IUPAC (1979) method. Iodine value, unsaponifiable matter, Reichert-Meissl value and acetyl value were determined by the methods depicted by Ranganna (1986), while the saponification value, percentage of free fatty acid (FFA) and peroxide value were determined according to the methods described by Williams (1966).

Separation of acylglycerols. The oil was separated into mono-, di- and triacylglycerols by silica gel (60-120 mesh) column chromatography. The solvent systems used to elute the column were similar to those described by Gofur *et al.* (1993a). For quantitative determination of acylglycerol classes, the sample (529.8 mg in 3 mL petroleum ether) was adsorbed on the top of the column; triacylglycerols were eluted with benzene, diacylglycerols with a mixture of diethyl ether and benzene (1:9, v/v) and monoacylglycerols with diethyl ether. Fractions were collected at approx. 1.5-2 mL/min. Elution was monitored by thin layer chromatography (TLC). Purity of the separated fractions was confirmed by TLC, using silica gel (HF_{254}) and hexane-diethyl ether 80/20 (v/v) as solvent system. Spots were visualized with chromic-sulphuric acid at 180 °C.

Fractionation of lipids. A total of 553 mg lipid extracted from the seeds by the method of Bligh and Dyer (1959) was fractionated into three major lipid groups: neutral lipid, glycolipid, and phospholipid by silica gel column chromatography (Gofur *et al.*, 1993b). Neutral lipids were eluted with chloroform, glycolipids with acetone and phospholipids with methanol. Approximately 0.5-1.0 mL fractions were collected per minute and elution was monitored by TLC. Solvents were evaporated in vacuum rotary evaporator and percentages of these fractions were determined by gravimetric method.

Fatty acid composition of oil. Fatty acids of *C. sativus* seed oil were determined as their methyl esters prepared by boron-trifluoride methanol complex method (Morrison and Smith, 1964). A GCD PYE Unicam gas chromatograph (PYE Unicam Ltd., Cambridge, UK) equipped with a flame ionization detector was used to determine the fatty acid methyl esters. Nitrogen carrier gas was used at a flow rate of 30 mL/min. Fatty acids were separated on a 1.8 m 2 mm i.d. glass column packed with 6% BDS (butanediol succinate polyesters) on solid support, Anakorm ABS (100/120) mesh.

Analysis was carried out at isothermal column temperature 190 °C; injector and detector temperature for all GLC analysis was 240 °C. The peaks were identified by comparison with standard fatty acid methyl esters.

Analysis of *C. sativus* seeds. Moisture, ash and crude fibre contents were determined by AOAC (1990) methods. Lipid content was estimated by the method of Bligh and Dyer (1959) using a solvent mixture of chloroform and methanol (2:1 v/v). The micro-Kjeldahl (Buchi Labortechnik AG, Switzerland) method of AOAC (1990) was employed to determine total nitrogen and the protein content was calculated from the total nitrogen, using N × 6.25. Water soluble protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. Determination of starch content was based on analytical method outlined elsewhere (Clegg, 1956). Total sugar content was determined by colorimetric method (Dubois *et al.*, 1951) and total carbohydrates were calculated by the difference (Rahim *et al.*, 1999).

Statistical analysis. All data were expressed as the mean and standard deviation (SD) of three experiments and were subjected to one way analysis of variance (ANOVA). Mean values were compared at $P<0.05$ significant level by Duncan's multiple range test using SPSS 11.5 software package.

Results and Discussion

The solvent extracts of three varieties of *C. sativus* seeds yielded on an average, 24.7% oil, which is similar to the value of 25.8% reported by Mariod *et al.* (2009). Information on detailed characteristics of oil and nutritional composition of seeds from *Cucumis* species are too scanty for meaningful comparisons.

The estimated specific gravities (Table 1) of *C. sativus* seed oils (0.9174-0.9192 at 23 °C) are in good agreement with the value 0.9140 at 30 °C for the same oil, but higher than the value 0.8840 at 30 °C for *C. prophetarum* seed oil reported by Mariod *et al.* (2009). Refractive indices of the oils were found to be 1.4645-1.4665 at 28 °C, being higher than 1.434 at 40 °C (Mariod *et al.*, 2009) for the same oil and 1.424 at 28 °C (Mian-Hao and Yansong, 2007) for *Cucumis melo* hybrid 'ChunLi' seed oil. Specific gravity and refractive index which are very stable parameters and used for checking the identity of oils, did not differ significantly ($P<0.05$) between the analyzed samples. Iodine values estimated for *C. sativus* (112.0-114.3) were much lower than the value 89.5 (Mian-Hao and Yansong, 2007) for *Cucumis melo* hybrid 'ChunLi' seed oil, but were consistent with the reported value of 114.0 for the same seed oil (Mariod *et al.*, 2009). Referring to Table 1, therefore, the oil samples of Type-1, in contrast to other samples in the present investigations have lower tendency to become rancid by oxidation. Saponification values of the three samples were in the range 189.8-194.8, being lower than the value 209.0 reported by Sadou *et al.* (2007) for *Cucumis metuliferus* seed oil. The estimated comparatively low saponification values indicate the presence of higher proportion of higher fatty acids. No significant difference ($P<0.05$) in iodine and saponification values were found between the sample means of Type-2 and Type-3. The content FFA (1.1-1.6%) of the *C. sativus* seed oils was similar to the value 1.3% cited in the literature (Mariod *et al.*, 2009), but lower than the value 0.75% (Mian-Hao and Yansong, 2007) reported for *Cucumis melo* hybrid 'ChunLi' seed oil. Results regarding FFA contents indicate more suitability of the oil sample of Type-2 for probably edible purpose as it contained significantly ($P<0.05$)

Table 1. Physical and chemical characteristics of *Cucumis sativa* seed oils

Characteristics	Type-1	Type-2	Type-3
Specific gravity at 23 °C	0.9174 ± 0.0021^{a}	0.9192 ± 0.0008^{a}	0.9185 ± 0.0018^{a}
Refractive index at 28 °C	1.4655 ± 0.0040^{a}	1.4645 ± 0.0042^{a}	1.4665 ± 0.0023^{a}
Iodine value (Hanus)	112.0 ± 0.85^{a}	113.6 ± 0.62^{b}	114.3 ± 1.25^{b}
Saponification value (mg KOH/g)	194.8 ± 0.66^{b}	189.9 ± 1.00^{a}	189.8 ± 1.41^{a}
Free fatty acids (%) as oleic	1.6 ± 0.15b	1.1 ± 0.15^{a}	1.4 ± 0.05b
Unsaponifiable matter (g/100g)	1.3 ± 0.20^{a}	1.2 ± 0.05^{a}	1.4 ± 0.10^{a}
Peroxide value (mEq/kg of oil)	3.7 ± 0.17^{a}	4.2 ± 0.26^{b}	4.0 ± 0.17^{ab}
Reichert-Meissl value	0.61 ± 0.07^{a}	0.81 ± 0.09^{b}	0.71 ± 0.06^{ab}
Acetyl value	2.5 ± 0.10^{a}	2.9 ± 0.34^{ab}	3.0 ± 0.20^{b}

Values are mean ± standard deviation of three experiments; means in the same row with different superscript are significantly ($P<0.05$) different.

lower percentage of FFA than those contained in the rest of the samples.

C. sativus seed oils contained unsaponifiable matter (Table 1) ranging from 1.2% in Type-2 to 1.4% in Type-3, being similar to 1.1% for the same oil reported by Mariod *et al.* (2009). No significant inter-variety differences ($P<0.05$) in the level of unsaponifiable matter were observed. The peroxide values in the samples of *C. sativus* ranging from 3.7 to 4.2 mEq/kg were slightly higher than 3.5 mEq/kg revealed by Mariod *et al.* (2009), but much lower than 8.0 mEq/kg reported by Fokou *et al.* (2009) for the same oil. Fresh oils usually have peroxide values well below 10 mEq/kg. The present experimental results indicate that *C. sativus* seed oils are quality oil. The low Reichert-Meissl values (0.61-0.81) as estimated for *C. sativus* indicate the low content of lower volatile soluble fatty acids, and this value is also in agreement with the low saponification value as obtained. Acetyl values of *C. sativus* seed oils were determined to be (2.5-3.0). Significant differences ($P<0.05$) in mean peroxide, Reichert-Meissl and acetyl values of the sample were noticed.

As shown in Table 2, the triacylglycerol contents varied from 82.1 to 83.7%, while diacylglycerols, from 10.9 to 11.5% and monoacylglycerols, from 1.1 to 1.9%. No significant differences ($P<0.05$) were observed in triacylglycerol and diacylglycerol composition to account for about 82.8% (average) and 11.2% (average), respectively, of the total weight of oil, in the three varieties. Total recovery of acylglycerols was more than 95% indicating that *C. sativus* seed oils contained lower amount of non-acylglycerols than that contained in *Mesua ferrea* seed oil (Sayeed *et al.*, 2004). Of the three samples, Type-3 contained significantly ($P<0.05$) higher amount (1.9%) of monoacylglycerols that can be separated easily by column chromatography and used as emulsifier. Mono- and particularly diacylglycerols occur naturally in oils and fats, where their presence is initially due to partial hydrolysis of the oil by enzyme action in the fruits or the seeds. Monoacylglycerols are surface-active materials, having both polar, water soluble, and non-polar, fat-soluble groups. It is for this reason that the higher monoacyl-glycerol are of great importance as emulsifier in the food industry. They are particularly valuable for producing stable oil-in-water emulsions and are also crystal promoters. Thus, fat containing small amount of monoacylglycerol will set quickly to a micro-crystalline matrix (Devine and Williams, 1961).

Table 2. Acylglycerol composition of *Cucumis sativa* seed oils (wt %)

Varieties	Monoacylglycerol	Diacylglycerol	Triacylglycerol
Type-1	1.5 ± 0.10^{b}	11.5 ± 0.60^{a}	82.1 ± 1.73^{a}
Type-2	1.1 ± 0.17^{a}	11.4 ± 0.40^{a}	83.7 ± 0.29^{a}
Type-3	1.9 ± 0.26^{c}	10.9 ± 0.45^{a}	82.7 ± 1.41^{a}

Values are mean ± standard deviation of three experiments; means in the same column with different superscripts are significantly ($P<0.05$) different.

Fractionation of *C. sativus* seed lipids by silica gel column chromatography into neutral lipids, glycolipids and phospholipids was quantified as 92.1-94.0%, 2.2-3.7% and 2.6-3.1%, respectively (Table 3). There were no significant differences ($P<0.05$) in the level of phospholipid contents of different sources. Results also indicated neutral lipids to be the most abundant component of seed lipids, recorded over 92% of the total weight of the lipid. However, the amounts of glycolipids and phospholipids found in *C. sativus* were lower than those of *Nicotiana tabacum*, whereas neutral lipids were higher (Ali *et al.*, 2008).

Table 3. Lipid composition of *Cucumis sativa* seed lipids (wt %)

Varieties	Neutral lipid	Glycolipid	Phospholipid
Type-1	93.3 ± 0.60^{ab}	3.4 ± 0.20^{b}	2.6 ± 0.26^{a}
Type-2	92.1 ± 0.45^{a}	3.7 ± 0.20^{b}	2.7 ± 0.34^{a}
Type-3	94.0 ± 0.79^{b}	2.2 ± 0.26^{a}	3.1 ± 0.45^{a}

Values are mean ± standard deviation of three experiments; means in the same column with different superscripts are significantly ($P<0.05$) different.

The fatty acid patterns (Table 4) of *C. sativus* seed oils were qualitatively similar to those of other plants; linoleic acid (61.9-62.2%) being the major fatty acids followed by oleic acid (15.6-16.5%). Linoleinic acid was detected in trace amount in all the samples. It was also noted that *C. sativus* oils contained mainly unsaturated fatty acids (77.8-79.1%), while saturated fatty acids were 20.9-22.2%. Saturated fatty acids, accounting more than 20%, were palmitic (10.1-10.7%) and stearic (10.2-12.1%). No significant ($P<0.05$) differences were detected in palmitic and linoleic acid contents of the seed oils. The most prominent feature of the fatty acid composition of *C. sativus* seed oils was the high amount of linoleic acid, being slightly lower than that reported by Mariod *et al.* (2009) for the same seed oil. But the amount of linoleic acid detected herein, was higher as compared to many other seed oils such as that of *Cucumis melo* var. *agrestis* (57.6%) (Mariod *et al.*, 2009), *Cucumis metuliferus* (56.21%) (Sadou *et al.*, 2007), *Cucurbita maxima* (43.0-50.3%), and *Cucurbita argyrosperma* (56.0%) (Applequist *et al.*, 2006), which is likely to satisfy the essential fatty acid requirement of humans. The nutritional value of linoleic acid is due to its metabolism at tissue level, which produces long chain polyunsaturated fatty acids and prostaglandins (Sayanova *et al.*, 2003). The saturated/unsaturated fatty acid ratio of the oils was found to be in the range of 0.2642 to 0.2853 in all varieties; however, Type-3 seed oil displayed higher unsaturation as compared to the others with a saturated/unsaturated fatty acid ratio of only 0.2642. These ratios indicate that the samples have a high content of unsaturated fatty acids, which may make them more attractive for the consumers who wish to ingest this type of acid.

Table 4. Fatty acid composition of *Cucumis sativa* seed oils (%)

Fatty acids	Type-1	Type-2	Type-3
Palmitic acid (C16 : 0)	10.1 ± 0.36^{a}	10.2 ± 0.51^{a}	10.7 ± 0.20^{a}
Stearic acid (C18 : 0)	12.1 ± 0.40^{c}	10.9 ± 0.10^{b}	10.2 ± 0.43^{a}
Oleic acid (C18 : 1)	15.6 ± 0.05^{a}	16.5 ± 0.43^{b}	16.3 ± 0.51^{ab}
Linoleic acid (C18 : 2)	61.9 ± 0.49^{a}	61.9 ± 0.45^{a}	62.2 ± 0.32^{a}
Linoleinic acid (C18 : 3)	0.3 ± 0.10^{a}	0.5 ± 0.17^{ab}	0.6 ± 0.20^{b}

Values are mean ± standard deviation of three experiments; means in the same row with different superscripts are significantly ($P<0.05$) different.

The nutrient contents of *C. sativus* seeds are reported in Table 5. The seeds contained moisture (6.7-7.3%), higher than the value of 4.41% reported by Mariod *et al.*, (2009) and 5.65 % reported by Achu *et al.* (2005) for the same seeds. The three samples did not show significant ($P<0.05$) differences in moisture content. For the preservation of a product for a long time and to diminish the probability of bacterial and fungal growth, that could alter the quality through decomposition, the content of moisture is important (Aguilera-Morales *et al.*, 2005). *C. sativus* seeds contained total lipids, 28.0-31.1%, higher than the value of 3.3-4.1% reported for *Castanea sativa* (Neri *et al.*, 2010). Ash contents was in the range 3.4-4.2%, similar to the reported values 4.0 (Mariod *et al.*, 2009) and 3.5 (Achu *et al.*, 2005), but lower than the values 8.3 (Mariod *et al.*, 2009) for *Cucumis prophetarum* and 5.7 (Mariod *et al.*, 2009) for *Cucumis melo* var. *flexuosus* seeds.

Table 5. Nutrient contents of *Cucumis sativa* seeds

Parameters (g/100 g)	Type-1	Type-2	Type-3
Moisture	7.2 ± 0.26^{a}	7.3 ± 0.36^{a}	6.7 ± 0.30^{a}
Lipid	28.0 ± 0.45^{a}	28.8 ± 0.26^{b}	$31.1\ 0.34^{c}$
Ash	4.2 ± 0.26^{b}	3.6 ± 0.17^{a}	3.4 ± 0.17^{a}
Total protein	14.8 ± 0.26^{a}	15.5 ± 0.50^{ab}	15.9 ± 0.34^{b}
Water soluble protein	9.5 ± 0.30^{a}	10.2 ± 0.20^{b}	9.7 ± 0.26^{ab}
Starch	5.1 ± 0.26^{a}	5.2 ± 0.20^{a}	5.2 ± 0.17^{a}
Crude fibre	5.1 ± 0.26^{b}	4.0 ± 0.20^{a}	4.7 ± 0.17^{b}
Total sugar	1.0 ± 0.20^{a}	1.3 ± 0.10^{a}	1.2 ± 0.20^{a}
Total carbohydrate	40.7	40.8	38.2

Values are mean ± standard deviation of three experiments; means in the same row with different superscripts are significantly ($P<0.05$) different.

Ash content is regarded a general measure of quality and often is a useful criterion in identifying the authenticity of food, with high ash figure suggesting the presence of an inorganic adulterant (Egan *et al.*, 1981). Total protein content was found to be 14.8-15.9% of which 9.5-10.2% was water soluble; these values for total protein were lower than 17.5% quantified by Mariod *et al.*, (2009) and 28.6% by Achu *et al.* (2005) for the same source. The protein content in the present results was lower than the values, 25.0% (Yanty *et al.*, 2008) for *Cucumis melo* var. *inodorus* and 29.9% (Mian-Hao and Yansong, 2007) for *Cucumis melo* hybrid 'ChunLi' seeds. Crude fibre content was 4.0-5.1%, similar to the value of 4.1% cited by Achu *et al.* (2005), but much lower than 23.3% for *Cucumis melo* var. *inodorus* and 19.0% for *Cucumis melo* hybrid AF-522 seeds (Yanty *et al.*, 2008). No significant differences ($P<0.05$) in mean starch content (5.1-5.2%) and total sugar content (1.0-1.3%) were observed in the sample means. Carbohydrate contents, in the range of 38.2-40.8%, were higher than those of 19.8% for *Cucumis melo* var. *inodorus* and 22.9% for *Cucumis melo* hybrid AF-522 seeds (Yanty *et al.*, 2008).

The findings imply that *C. sativus* seeds may be used as a potentially attractive source of lipids and some common nutrients. The protein content also commends *C. sativus* seeds as a nutritive complement. The present study, moreover, highlights the importance of understanding the cultural context and uses of cultivated plant foods. It may be that not all cultivated plant foods are consumed by all the members of a community. Consumption patterns, for example, can vary by gender or age, or even physiological state (e.g. pregnancy). Nutrient information would be critical to the success of efforts for promoting the wider use of indigenous plant foods as part of a broader program aimed at educating local populations with regard to the nutritional benefits of many cultivated plant foods that exist in their environment.

Conclusion

Improved knowledge on the analysis of *Cucumis sativus* seeds would assist in efforts to achieve industrial application of this plant. The physicochemical constants of the oils studied herein can be helpful in identifying the quality of oil and oil products for commercial exploitation. The quality of *C. sativus* seed oil is comparable to that of other oils and can be utilized in the paint, varnish and ink industries and is also recommended for human consumption after proper refining. In terms of both quantity and quality, all three varieties of *C. sativus*, herein reported, are potentially useful and important nutritional sources. The results agree with the data reported in the literature and these analytical data will also be helpful for the selection of variety.

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Removal of Copper from Aqueous Solutions Using Sawdust, Zeolite and Activated Carbon: Equilibrium Time Convergence

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Abstract. In this study, adsorption of copper(II) ions from aqueous solutions on sawdust, zeolite and activated carbon was studied using batch experiment techniques. The effect of different parameters such as pH of solution, adsorption time and amount of adsorbents were evaluated. Favourable pH for maximum copper adsorption was 6, 6 and 4 for sawdust, zeolite and activated carbon, respectively. The equilibrium of copper adsorption has been described by the Langmuir, Freundlich and Temkin isotherms and their fitness for adsorption was compared. The Temkin model had a better fitness than the other models. Maximum adsorption capacity of sawdust, zeolite and activated carbon were 96.11%, 96.2% and 97.02% (mg/g), respectively. The study of applicability of kinetic models of Lagergren (1898) and Ho *et al.* (1996) showed the latter model to be better fit than the former. The rates of adsorption obey the rules of A-order model with good correlation.

Keywords: adsorption efficiency, sawdust; zeolite, activated carbon, equilibrium time, copper

Introduction

Presence of heavy metals in water and wastewater beyond a certain limit motives toxicity in natural sources. Several methods are used to decrease heavy metal concentration in water and wastewater (Esteves *et al.,*2000). Among heavy metals, Pb, Zn, Cu and Cd head the list of environmental threats because even at extremely low concentrations they can cause brain damage in children (Ahmedna *et al.,* 2004). Treatment of aqueous wastes containing soluble heavy metals involves decreasing concentration of metals followed by their recovery or secure disposal of the waste (Ertugay and Bayhan 2010).

Copper is among the common toxic pollutants found in industrial effluents. Efficient removal of copper from wastewater by appropriate treatments has long been a crucial issue (Da'na *et al.,* 2010). There is wide usage of copper in industry such as relating to electroplating, electrical appliances, machinery, transportation and military supply sites etc. Copper is an essential substance to human life, but in high doses it can cause anemia, liver and kidney damage and stomach and intestinal irritation (Kabra *et al.,* 2008). The accumulation of Cu^{2+} in human body also causes brain, skin, pancreas and heart diseases (Veli and Aly¨uz, 2007).

Several adsorbents are suggested for removing heavy metals from aqueous media. Zeolite exhibited high selectivity for certain heavy metal ions such as Pb^{2+}, Cd^{2+}, Zn^{2+} and Cu^{2+} (Sljivic *et al.,* 2009; Sreejalekshmi *et al.,* 2009; Camilo *et al.,* 2005; Babel and Karniawan, 2003). Sawdust is one of the cheapest and abundantly available adsorbents and has the capacity of adsorbing heavy metals from water and wastewater. Metal ions connect to functional groups of sawdust such as COOH and OH and release H^+ ions (Asadi *et al.,* 2008). Several studies are available relating to the preparation of activated carbon from agricultural wastes such as *Phaseolus aureus* hulls (Rao *et al.,* 2009), walnut, hazelnut, almond, pistachio shell and apricot stone (Kazemipour *et al.,* 2008), maize cob husk (Igwe *et al.,* 2005), nut shells (Ahmedna *et al.,* 2004), black gram husk (Saeed and Iqbal, 2003), peanut husks (Ricordel *et al.,* 2001), sugarcane bagasse pith (Krishnan

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and Anirudhan, 2002 a;b) and their application for the removal of copper from water and wastewater. Still, there exists the need to develop suitable low-cost indigenous adsorbents for the removal of metal ions from aqueous solutions (Rao *et al.,* 2009). Also there are a few studies available on removal of Cu(II) from water and wastewater such as zeolite, clay and a diatomite (Sljivic *et al.,* 2009), three species of dead fungal biomasses (Li *et al.,* 2009) and sawdust (Asadi *et al.,* 2008; Meena *et al.,* 2007).

For treating water and wastewaters, adsorption was found to be a promising technique for removal of trace amount of adsorbates from aqueous solutions. Moreover, waste materials and their value added products used as adsorbent for treating heavy metals can improve the economic viability of the adsorption process (Sreejalekshmi *et al.,* 2009). At low concentrations, pollutants can be more effectively removed by ion-exchange or adsorption on solid sorbents such as activated carbon (Nelson *et al.,* 1974; Sigworth and Smith,1972), activated carbon from rice hulls and coal fly ash (Sen and De, 1987; Prabhu *et al.,* 1981). Presently, household water purification systems in south of Iran (Heidari and Moazed, 2008; Heidari *et al.,* 2008) use activated carbon which is very expensive. The aim of this study is checking the feasibility of using zeolite and sawdust for removal of copper (II) in low concentrations in order to choose the optimal economical adsorbent. In this paper, the sorption kinetics and the convergence of equilibrium time for removal of Cu(II) from aqueous solutions using sawdust, zeolite and activated carbon in low concentrations are reported.

Materials and Methods

Preparation of adsorbents. Sawdust was obtained from a local factory near Zabol, Iran. It was passed through 1.5 mm mesh sieve, washed with hot distilled water and finally dried in oven at 90 °C for 3 h. Zeolite was collected from Firoozkouh village in the city of Tehran, Iran. Sawdust, zeolite and activated carbon particles of 1.5 mm diameter were used. The activated carbon used was a commercial product of the Merck Chemicals Company prepared from wood.

Adsorbate solution supply. Copper (II) stock solution was prepared from analytical grade copper nitrate $Cu(NO_3)_2$ in concentration of 1000 mg/L. For aqueous solution of copper nitrate, double distilled water was used. Test samples of various concentrations were prepared from this stock solution (APHA, AWWA, WPCF, 1989).

Instrumentation. The measurements were made with a Spectrophotometer (model AA220 made by Varian Co., Australia). pH was determined with model 630 metrohm pH meter with combined glass calomel electrode.

Batch mode adsorption studies. For determining the optimum adsorption pH for all the 3 adsorbents, separate 250 mL Erlenmeyer flasks were used which were washed with dishwashing liquid and then with acid and deionized water. For each experiment, 100 mL of copper solution of 10 mg/L concentration and 1 g of adsorbent were placed in each flask. The pH of solution was adjusted, at 2, 3, 4, 5, 6, 7, 8 and 9. The samples were agitated at room temperature (30±1 °C) using a mechanical shaker at a constant rate of 150 rpm for 12 h to attain equilibrium (Heidari *et al.,* 2008). Then solutions were passed through Whatman filter paper No. 45. The pH of solutions was adjusted at 2 using nitric acid and the adsorption experiments were performed immediately. As pH of the solution approaches 2, cations remaining in the solution will have a lower chance of being adsorbed by the glass surface because the competition between cations and H^+ ions increases. Thus, by reducing the pH to 2, experimental errors will be lowered to minimum. While running kinetic adsorption experiment, 1 mg of sawdust adsorbent and 100 mL of copper solution at 1 mg/L concentration were added to each flask and their pH was adjusted at 6. The kinetics and adsorption contact time were determined for 5-150 min then the samples were removed from the shaker and the concentration of solution was measured. The experiments were repeated, varying the initial metal ion concentration from 10 to 300 mg/L. The experiments were performed similarly with zeolite and activated carbon. It is notable that in all the experiments, pH adjustments were made using dilute aqueous solutions of HCl and NaOH. pH of solution was measured at each stage of the experiment with pH meter (Model: PTR 79).

Kinetic models. The two adsorption kinetic models used in this study are pseudo-first-order and pseudo-second-order equations developed by Lagergren (1898) and Ho *et al.* (1996), respectively. The Lagergren pseudo-first-order equation is as below:

$$\log(q_e \quad q_t) = \log q_e - \frac{\mathbf{k}_1}{2.303} \tag{1}$$

Where k_1 is the rate constant of pseudo-first-order adsorption and q_e and q_t denote the amount of adsorption at equilibrium and at time t, respectively. The pseudo-second-order equation is expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{2}$$

Where q_e and q_t are the amount of metal ion adsorbed (mg/g) at equilibrium and at time t, respectively. k_2 is the adsorption constant (g/mg/min). The product $k_2 q_e^2$ is the initial sorption rate represented as $h = k_2 q_e^2$. Kinetic parameters of these models were calculated from the slope and intercept of the linear plots of log $(q_e - q_t)$ *vs* t and t/q_t *vs* t (Asano, 1998).

Adsorption isotherm models. The sorption equilibrium data of copper ion onto activated carbon, zeolite and sawdust, were analyzed in terms of Langmuir, Freundlich and Temkin isotherm models (Pehlivan *et al.*, 2008). For performing the isotherm experiments, the flasks were washed with dish washing liquid, abundant water and finally with deionized water. The experiments were conducted at the optimum pH and room temperature. Adsorption isotherms were studied at different initial metal ion concentrations over a range of 10-120 g/L in 100 mL metal solution of 50 mg/L concentration and the samples were placed on mechanical shaker at a constant rate of 150 rpm for 2 h. Afterwards, the samples were filtered and amount of copper ions was measured; this process was repeated for all the adsorbents. All the experiments were carried out in duplicate and the values were reported as average of two readings.

Langmuir isotherm. The Langmuir adsorption isotherm is probably the most widely applied one. A basic assumption of the Langmuir theory is that adsorption takes place at specific homogeneous sites of the adsorbent. The saturated monolayer isotherm can be represented by the below given equation (Runping *et al.*, 2005).

$$q_e = \frac{abC_e}{1 + aC_e} \tag{3}$$

Where q_e is the amount of metal ions adsorbed (mg/g), C_e the equilibrium concentration (mg/ L), b is the monolayer adsorption capacity of the adsorbent (mg/g) and a is the Langmuir adsorption constant (L/mg) related to the free energy of adsorption.

Freundlich isotherm. The Freundlich isotherm equation $q_e = kC_e^{1/n}$ can be written in the linear from as given below:

$$\log q_e = \log k + \frac{1}{n}\log C_e \tag{4}$$

Where q_e and C_e are the equilibrium concentrations of metal ions in the adsorbed and liquid phases in mmol/g and mmol/L, respectively, k and n are the Freundlich constants which are related to the sorption capacity and intensity, respectively. The Freundlich constants k and n can be calculated from the intercept and slope of the linear plot of log q_e *vs* log C_e.

Temkin isotherm. Temkin isotherm was derived assuming that the fall in the heat of sorption is linear rather than logarithmic, as implied in the Freundlich equation. It is expressed as:

$$q_e = \frac{RT}{b}\ \mathrm{In}(k_T C_e) = B_1 \mathrm{In}(k_T C_e) \tag{5}$$

Where q_e and C_e are the equilibrium concentrations of metal ions in the adsorbed and liquid phases in mmol/g and mmol/L, respectively, and constant $B_1 = RT/b$ is related to the heat of adsorption, R the universal gas constant (J/mol/K), T the temperature (K), b the variation of adsorption energy (J/mol) and k_T is the equilibrium binding constant (L/mg) corresponding to the maximum binding energy.

Adsorbent characterization. Activated carbon is a form of carbon that has been processed to make it extremely porous and thus having a very large surface area available for adsorption or chemical reactions. Due to its high degree of microporosity, just one gram of activated carbon has a surface area in excess of 500 m². Activated carbon does not bind well to certain chemicals, including alcohols, glycols, ammonia, strong acids and bases, metals and most inorganics, such as lithium, sodium, iron, lead, arsenic, fluorine and boric acid. Therefore, it has been used in adsorption processes as an efficient adsorbent. Zeolite has received much attention due to the fact that it has a wide range of structure having different chemical composition and physicochemical properties. In general, zeolite structure consists of alumino-silicate crystalline material. The zeolite crystal is made up of the interlocking tetrahedrons of SiO^4 and AlO^{4-}. Due to these properties, zeolite has been used for heavy metal adsorption. Sawdust is one of the low-cost adsorbents which consists of lignin, cellulose and hemicellulose, with polyphenolic groups playing important role for binding dyes through different mechanisms (Gupta and Suhas, 2009). In order to characterize the adsorbents, their surface area was determined using the methylene blue adsorption method described by Viladkar *et al.* (1996). Other properties of the adsorbents are shown in Table 1.

Table 1 Characteristics of adsorbents

Parameter	Sawdust	Zeolite	Activated carbon
Surface area (m^2/g)	600 - 750	640 - 800	850 - 1000
Bulk density (kg/m^3)	1020	2100	763.5
Moisture (%)	8.50	7-8	1-2

Results and Discussion

Studies on pH. pH is one of the most important parameters controlling the adsorption. The effect of pH on the adsorption of copper ions from water samples was investigated in the pH range of 2 to 8. The pH of the solution sample was adjusted to the required value by addition of sodium hydroxide and hydrochloric acid. The results showed (Fig. 1) that the extraction percentage was almost constant and quantitative at pH 6, 6 and 4 for sawdust, zeolite and activated carbon, respectively. Since adsorption process is pH sensitive and also due to different binding conditions of the adsorbent groups, the maximum adsorption efficiency for activated carbon occurred at pH 4. Results obtained by others are the same as the study of Sreejalekshmi *et al.* (2009) which revealed that this difference may be due to the utilization of new adsorption sites offered by –COOH groups on the adsorbed citric acid. At higher pH, the adsorption efficiency decreased a little. The adsorption capacity of copper for sawdust and zeolite increased from pH 2-5 but it decreased above pH 6, whereas for activated carbon, it increased from 2-4 and decreased above pH 4. Fan *et al.* (2009) stated that the removal of Ni(II) in 0.01M $NaNO_3$ solutions was more at pH 2-8 than at other pH. The rapid increase in adsorption efficiency is due to two reasons; first at low pH, H^+ ions existed in solution which competed with copper ion for surface adsorption, second is that there is a critical pH range for each hydrolysable metal ion; at which the metal adsorption efficiency reaches from low level to maximum level. This pH range is called surface adsorption edge (Kumar and Bandypadhyay, 2006). Decrease in adsorption of copper ions is due to formation of soluble carboxylic groups (Krishnan and Anirudhan, 2002 a; Raji *et al.*, 1997). The little decrease in adsorption in this study, at pH levels higher than optimum pH, agrees with other studies (Pehlivan *et al.*, 2008; Gupta *et al.*, 2003; Krishnan and Anirudhan, 2002 b; Mathialagan *et al.*, 2003).

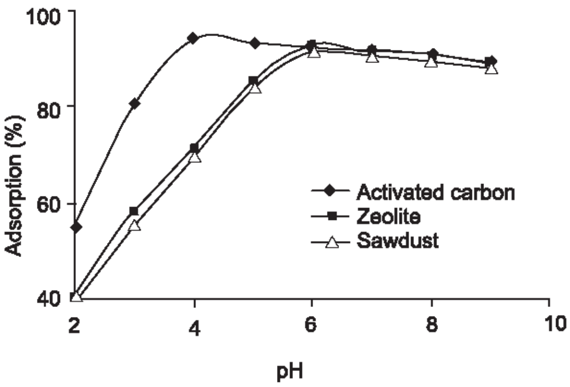

Fig. 1. Entrance pH effect on copper adsorption efficiency of sawdust, zeolite and activated carbon.

Kinetic adsorption studies. ***Effect of contact time and initial concentration.*** Figures 2-4 show the effects of contact time and initial concentration on copper

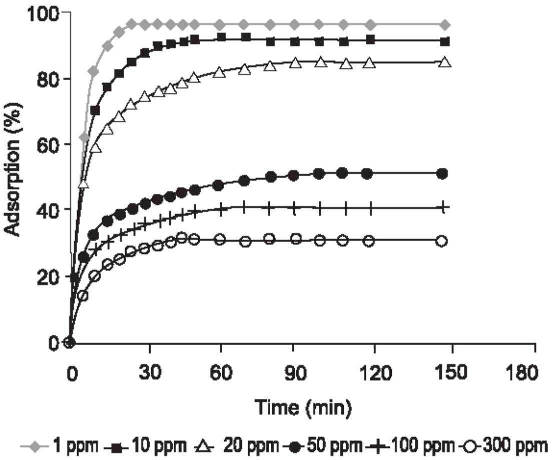

Fig. 2. Effect of contact time and copper initial concentration on sawdust adsorption efficiency.

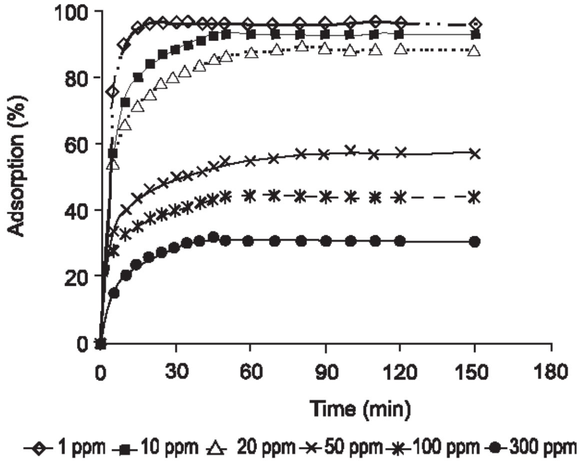

Fig. 3. Effect of contact time and copper initial concentration on zeolite adsorption efficiency.

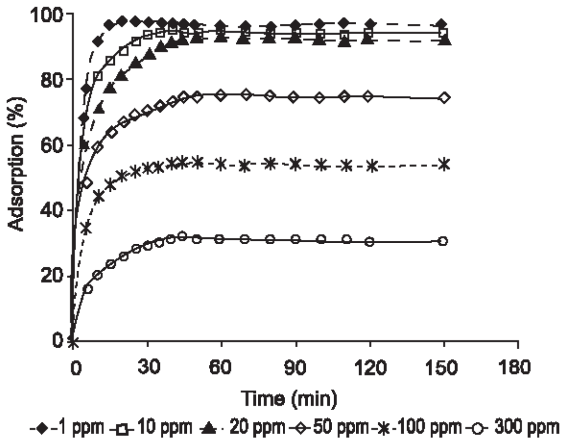

Fig. 4. Effect of contact time and copper initial concentration on activated carbon adsorption efficiency.

adsorption efficiency for sawdust, zeolite and activated carbon. It can be seen that with increase in the contact time, adsorption efficiency of all the 3 adsorbents increased. Therefore, for each of the 3 adsorbents, minimum equilibrium time was for concentration 1 mg/L and maximum equilibrium time for concentration 50 mg/L. In other words, the adsorption speed was maximum in concentration of 1 mg/L. Li *et al.* (2009) found that copper biosorption increased with the rise of initial Cu(II) ion concentration at un-perceptible pH. Under these conditions since more copper ions were available for adsorption at higher concentration, adsorption efficiency increased. The adsorption kept constant because the adsorbent became saturated after 50 mg/L metal ion concentration.

Effect of initial concentration on equilibrium time. Figure 5 shows the variation in equilibrium time of adsorption of copper in different concentrations by sawdust, zeolite and activated carbon. The maximum equilibrium time for sawdust, zeolite and activated carbon was 110, 100 and 70 min, respectively, in 50 mg/L concentration. With the increase in the concentration of solution from 50 to 300 mg/L, the equilibrium time of adsorption decreased. At higher concentrations, due to accumulation of metal ions around the adsorbent and there being more chances of metal contact with the adsorbent, the adsorbance is completed in less time and the process attains equilibrium, sooner. Also it was observed that by decreasing the concentration of solution from 50 to 1 mg/L, the equilibrium time for sawdust, zeolite and activated carbon decreased to 25, 20 and 20 min, respectively. With decreasing the initial concentration, the equilibrium time is expected to increase due to less chances of metal contact with the adsorbent. But due to the low quantity of metal ions in solution at low concentrations, this small number of ions is adsorbed in less time; thus with the decrease in the initial concentration of copper, the equilibrium time also decreased. Hence, the concentration of solution is the determining factor of equilibrium time and not the adsorbent capacity. Also with decreasing the concentration of solution from 50 to 1 mg/L, the difference between the equilibria of all the 3 adsorbents gradually decreased and then converged (Fig. 5). Convergence of equilibrium time at higher concentrations was also observed. It is evident from Fig. 5 that at median concentrations, the difference between the equilibrium time reached its maximum level, hence the effect of adsorbent capacity then was more obvious.

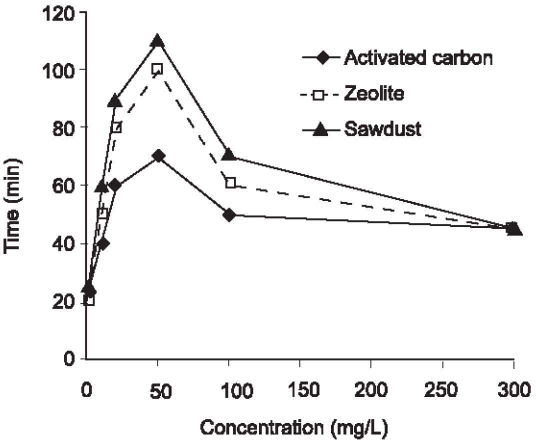

Fig. 5. Effect of initial concentration of copper on equilibrium time.

Effect of initial concentration on adsorption efficiency. Variation in adsorption efficiency (adsorption efficiency in equilibrium time) of sawdust, zeolite and activated carbon for copper in different concentrations is shown in Fig. 6. It is obvious that with decreasing the concentration of copper solution from 300 mg/L to 1 mg/L, the adsorption efficiency of sawdust, zeolite and activated carbon increased. Thus the maximum adsorption efficiency in 1 mg/L concentration was 97.02% for activated carbon, 96.2% for zeolite and 96.11% for sawdust. With the decrease in the initial concentration of solution (and thus the number of metal ions in the solution) from 100 to 50 mg/L, the difference in the adsorption efficiency of all the adsorbents increased; with further decrease of the concentration from 50 mg/L to 1 mg/L, the difference between the adsorption efficiency of all the adsorbents decreased and became closer and closer

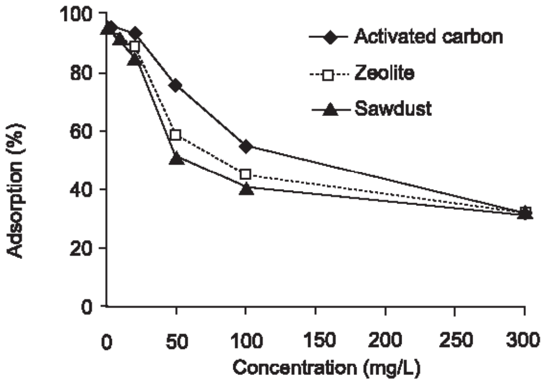

Fig. 6. Variation of adsorption efficiency with copper initial concentration in equilibrium time.

to each other till they converged at the concentration of 1 mg/L. This conforms with the other studies. Mukherjee *et al.* (2007) showed that phenol removal efficiency decreased with the increase of the initial phenol concentration. At low concentrations, the limiting parameter of adsorption efficiency is the concentration of solution and adsorbent capacity does not have an effective role (Heidari *et al.*, 2008). Thus with decreasing the initial concentration of metal ions, the difference of the adsorption efficiency between the adsorbents decreases and ultimately they converge with each other. With increasing the concentration of solution from 50 to 300 mg/L, the difference of the adsorption efficiency between the adsorbents decreases as the adsorption efficiencies of all the sdsorbents become almost equal.

Kinetic adsorption studies. The models of Lagergren (1898) and Ho *et al.* (1996) fitted with the data obtained from the kinetic adsorption experiments for initial concentration. For determining the Lagergren coefficients, log (q_e-q_t) was plotted *vs* t (Fig. 7).

Ho *et al.* (1996) model correlation coefficients for sawdust, zeolite and activated carbon resulting from plotting t/q_t *vs* t are shown in Fig. 8.

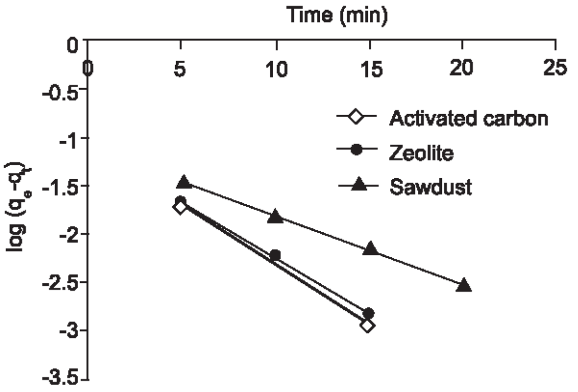

Fig. 7. Fitting Lagergren model for sawdust, zeolite and activated carbon adsorbents.

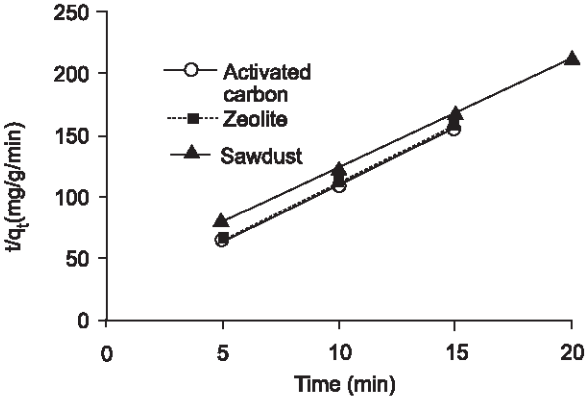

Fig. 8. Fitting Ho *et al.* (1996) model for sawdust, zeolite and activated carbon adsorbents.

As shown in Table 2, correlation coefficient of Ho *et al.* (1996) model is greater than the correlation coefficients obtained for Lagergren model for all the adsorbents. It, therefore, follows that Ho *et al.* (1996) model describes the data better than Lagergren model. Also the former model has a good compatibility with the present results. Its application to all the adsorbents showed that the speed of surface adsorption of copper by activated carbon is more than by zeolite and sawdust adsorbents. Kurniawan *et al.* (2006), in their study on different adsorbents, stated that among the adsorbents

Table 2. Constant coefficient of kinetic adsorption models for copper by sawdust, zeolite and activated carbon adsorbents

	Ho *et al.* model			Lagergren model			
Absorbent	R^2	K_2	$q_{e\,cal}$	R_2	K_1	$q_{e\,cal}$	$q_{e\,cal}$
Sawdust	0.9997	2.24580	0.11288	0.9982	0.16074	0.07785	0.09611
Zeolite	0.9991	4.278184	0.10761	0.9975	0.25885	0.06989	0.0962
Activated carbon	0.9996	4.471541	0.10931	0.9971	0.28764	0.08824	0.09702

derived from agricultural waste, hazelnut shell activated carbon (Cr(VI): 170 mg/g), orange peel (NiII): 158 mg/g and chemically modified soybean hull (CuII): 154.9 mg/g) had significantly higher metal adsorption capacities, compared to those from natural materials such as clay Ni(II): 81 mg/g; Cu(II): 83 mg/g; Zn (II): 63 mg/g). The kinetic studies of Lu *et al.* (2009) showed that correlation coefficients of the pseudo-first-order model were in the range 0.8507-0.9990 and for the pseudo-second-order model were in the range 0.9446-0.9988.

Figure 9 shows the variation of Ho *et al.* (1996) model coefficients with change in concentration of activated carbon; maximum value of k_1 was attained at the concentration of 1 mg/L and equals 0.287/min. With increasing the concentration of solution from 1 to 10 mg/L, the value of k_1 decreased down to 0.115. At concentration of 20-300 mg/L, the value of k_1 becomes very low and approximately constant. This proves that with the decrease in concentration, the speed of adsorption increases, hence the equilibrium time will decrease. Sreejalekshmi *et al.* (2009) in their studies showed that between the pseudo-first-order and the pseudo-second-order kinetic models, the former one was found to be the best-fit kinetic model for adsorption.

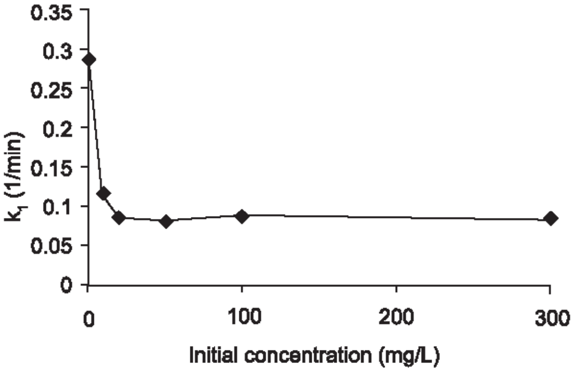

Fig. 9 . Variation of Ho *et al.* (1996) model with initial concentration of copper for activated carbon adsorbent.

Adsorption isotherm studies. The results of adsorption isotherm were analyzed using correlation method by SPSS (version 14). Model parameters and their statistical characteristics were accomplished using the nonlinear correlation for Langmuir, Freundlich and Temkin isotherm models. The results were calculated under 95% level of significance (Table 3). Regression coefficients showed that Temkin model had better fitness than the Langmuir and the Freundlich ones.

Dimeionless separation factor R_L can be calculated from the Langmuir constant using the equation as below:

$$R_L = 1/(1 - a.\ C_0) \qquad (6)$$

R_L is related to the nature of the interaction between adsorbent and adsorbate and also isotherm type: unfavourable (R_L>1), linear (R_L=1), favourable (0<R_L<1), or irreversible (R_L= 0) (Sljivic *et al.*, 2009). The calculated R_L values for various initial Cu(II) concentrations were 0.0340 (activated carbon), 0.0340 (zeolite) and 0.0769 (sawdust). Thus, the adsorption process in all the investigated systems was favourable. These results correspond with those of Madhava *et al.* (2009) and Sljivic *et al.* (2009) relating to copper removal studies. Freundlich coefficient value (n), less than 1 also matches with the values of Mukherjee *et al.* (2007) and Mathialagan *et al.* (2003). The value of 1/n > 1, shows that the adsorbent has suitable capacity for metal ion adsorption because curve gradient is intensive. The copper adsorption efficiency for activated carbon was more than that of other adsorbents; coefficient *k* was 7.42, 5.53 and 5.32 for activated carbon, zeolite and sawdust, respectively.

Table 3. Copper adsorption parameters calculated by Langmuir Freundlich and Temkin equations

Model	Parameters	Activated carbon	Zeolite	Sawdust
Langmuir	R^2	0.902	0.932	0.880
	a (L/mg)	0.57	1.20	0.24
	b (mg/g)	23.72	13.55	11.07
Freundlich	R^2	0.832	0.786	0.772
	k	7.42	5.53	5.32
	n	0.67	0.79	0.696
Temkin	R^2	0.977	0.965	0.993
	b (J/mol)	10.73	9.26	7.39
	K_T(L/mg)	4.66	5.71	3.79

The Temkin isotherm assumes that the heat of adsorption of all the molecules in the layer decreases linearly with coverage due to adsorbent-adsorbate interaction (Mathialagan *et al.,* 2003). With respect to the experimental values, the adsorption capacities predicted by Temkin model were higher (Table 3). The adsorption energy (b), obtained from Temkin isotherm was the highest for activated carbon and the lowest, for sawdust. The positive values of parameter b indicated that the processes were exothermic (Rao *et al.,* 2009; Sljivic *et al.,* 2009).

Optimum adsorption occured at about pH 6 for all the adsorbents. In the range of 25-55 the adsorption efficiency of all the adsorbents was high. Obviously, at lower concentrations, the adsorption was higher. At concentration of 50, the equilibrium time was the highest; showing that at concentrations higher and lower than 50, there were some conditions at which the adsorption occured more rapidly. At extreme concentrations, the adsorption rate of each of the 3 adsorbents was the same indicating that there is not any difference between the adsorbents at these concentration ranges.

Conclusion

With decreasing the initial concentration of solution from 50 to 1 mg/L, the equilibrium time for sawdust, zeolite and activated carbon decreases and ultimately become closer to each other. Also with decreasing the initial concentration of copper in solution, the adsorption efficiency of all the adsorbents increases. Thus the less the copper concentration is, the less becomes the adsorbent capacity and *vice versa.*

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Investigation of Carbon Monoxide at Heavy Traffic Intersections of Karachi (Pakistan) using GIS to Evaluate Potential Risk Areas for Respiratory and Heart Diseases

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Abstract. Measurement of carbon monoxide in the ambient air at 36 locations on the busy roads of Karachi showed peak values of CO at 18 sites to be within the permissible limit of 10 ppm whereas up to 70 ppm at the other 18 sites. The evaluated carboxy haemoglobin (COHb) level was in the range of 1.1 to 15.8 %.

Keywords: carbon monoxide, air pollution, GIS, vehicular pollution

Introduction

Recent evidence in respect of air pollution indicates that transportation is the major source of air pollution in urban areas (Ghose *et al.,* 2004) and that traffic is the leading cause of pollutant emissions especially those of CO and NOx, VOCs, SOx and particulate matter (Harrop, 2002). It is estimated that road traffic contributes 60% of air pollution in urban areas (Anjaneyulu *et al.*, 2006).

Carbon monoxide is one of the major air pollutants in metropolitan cities. In central part of cities and during traffic jams, its concentration shoots up. CO discharged from motor vehicles and other sources has indirect effects on climatic change and adverse effects on the health of the exposed humans (Khan *et al.,* 1996; Khalil, 1995).

Carbon monoxide is a highly toxic gas but it is not easily detected by olfactory senses. It can seriously affect human aerobic metabolism owing to its high affinity for haemoglobin, forming carboxy haemoglobin (COHb), reducing the capability of the blood to carry the oxygen to body tissues such as heart and brain. Reactivity of CO with haemoglobin is 240 times greater than that of oxygen (Harrop, 2002; WHO, 1999). The effects of CO depend on its concentration, exposure time and health status of people, their age and activities. Long-term exposure to low concentrations of CO can have effects similar to short-term exposure to high concen-trations. The symptoms of exposure to CO include headache, tiredness, dizziness, nausea, vomiting and drowsiness and in very acute situations, unconsciousness and even death (Malakootian and Yaghmaeian, 2004).

The present study was focused on examining the status of CO pollution due to traffic congestions in Karachi city with some specific significance for urban planners. The main objective of this study was collection of data relating to CO concentrations at heavy traffic locations of Karachi, and assessment of human blood carboxy-haemoglobin (COHb) concentrations at these locations showing the spatial clusters and patterns of CO with its impact on respiratory and heart diseases and evaluating potential risk areas. Such a study was not made earlier.

Materials and Methods

Sampling map. Sampling of CO was carried out at 36 identified locations in different towns of Karachi, selected on main traffic congested areas as shown in Fig. 1 with town boundaries.

Sampling. Concentration of carbon monoxide was measured by Snift CO analyzer (Model 50). The analyzer

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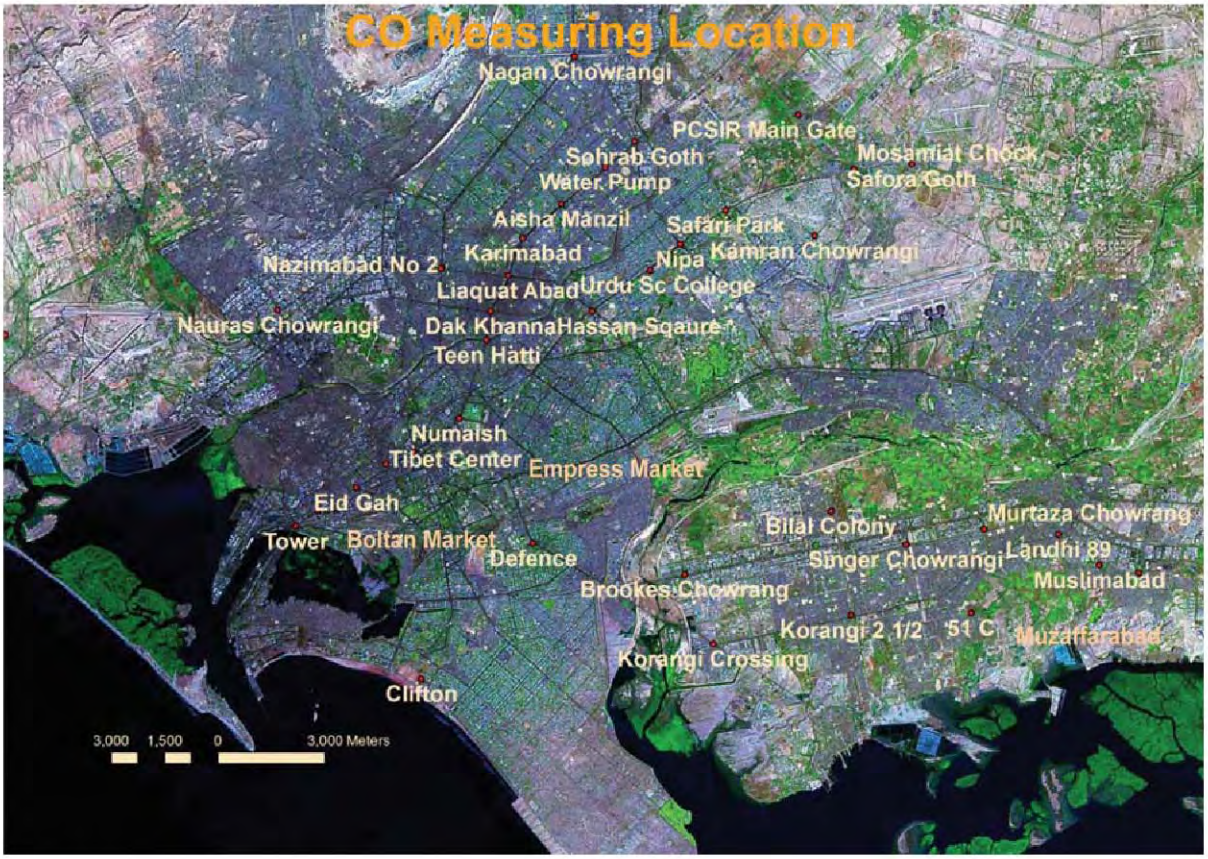

Fig. 1. CO measuring locations.

is ideal for measuring (in ppm) the level of carbon monoxide in ambient air. During all the measurements, the meter was kept at about 1.2 m above the ground level. At each site, level of CO in the ambient air was taken at intervals of 10 min and a set of ten readings was recorded during a period of about 2 min with repetition after every 8 min. The data was generated from 08 AM to 18 PM, at each site. Thus 60 CO spot readings were taken at each location, making a total of 2160 readings. From these readings, 8 h TWA average values were calculated.

GIS techniques. GIS is a computer system capable of assembling, storing, manipulating and displaying geographically referenced information, identified according to their locations.

GEO reference. Geo refers to the process of assigning map coordinates to image and non-image data (met data). The geo referenced map can be used as a planimetric map, for on-screening digitization with GIS. Fig. 2 shows interpolated surface of CO.

Digitization. In the broadest sense, digitization refers to any process that converts non-digital data into the numbers. There are two types of digitization namely tablet digitization and screen digitization. In the present study, screen digitization method was used wherein vector data are drawn in the viewer with a mouse using the displayed image as a reference. These data are then written on to the vector layer.

Cartographic techniques. With the help of cartographic and geographical information system (GIS) techniques, town boundaries were demarcated and record of CO at different locations of Karachi was made. Locations having different potential of CO and areas of different towns were computed; GIS technique was used for plotting and mapping the information retrieved.

Results and Discussion

Table 1 shows the time weighted everage values of CO. The time weighted average (TWA) values of carbon

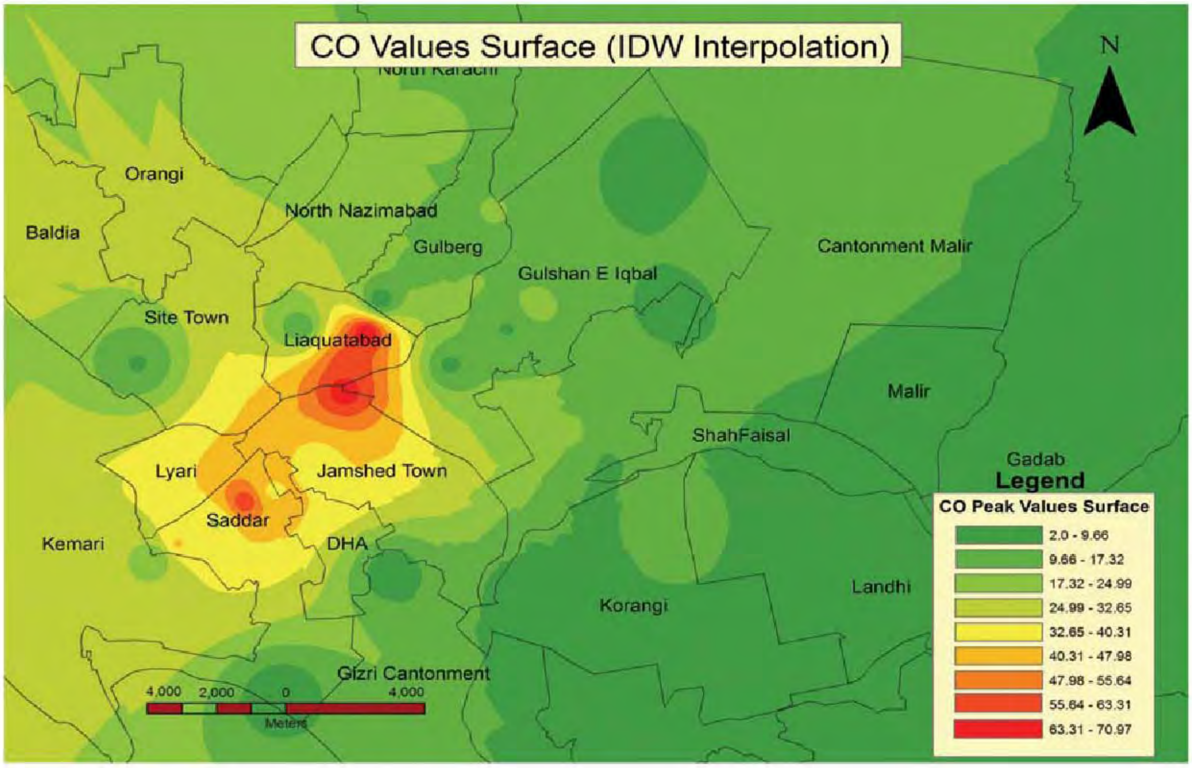

Fig. 2. Interpolated surface of CO

monoxide for 8 h average ranged from 3 to 71 ppm, whereas equilibrium concentration of blood COHb - air CO level (%), varied from 0.7 to 15.8% (Table 1). The results suggest that TWA 8 h average concentration of CO at 20 locations (location no. 1-17, 23,33 and 35) out of thirty six locations was very high and much above the permissible limit of 9 ppm (10 mg/m^3) recommended by the WHO (1999, 1998). The reasons of high concentration of carbon monoxide pollution on the streets include old vehicles with poor maintenance, narrow roads with uneven surfaces, rash driving, lake of education of vehicle drivers, especially commercial vehicle drivers, frequent traffic jams and congestion.

During traffic jams, the concentration of carbon monoxide shoots up abruptly within fraction of a minute becoming health hazard for human beings. Similarly the concentration of carbon monoxide varies with traffic density and types and condition of vehicles in the given traffic stream. Other factors such as wind velocity, wind direction, humidity and temperature are also important. Higher wind velocity and more open area around the location lower the concentration of carbon monoxide, whereas high temperature along with higher wind velocity would increase the rate of diffusion, which could enhance the dissipation of carbon monoxide soon after the emission.

Locations No.5, 6,7,12,14,15,16 and 17 (Table 1) are highly congested areas having narrow roads, slow moving traffic and high traffic density as compared to the rest of locations, resulting in high concentration of CO. Generally, the concentration of carbon monoxide increases between 8:00 and 9:00 h local time, at the time when people go to their offices and students to their institutions. Commercial activities reach their peak leves between 11:00-15:00 h; the traffic density gets lower at 16:00 h thus reducing the CO emission. After 16:00 h, the traffic density again increases subsequently raising the CO level.

When carbon monoxide is inhaled, it combines with the blood haemoglobin forming carboxy haemoglobin. Normal or background level of blood carboxy haemoglobin (COHb) is about 0.5% originating from the destructive metabolism of haem, a component of haemoglobin and ambient air CO (Khan *et al.,* 1996).

The equilibrium percentage of COHb in blood stream of a person exposed to less than 100 ppm can be estimated by the equation:

COHb in blood (%) = 0.16 (CO conc. in ppm + 0.5); (0.5% is the normal background COHb concentration in blood).

Using this equation, blood COHb -air CO equilibrium data for different locations were estimated in the range of 0.7 to 15.8 % (Table 1).

Table 1. Time weighted average of carbon monoxide and calculated values of carboxyhaemoglobin (COHb) at the selected sites for 8 hours.

ID	Location	TWA CO values (ppm)	Calculated values of COHb (%)
1	NIPA	24.5	4.4
2	Sohrab Goth	20	4.0
3	Water Pump	13	2.6
4	Aisha Manzil	15	3.0
5	Teen Hatti	69	11.5
6	Dak Khana	63	10.6
7	Liaquatabad	71	12.0
8	Karimabad	11	2.2
9	Mosamiat Chock	14	3.0
10	Safora Goth	15	3.0
11	Safari Park	13	2.6
12	Numaish	37	6.4
13	Tower	20	4
14	Empress Market	39	6.7
15	Eid Gah	32	5.6
16	Bolton Market	41	7.1
17	Tibet Center	61	10.3
18	Korangi 51 C	4	1.1
19	Korangi 2 1/2	4	1.1
20	Korangi Crossing	7	1.6
21	Singer Chowrangi	3	0.9
22	Murtaza Chowrangi	5	1.3
23	Bilal Colony	16	3.1
24	Landhi 89	4	1.1
25	Muslimabad	7	1.6
26	Muzaffarabad	4	1.1
27	Brookes Chowrangi	3	0.9
28	PCSIR Main Gate	3	0.9
29	Defence	2.5	0.9
30	Clifton	3	0.9
31	Hassan Sqaure	8	1.8
32	Urdu Sci. College	9	2
33	Nagan Chowrangi	20	4
34	Kamran Chowrangi	5	1.3
35	Nazimabad No. 2	11	2.2
36	Nauras Chowrangi	9	2

Table 2 shows adverse effects of CO on human health, (Wolf, 1971). It is obvious that upto 1% CO in the haemoglobin may be considered ideal whereas above 1% is detrimental. However, it is the level above 2% where physiological effects become evident.Thus 2-5% blood COHb level may affect the central nervous system, impair time interval discrimination, cause visual acuity, affect discrimi-nation of brightness and may change certain other psychomotor functions. When this situation is supplemented with other gaseous pollutants of automobile exhaust like hydrocarbons, oxides of sulphur and nitrogen dust, etc., a bleak picture can be visualized.

Table 3 shows town-wise sites selected for CO measurement and also presents minimum, maximum, range, mean, standard deviation and sum values of CO through GIS interpolation technique. The results suggest that Liquatabad, Jamshed Town, Gulshan-e-Iqbal and Saddar (Table 3) are the highly vulnerable areas where people are at great risk of developing respiratory and heart related diseases.

Table 4 shows town-wise risk areas with reference to health effect of COHb. Exposure to elevated CO level is associated with impairment of visual perception, working capacity, manual dexterity, learning ability and performance of work task (Aziz and Qureshi, 2003).

Table 2. Effect of COHb blood level on humn health

COHb blood level (%)	Demonstrated effects
Less than 1.0	No apparent effects
1.0 to 2.0	Some evidence of effect on behavioral performance
2.0 to 5.0	Central Nervous System effects impairment of time interval discrimination, visual acuity, brightness discrimination and certain other psychomotor function.
> 5.0	Cardiac and pulmonary functional change
10.0 to 80.0	Headache, fatigue, drowsiness, coma, respiratory failure, death

Table 3. Town wise area and CO concentration through GIS interpolation techniques

Town name	Area (km^2)	Min (ppm)	Max (ppm)	Range (ppm)	Mean (ppm)	STD (ppm)	Sum (ppm)
Baldia	28595000	18.51	28.65	10.14	26.14	1.55	299040
Bin Qasim	114185000	2	7.74	5.74	5.4	0.97	246604
Cantonment Malir	119152000	6	24.63	18.63	11.47	2.53	546764
DHA	7650000	8.79	42.74	33.94	28.83	9.15	88221
Gadab	441369984	2	27.25	25.25	11.51	6.11	2032680
Gizri Cantonment	38302500	4	18.28	14.27	12.05	2.73	184639
Gulberg	13820000	4.07	32.75	28.68	16.88	3.03	93288
Gulshan-e-Iqbal	53690000	3.01	68.99	65.99	15.2	8.87	326507
Jamshed Town	23417500	7.94	68.93	60.99	33.26	12.97	311536
Kemari	113568000	2	35.83	33.83	24.11	5.7	1095140
Korangi	41472500	3	15.99	12.99	7.13	2.46	118290
Landhi	39160000	3	15.9	12.9	6.46	1.93	101145
Liaquatabad	10855000	11.02	70.98	59.95	39.3	14.85	170625
Lyari	7980000	27.48	46.63	19.15	37.04	3.58	118235
Malir	17792500	6.03	10.49	4.45	7.81	0.86	55605
North Karachi	20457500	11.5	19.6	8.1	16.16	1.87	132239
North Nazimabad	16702500	16.31	28.25	11.94	21.77	3.38	145467
Orangi	23475000	23.64	29.54	5.9	26.01	1.44	244227
Saddar	24155000	3.01	60.97	57.96	28.06	12.54	271083
ShahFaisal	11710000	6.11	11.73	5.63	8.61	1.49	40309

Table 4. Town wise risk areas on the basis of blood COHb level through GIS techniques

Town name	Blood COHb level (%)	Health effects of blood COHb level	Level of risk areas
Bin Qasim	1.364	Some affects on behavioural performance	Low risk areas
Landhi	1.5336	Some affects on behavioural performance	Low risk areas
Korangi	1.6408	Some affects on behavioural performance	Low risk areas
Malir	1.7496	Some affects on behavioural performance	Low risk areas
Shahfaisal	1.8776	Some affects on behavioural performance	Low risk areas
Cantonment Malir	2.3352	Some affects on behavioural performance	Medium risk areas
Gadap	2.3416	Some affects on behavioural performance	Medium risk areas
Gizri Cantonment	2.428	Some affects on behavioural performance	Medium risk areas
Gulshan-e-Iqbal	2.932	Some affects on behavioural performance	Medium risk areas
North Karachi	3.0856	Nervous and psychomotor function	Medium risk areas
Gulberg	3.2008	Nervous and psychomotor function	Medium risk areas
North Nazimabad	3.9832	Nervous and psychomotor function	Medium risk areas
Kemari	4.3576	Nervous and psychomotor function	Medium risk areas
Orangi	4.6616	Nervous and psychomotor function	Medium risk areas
Baldia	4.6824	Nervous and psychomotor function	Medium risk areas
Site Town	4.7048	Nervous and psychomotor function	Medium risk areas
Saddar	4.9896	Nervous and psychomotor function	Medium risk areas
DHA	5.1128	Cardiac and pulmonary functional change	High risk areas
Jamshed Town	5.8216	Cardiac and pulmonary functional change	High risk areas
Lyari	6.4264	Cardiac and pulmonary functional change	High risk areas
Liaquatabad	6.788	Cardiac and pulmonary functional change	High risk areas

Conclusion

GIS-based this study shows that the concentration of carbon monoxide in the ambient air on the busy roads of Karachi is very high and almost the entire pollution in the environment is being generated by automobile exhaust. Growing number of vehicles, used leaded gasoline, poor condition and maintenance of vehicles, use of defective silencers, poor road conditions, rash driving etc. are the major causes of high concentration of CO in the environment of Karachi.

Thus, there is a growing need to formulate proper regulatory laws to limit emission of gaseous pollutants from individual vehicles and to implement the regulations forcefully by on- the-spot checking. Traffic geometry also plays important role in avoiding congestion on the roads and local route modification could prevent major causes of pollution. Faulty and worn out vehicles should be removed from the roads.

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Petroleum Hydrocarbon Pollution after the Tasman Spirit Oil Spill of Coastal/Deep Sea Sediment along the Clifton Beach Karachi, Pakistan

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Abstract. An oil tanker, Tasman Spirit, carrying 67000 tons crude oil, got damaged near the Clifton Beach of Karachi, Pakistan and approx. 31,000 ton oil spilled into the sea. The distribution of aliphatic and aromatic hydrocarbons was determined in deep sea and surface sediment collected at 12 stations along the Clifton beach of Karachi, following the oil spill. Sampling was performed during 2003-2006, starting just after the accident of the oil tanker. Concentrations of PAHs (Σ16 parent components) and aliphatics were in the range of 0.09-560 µg/kg dw and 0.12-685 µg/kg dw, respectively, since the date of accident and after bioremedial measures. The highest concentrations were found within the radius of 50 km around the site, the area most heavily impacted by the spill, whereas at the stations, away from the ship, the concentrations were in the lower range without alkylated compounds. Addition of increasing amounts of ship fuel oil (taken from a Pakistani ship) to a representative sediment sample showed that measurable concentration of the Tasman Spirit oil was > 1 g/kg of sediment. The toxicity of selected samples of surface sediment from the coastal area near oil spill showed higher PAH concentrations; the average number of dead fauna was 90-95% within 3 days of oil spill which gradually decreased with the time.

Keywords: Tasman Spirit oil spill, Clifton beach, sediment, hydrocarbons, toxicity

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Introduction

The accident of grounding of oil tanker Tasman Spirit, transporting 67000 tons of Iranian crude oil, occurred near Karachi harbour along the Clifton beach, on the 27th July, 2003. The hull got perforated and approximately 31000 tones of crude oil spilled into the sea at the Karachi coast .The ship sank at 50 km offshore after leaking oil for 12 days. Being broken into two parts, it caused the largest oil spill that ever occurred at Karachi beach in the history of Pakistan.

Petroleum hydrocarbons (PHs) are composed of small and long chain aliphatic and aromatic hydrocarbons varying in molecular weight and toxicity. PAHs are mostly produced by incomplete combustion of organic matter or spillage of petroleum or petroleum by-products (Stark *et al.,* 2003; Mazeas and Budzinski, 2001; McRae *et al.,* 2000; Yunker *et al.,* 1999; Hammer *et al.,* 1998; Neff, 1995; 1979; O'Mally, 1994; Volkman *et al.,* 1992; Bjorseth and Ramdhal, 1983; Wakeham *et al.* 1980 a;b; Laflamme and Hites, 1978; Youngblood and Blumer 1975; National Academy of Science, 1972).

Immediately after oil spill more volatile compounds begin to evaporate increasing the viscosity and density of residues (Michel, 1992). Polyaromatic hydro-carbons (PAHs) are naturally present in crude oil (WHO, 1998) some of which are highly persistent in environment (Wong *et al.,* 2004; Machala *et al.,* 2001; Menzie *et al.,* 1992) and have strong bio-concentration capacity (Connell and Schuumann, 1988), toxicity, and sublethal effects on some aquatic organisms (Hartmann and Quinn, 1999) and creating diseases (Grimmer, 1983). Through bio-accumulation in the edible fishes and shell fishes, PAHs reach humans (Hartmann and Quinn, 1999) and due to their carcinogenic nature, may cause cancer (Freitag *et al.,* 1985). Sediment and biota have relatively strong affinity for lipophillic hydrocarbons and are their valuable indicators, (Kayal and Connell 1990; Connell and Hawker, 1986) in evaluating the petroleum effects; these are not affected by weathering conditions and seasonal changes (Zann, 1996).

In consideration of all the mentioned factors, the distribution of aliphatic and aromatic hydrocarbons was determined in deep sea and surface sediment collected at 12 stations along the Clifton beach of Karachi following the Tasman Spirit oil spill. Sampling was carried out during July 2003- December 2005. The sedimentary aromatic fractions were also analysed for

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*Author for correspondence; E-mail: aliamunshi@gmail.com possible effects of polyaromatic hydrocarbons (PAHs) on benthic biota.

Materials and Methods

Sampling. Sediment samples were collected from 12 sampling stations of the effected coastal area of Karachi (Fig. 1) located at five different points as indicated in Fig. 2; their GS values are given in Table 1. Samples were collected with stainless steel grab, deposited into new properly labelled plastic bags, placed in ice and brought to laboratory in intact condition. At laboratory all the samples were stored at -20 °C before processing and analysis.

Chemicals and reagents. Standard PAHs (16 compounds, each at 100, 200, 1000 or 2000 mg/mL), surrogate (d_{12}-chrysene) and internal standards (d_8-naphthalene) were all obtained from Dr. Ehresnstorfer's laboratory, Germany, and CRM from IAEA. Working standards of the micro-pollutants were prepared by combining the standard mixture with the corresponding IS stock solution. These solutions were further diluted with hexane to prepare calibration solution in the range of 0.001-10 ng/mL. All solvents used for sample processing and analyses (dichloromethane, hexane and methanol) were HPLC grade.

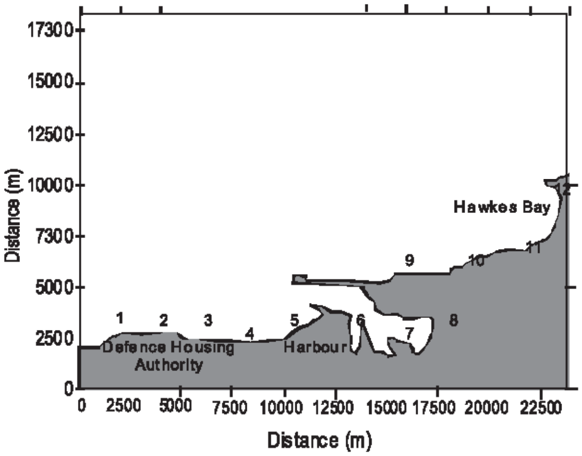

Fig. 2. Distance of selected sampling stations from the accident site and their location along the Clifton Beach.

Analytical procedure. Sample preparation (pre-GC). At the laboratory, samples were dried for 24 h at 60 °C, then copper powder was added and mixed well to remove sulphur from the sediment. Sediment sample

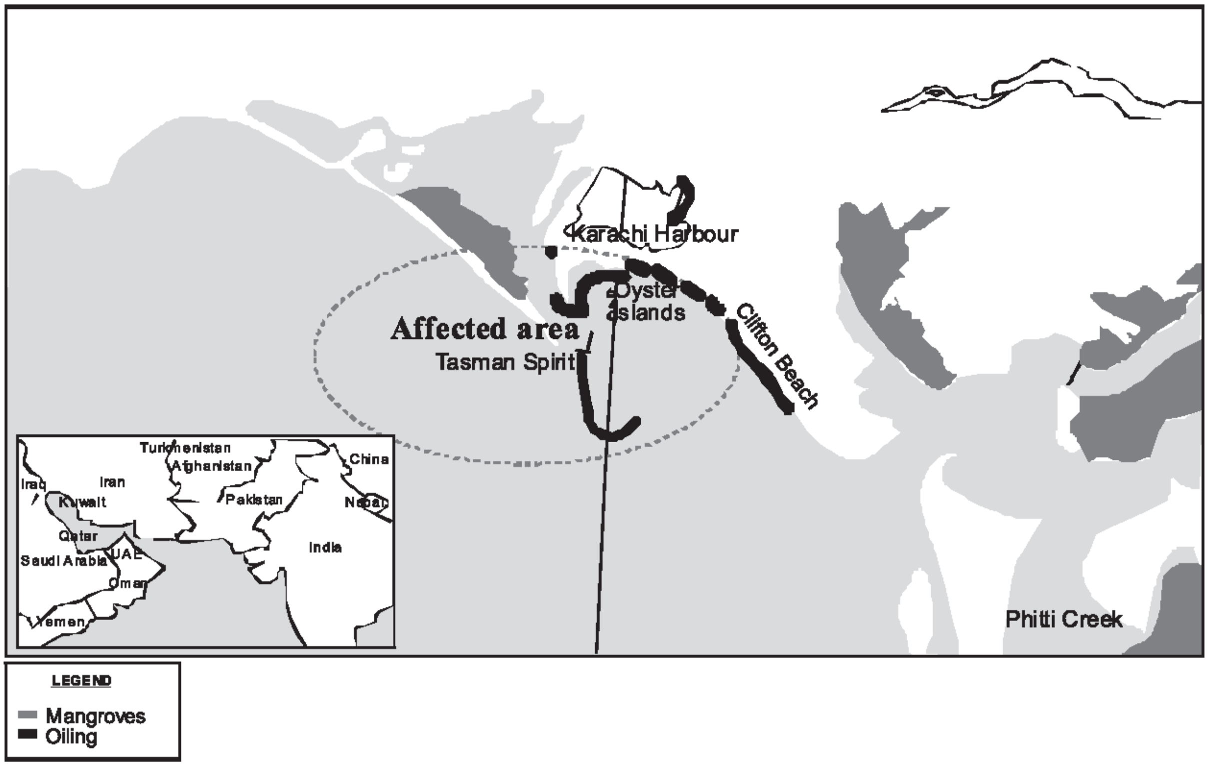

Fig. 1. Location of Tasman Spirit oil spill incident at Clifton Beach Karachi (Pakistan) on July 27, 2003.

Table 1. Sampling stations according to GPS

Sampling station	Longitude	Latitude
Sampling station # 1	65.58.46 E	23.47.25 N
Sampling station # 2	66.59.48 E	24.48.26 N
Sampling station # 3	67.01.43 E	24.47.53 N
Sampling station # 4	67.01.43 E	24.47.36 N
Sampling station # 5	66.59.24 E	24.47.16 N
Sampling station # 6	66.01.43 E	21.47.53 N
Sampling station # 7	77.01.43 E	30.47.36 N
Sampling station # 8	60.59.24 E	22.57.16 N
Sampling station # 9	67.59.48 E	23.48.26 N
Sampling station # 10	57.59.48 E	22.48.26 N
Sampling station # 11	57.01.43 E	29.47.36 N
Sampling station # 12	66.59.48 E	24.48.26 N

was spiked with surrogate (d12-cheysene) standard solution and homogenized well.

Saponification. All dried samples were saponified with 50 mL of 0.5 N methanolic potassium hydroxide solution for 10 h, filtered and evaporated to 1-2 mL on rotary evaporator and passed through silica gel column for purification and fractionation.

Purification/isolation. Saponified samples were purified through removing the interfering substances by adsorption column chromatography using silica gel as sorbent (WHO, 1998).

The concentrated extracts of saponification were fractionated by a deactivated silica gel/alumina column. The column was eluted first with 100 mL hexane containing aliphatic hydrocarbons then with 100 mL benzene containing aromatic hydrocarbons. Prior to injection, d8-napthalene was added as an internal standard. Finally, both fractions of aliphatic and aromatic hydrocarbons were evaporated down to 1-2 mL separately and run on GC.

Sample analysis. The samples were analyzed by the gas chromatograph (Perkin-Elmer Clarus 500) equipped with a fused silica capillary column (Elite-1; 30 m long, 0.53 mm i.d., 1.5 mm film thickness). FID was used as detector. The oven temperature programme was set at 60 °C (initial time, 10 min) to 140 °C at a rate of 2 °C/min and held for 10 min, 140 °C to 230 °C at a rate of 3 °C/min for 10 min. The inlet mode chosen was split-less and helium was used as carrier gas. Inlet and detector temperatures were 270 °C and 285 °C, respectively.

Quality control. The quality of data was assured through the analyses of appropriate certified reference materials. Deutrated surrogates and internal standards were used to compensate for losses involved in the sample extraction. The surrogate standard was used to monitor the method performance for each sample, while the internal standard was used to compensate for variance in final extracted volume, injection volume and instrument sensitivity. Analysis of blank samples was also carried out with each batch. Compounds were identified mainly by retention time. All the results for fishes and shellfishes were reported on wet-weight basis.

Results and Discussion

A total of 100 samples were collected at different times of the day from 12 sampling stations located at 5 points of the affected area. Complete sampling schedule and site information are given in Table 2.

Table 2. Sampling schedule and details

Position/location from the effected area	Distance	No. of samples	Sample description
Near the beach	5 km	40	Fishes
Latitude 24.48.26° N, Longitude 66.59.48° E	23.48 miles 37.7 km	15	Crabs, sediment, water and snails
Latitude 24.47.53° N, Longitude 67.01.43° E	25.64 miles 41.2 km	15	Crabs, sediment, water and snails
Latitude 24.47.36° N Longitude 67.01.43° E	27.3 miles 43.9 km	15	Crabs, sediment, water and snails
Latitude 24.47.16° N, Longitude 66.59.24° E	28.08 miles 45.1 km	15	Crabs, sediment, water and Snails

The study is spread over 4 years, from the year 2003 to 2006; yearly data are given in order in Table 3-6. In 2003 and 2005, 5 stations were covered while 12 stations were covered in 2004 and 2006 along those 5 points. Total concentration of 16 parent sedimentary PAHs were determined for comparison with the other data of incidents similar to that of Tasman Spirit that occurred in other parts of the world and a general ecotoxicity assessment was made. The GC profile of the Σ PAH fractions recovered from sediment samples exhibited general features of the coastal environment, with the predominance of aliphatic and polyaromatic compounds based on dissolved and unsolved hydrocarbons.

PAHs concentration in the analyzed sediment samples collected from different sampling stations was different with respect to individual components and concentration which may be due to the spreading pattern of the spilled oil as well as adsorbing properties of the sediment for

Table 3. PAHs contamination level in sediment samples in 2003 (ppm)

Components	St 1, A	St 2, A	St 3,A	St 1, B	St 2, B	St 3,	St 4
Naphthalene	0.176	4.862	0.242	0.623	<LOR	<LOR	<LOR
Acenaphthylene	<LOR	4.118	<LOR	<LOR	<LOR	<LOR	<LOR
Acenaphthene	<LOR	3.569	<LOR	<LOR	0.481	<LOR	1.181
Fluorene	<LOR	3.841	<LOR	<LOR	<LOR	<LOR	<LOR
Phenanthrene	0.508	3.504	<LOR	<LOR	0.096	<LOR	0.237
Anthracene	<LOR	3.518	0.571	<LOR	<LOR	0.979	<LOR
Fluoranthene	0.254	3.474	<LOR	<LOR	0.154	<LOR	0.378
Pyrene	<LOR	3.682	0.349	0.257	0.568	0.742	1.396
Benzo(a)anthracene+Chrysene	<LOR	6.886	<LOR	<LOR	<LOR	<LOR	<LOR
Benzo(b)fluoranthene+Benzo(k) fluoranthene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR
Benzo(a)pyrene+Dibenzo(a,h)anthracene	<LOR	6.214	<LOR	<LOR	<LOR	<LOR	<LOR
Benzo(ghi)perylene+Indeno(1,2,3-c,d)pyrene	<LOR	5.034	<LOR	<LOR	<LOR	<LOR	<LOR
Sum	0.937	48.704	1.163	0.880	1.299	1.721	3.191

*LOR= Limit of reporting; ** A & B= are the two points at a station at a distance of 5 km.

Table 4. PAHs contamination level in sediment samples in 2004 (ppm)

Components	St 1	St 2	St 3	St 4	St 5	St 6	St 7	St 8	St 9	St 10	St 11	St 12
Naphthalene	0.225	0.255	0.255	0.236	0.173	0.247	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR
Acenaphthylene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR
Acenaphthene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	0.003	<LOR	0.134
Fluorene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	0.002	<LOR
Phenanthrene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	0.20
Anthracene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR
Fluoranthene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR
Pyrene	0.322	0.365	0.365	0.267	0.23	0.297	0.227	0.210	0.24	<LOR	0.002	0.25
Benzo(a) anthracene + Chrysene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	0.006	<LOR	<LOR
Benzo(b)fluoranthene+ Benzo(k) fluoranthene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR
Benzo(a)pyrene+ Dibenzo(a,h) anthracene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	0.108
Benzo(ghi)perylene+ Indeno(1,2,3-c,d)pyrene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR
Sum	0.548	0.620	0.620	0.503	0.376	0.544	0.227	0.210	0.204	0.009	0.005	0.721

*LOR= Limit of reporting.

PAHs. Average concentrations of Σ PAH and Σ C_5-C_{25} in the sediment (in ng/g of dry weight) of the most significant and representative 8 stations during the years 2003 and 2004 are shown in Table 7.

The GC profile of the Σ aliphatic and polyaromatic fractions represented the level of the individual PAH and AH analyzed not only in sediments but also in samples of marine fauna as well as water samples collected from the affected area during the study; their comparison is given in Fig. 3. High levels of PAHs were observed in all sediment samples collected nearest to the oil impacted area after 28 months of the spill comparative to the previous monitoring made after 18 months at the same site which is the evidence of bio-accumulation of PAHs. Unfortunately, any background data for the area prior to the Tasman Spirit oil spill is not available. It is, therefore, suggested that the contamination found in the marine environment is not solely but mainly due to this spill. Intact hydrocarbons enter the oceans from a variety of sources, including decaying phyto- and zoo-planktons, routine tanker and shipping operations, terrestrial runoffs, atmospheric fallouts,

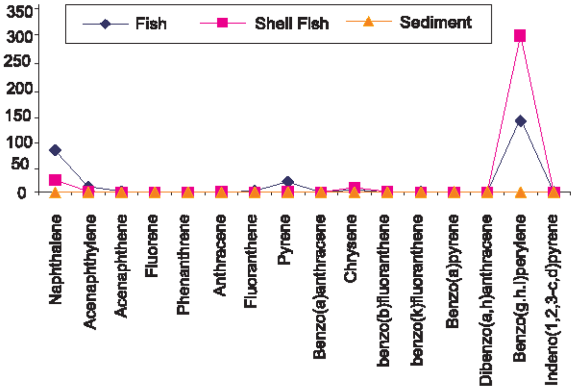

Fig. 3. Comparison of oil pollution status due to Tasman Spirit: PAHs trend in fish, shell fish and sediment.

Table 5. PAHs contamination level in sediment samples in 2005 (ppm)

Components	St 1	St 2	St 3	St 4	St 5
Naphthalene	0.009	<LOR	0.139	<LOR	<LOR
Acenaphthylene	<LOR	<LOR	<LOR	<LOR	<LOR
Acenaphthene	<LOR	<LOR	<LOR	<LOR	<LOR
Fluorene	<LOR	<LOR	<LOR	<LOR	<LOR
Phenanthrene	0.023	<LOR	<LOR	<LOR	<LOR
Anthracene	<LOR	<LOR	<LOR	<LOR	<LOR
Fluoranthene	0.026	<LOR	<LOR	<LOR	<LOR
Pyrene	<LOR	0.028	<LOR	0.164	0.172
Benzo(a)anthracene+Chrysene	<LOR	<LOR	<LOR	<LOR	<LOR
Benzo(b)fluoranthene+Benzo(k) fluoranthene	<LOR	<LOR	<LOR	<LOR	<LOR
Benzo(a)pyrene+Dibenzo(a,h)anthracene	<LOR	<LOR	<LOR	<LOR	<LOR
Benzo(ghi)perylene+Indeno (1,2,3-c,d)pyrene	<LOR	<LOR	<LOR	<LOR	<LOR
Sum	0.0586	0.028	0.139	0.164	0.172

*LOR= Limit of reporting.

Table 6. PAHs vontamination level in sediment samples in 2006, (ppm)

Components	St 1	St 2	St 3	St 4	St 5	St 6	St 7	St 8	St 9	St 10	St 11	St 12
Naphthalene	0.001	<LOR	<LOR	0.002	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR
Acenaphthylene	<LOR	<LOR	<LOR	<LOR	<LOR	0.009	<LOR	<LOR	<LOR	0.001	<LOR	0.006
Acenaphthene	<LOR	0.003	<LOR	<LOR	<LOR	<LOR	<0.005	<LOR	0.003	<LOR	<LOR	<LOR
Fluorene	<LOR	<LOR	<LOR	0.006	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	0.008	<LOR
Phenanthrene	<LOR	<LOR	0.005	<LOR	<LOR	0.004	<LOR	0.005	<LOR	<LOR	<LOR	<LOR
Anthracene	0.002	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	0.005
Fluoranthene	<LOR	<LOR	<LOR	<LOR	0.006	<LOR	<LOR	<LOR	<LOR	0.003	<LOR	<LOR
Pyrene	<LOR	<LOR	0.004	<LOR	<LOR	<LOR	<LOR	<LOR	0.004	<LOR	0.004	<LOR
Benzo(a)anthracene+Chrysene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	0.008	<LOR	<LOR	<LOR	<LOR	<LOR
Benzo(b)fluoranthene+Benzo(k) fluoranthene	<LOR	0.001	<LOR	<LOR	<LOR	<LOR	<LOR	0.0061	<LOR	<LOR	<LOR	<LOR
Benzo(a)pyrene+Dibenzo(a,h)anthraene	<LOR	<LOR	<LOR	<LOR	0.012	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR
Benzo(ghi)perylene+Indeno(1,2,3-c,d)pyrene	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR	<LOR
Sum	0.003	0.004	0.009	0.014	0.018	0.013	0.013	0.011	0.007	0.004	0.012	0.011

*LOR = Limit of reporting.

natural seepage and from shipping and offshore well disasters.

Individually, minimum concentration (0.014 ppm) was found to be that of naphthalene while maximum (11.923 ppm), that of dibenzo (a,h)anthracene; concentration of the 7 of 14 compounds increased in the order of fluorene (0.012), anthracene (0.027), chrysene (0.057), pyrene (0.188), benzo(a)anthracene (0.012), phenanthrene (0.027), and benzo(a) pyrene (0.612 ppm). Also the average minimum concentration (0.008 ppm) was that of naphthalene and maximum (1.325 ppm) that of dibenzo (a,h)anthracene (Fig. 4).

It was observed that the spilled oil rarely dispersed in the water column and mainly sedimented in the form of tar balls in the deep sea sediments which was attributed

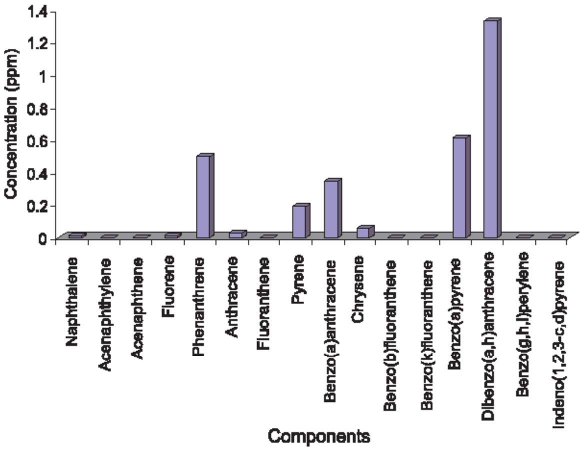

Fig. 4. Average PAH concentration of individual component.

to the heavy nature of the spilled oil. Concentrations in the range of 3-450 mg of total PAHs per kg of dry wt sediment were reported in subtidal sediments (Pastor *et al.,* 2001) from the Arabian Gulf following War (Readman *et al.,* 1992). However, average concentration of individual component of PAHs in this study (Fig. 4) was found lower than the value reported ten years later in 1999; the median sediment concentration was of 117 mg/kg (Page *et al.,* 2002).

After analysis of a number of sediment samples, average concentrations of polyaromatic and aliphatic hydrocarbons from different sample stations during 2003-2006 are given in Table 3-6 in order and level of the oil pollution is given year-wise given in Fig. 5.

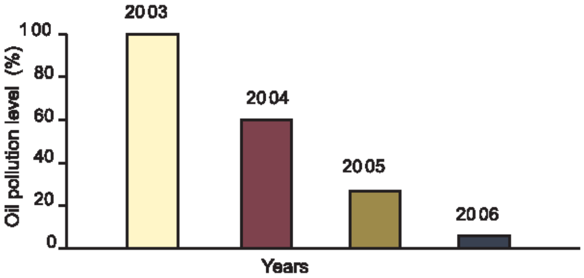

Fig. 5. Level of oil pollution at Clifton beach, during the period of study.

Table 7. Average concentrations of ΣPAH and ΣC5-C25 in sediments (ng/g dry weight) at 8 representative stations

Area	Survey year	ΣPAHs (ng/g)	ΣAliphatic (ng/g)
Clifton beach,			
Station # 1	August, 2003	10.9–5.4	5.0–7.0
Station # 2	March , 2004	<0.04–109	<0.04–5.0
Station # 3	August, 2003	8.2–7.2	n.d
Station # 4	March , 2004	35–65	4.3–2.3
Station # 5	August, 2003	11.6–72	0.2–40
Station # 6	March. , 2004	23.3–12	n.d*
Station # 7	August, 2004	10.56–13.4	0.20–1.5
Station # 8	March , 2004	20.06–3.9	0.03–0.7

* n.d. = not detected.

The components of Tasman Spirit oil, found in sediment samples collected during 2003, varied from those found in 2006. Concentration of components also varied with respect to the sampling stations, the lowest PAHs concentration 0.089 ppm being at sampling station 2 and the highest 0.556 ppm, at sampling station 4.

Physicochemical properties of the oil were measured and showed high density (0.97 kg/L) and viscosity (29, 500 cSt at 15 °C) as well as a high content of resins. The stable emulsions with water were broken-up into fragments of enough density to sink during its way towards the shore, favoured by the humid weather conditions following the spill. These conditions helped wash the beached oil back into the sea, mixed with sand. In fact, tar aggregates of 1-20 cm in diameter, amounting upto 300 kg/km^2 were found in January 2003, during sampling of bottom fauna by beam trawling, in areas of the continental shelf below the main drifting path of the spill (Fig. 1). The oil spill, immediately after the accident caused colossal damage to the environment, marine life and human beings covering approx. 2062 km marine area exposing about 300,000 persons to toxic VOCs released by evaporation into the air from the oil spill.

Conclusion

Studies have demonstrated that levels of PAHs and AHs as low as 1 μg/g in sediments cause toxic and long-term effects. The present detailed study was spread over four years; the monitoring of the levels of PAHs and AHs, from July 27,2003 up to December 2006, started with making an initial survey so as to know the current situation concerning PAH levels at the spill affected area. Though it is concluded that no harmful oil pollution persisted up to 2006 due to considerable bioremedial measures that were taken in this area, regular monitoring for at least the next 10 years is recommended for recording the decrease and/or the increase in hydrocarbon level. The studies should be focused on determining the effects of oil on the changes in surface and deep sea sediments and strict improvements in the existing environmental legislation should be made to cover all aspects of pollution control management, within the country.

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Lead Concentration in Road Side Dust and Selected Vegetables of Lahore City, Pakistan

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Abstract. The contents of lead as a pollutant in road side dust and vegetable samples of urban and rural areas of Lahore city, Pakistan, were monitored with the help of atomic absorption spectrophotometer. Average concentration of lead in dust samples collected from residential areas was 25 to 165 mg/L, from rural areas 9.5 to 13.0 mg/kg, from commercial areas 201 to 1685 mg/kg, and from canal side 105 to 220 mg/kg. In most of the vegetable samples, lead level was in the permissible range, whereas in some samples, the average lead concentration ranged from 0.13 to 5.0 mg/L.

Keywords: lead, vegetables, soil

Introduction

Lead is distributed widely in nature in different forms because of its easy working qualities and permanence. It is used in batteries, cable sheaths, solder, radiation shielding etc. The widespread uses of lead in manufacturing of tetraethyl antiknock agents and paint pigments such as white lead, red lead and chrome yellow etc. led to environmental and health problems. It is difficult to know the natural lead level in the presence of human activities. In natural conditions lead is estimated to be 0.01 mg/kg in food, 0.0005 mg/kg in water,and 0.15 mg/m^3 in air (Maharachpong *et al*., 2006; De, 1996). Lead levels in air vary with location and weather. In water, lead is derived from soil and rocks, fallout, dust and vehicular exhausts. Lead is widely distributed in food, with the widest variation in root and green vegetables. This variation reflects the contamination of soil by domestic and industrial fallout. Lead in vegetables and fruits cannot be removed by washing (Beg *et al.*, 1987). In soil and dust, it is recognized as a public health problem and permissible lead levels in the soil have been proposed (Rodriguez-Flores and Rodriguez-Castellon, 1982). Lead is cumulative body poison (Wahlin *et al*., 2006). There are three means, by which lead enter our body system through air, water and food and produce toxic symptoms. The toxicity of lead has been studied extensively in recent years at trace and sub trace levels, by many countries on continuous basis to keep a check on their natural food supply before use (Wei and Yang, 2010). Mild level lead poisoning causes anemia, headache, fatigue and irritation. Acute lead poisoning in humans leads to severe damage of kidney, liver, brain, central nervous system, reproductive organs and sometimes to death. However, toxicity of lead compounds depends on their solubility in body fluids. The smaller is the particle size, the greater is its solubility and hence the toxicity. All these facts cause great concern for public health, demanding accurate determination of this metal ion at different sites, in different vegetables and items of human use so as to be able to avoid potential human health risk (Shi *et al*., 2011).

Techniques principally used for the determination of trace concentrations of lead are atomic absorption spectroscopy, colorimetry, polarography and emission spectroscopy and inductively coupled plasma. In this presentation lead is determined in roadside dust and vegetable samples by atomic absorption spectrophotometry as it is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis, due to it rapidness, high sensitivity, specificity and being relatively free from interferences (Khairy *et al*., 2011).

Materials and Methods

Sampling. Forty samples of road side dust were collected along the intersection of major roads, residential, commercial and rural areas. Samples were also collected from pavements, side gutters and sidewalks, where traffic density was high or low. Twenty three samples

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*Author for correspondence; E-mail: pcsir322@gmail.com of various vegetables were collected from different rural and urban areas of Lahore city including high ways, motor way and bund road (Brown, 1986).

Sample digestion. Soil/dust samples were collected and pulverized to pass through a 100 mesh sieve and dried at 105 to 110 °C to constant weight. Sample (2-5 g) was mixed with 10 mL of concentrated nitric acid in a beaker then heated gently. Again 10 mL of concentrated nitric acid and 10 mL of 70% perchloric acid were added and the mixture was boiled until the solution became almost colourless. The mixture was heated to reduce the volume down to 2-3 mL then cooled. 10 mL of water was added and warmed to dissolve the salts. Samples were filtered, volume was made 100 mL using distilled water and the so prepared solution was used for analysis.

All vegetable samples were oven dried at 130- 150 °C, grinded and 2 g of each sample in 250 mL beaker was treated with 5 mL of nitric acid. The beaker was covered with watch glass and heated on hot plate. After 1 h, the contents of beaker were treated with further 5 mL of nitric acid and 1-2 mL of 35% hydrogen peroxide was added while swirling the contents. The heating was continued until the volume of the sample was reduced down to 2-3 mL. It was then cooled, filtered using Whatman filter paper # 42 and volume was made 100 mL with double distilled water in a 100 mL volumetric flask. A blank was prepared using the same procedure (Brown, 1986). The samples were analyzed by atomic absorption spectra 40 Varian Australia (Lu *et al.*, 2009). Calibration graph was prepared using stock solutions of lead.

Results and Discussion

The samples were analyzed using atomic absorption spectra and calibration graph was prepared using stock solution of lead which was linear passing through the origin (Fig. 1), in the range of 0.1 to 10 mg/kg of lead (Paoletti *et al.*, 1991). WHO limit of lead in soil as a reference value is 13 mg/kg (Faiz *et al.*, 2009). Comparison of various areas shows average concentration of lead to increase from 25 ± 0.17 to 165 ± 0.20 mg/kg in the residential areas, far away from the freeways (Table 1). In the rural areas, lead was the lowest (Fig. 2) ranging from 9.5 ± 0.20 to 13 ± 0.12 mg/kg within the WHO limits (Table 2); the reason is the low traffic density corresponding to the distance of the rural areas from the main city (Wahlin *et al.,* 2006). Maximum concentration of lead was found in the commercial areas of Lahore ranging from 201 ± 0.41 to 1685 ± 0.94 mg/kg (Table 3). Commercial areas of Lahore are mostly surrounded by multi-storey buildings which act as sink

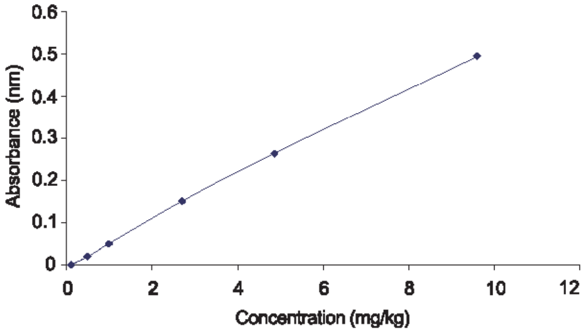

Fig. 1. Pb(II) calibration curve.

Table 1. Lead concentration in dust samples of residential areas of Lahore

Sample no.	Pb concentration (mg/kg)	Sampling areas
S-1	50.0 ± 0.26	Muslim Town, Ferozepur Road
S-2	129.0 ± 0.24	Qaudri Cowk Township
S-3	75.0 ± 0.16	Johar Town
S-4	25.0 ± 0.17	College Road, Township
S-5	156.0 ± 0.21	Garden Town
S-6	< 1 ± 0.02	Blank
S-7	135.0 ± 0.17	Model Town
S-8	165.0 ± 0.20	Johar Town, Main Road
S-9	156.0 ± 0.17	Misri Chowk
S-10	115.0 ± 0.17	Main Defence Road
S-11	120.0 ± 0.26	Defence Road Hospital
S-12	130.0 ± 0.26	Defence Road School

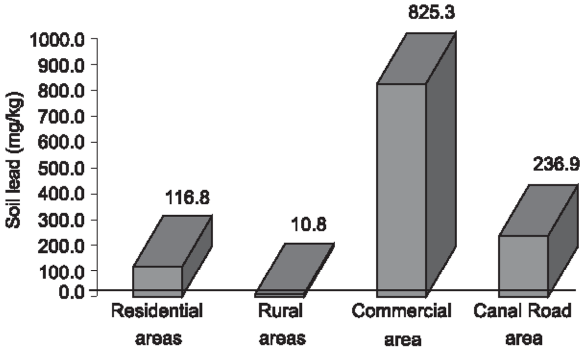

Fig. 2. Average Pb concentration in different areas of Lahore.

Table 2. Lead concentration in dust samples of rural areas of Lahore

S. no.	Pb concentration (mg/kg)	Sampling area
S-1	10.0 ± 0.20	Rice field, near Rohi Nallah
S-2	13.0 ± 0.25	Wheat field, village Kamahan
S-3	9.5 ± 0.20 Harpalkey	Rice field, village
S-4	11.0 ± 0.26	Wheat field, village Leel
S-5	< 1 ± 0.02	Blank
S-6	13.0 ± 0.12	Corn field, badian
S-7	9.5 ± 0.17	Defence Bhatta Chowk
S-8	9.7 ± 0.09	Sugar cane from State Life Agricultural land

Table 3. Lead concentration in dust samples of commercial area of Lahore

S. no.	Pb concentration (mg/kg)	Sampling area
S-1	850.0 ± 1.24	Ichhra School Ferozepur Road
S-2	895.0 ± 0.81	Ichhra Bus stop
S-3	870.0 ± 0.81	Garden Town Bus stop
S-4	790.0 ± 0.82	Model Town Bus stop
S-5	710.0 ± 0.82	Ittifaq Hospital
S-6	201.0 ± 0.41	PCSIR Bus stop
S-7	670.0 ± 0.47	Waltan Road R.A Bazar
S-8	795.0 ± 0.94	Sadar Road
S-9	< 1 ± 0.02	Blank
S-10	806.0 ± 0.47	Dharampura Main Road
S-11	816.0 ± 0.41	Railway Station
S-12	1685. ± 0.94	Ravi Road

of pollutants. The increase in lead pollution may be due to the large traffic volume, emitting substantial quantity of lead along with smoke and other unwanted gases. It is concluded that soil along the roads having heavy traffic load contains more lead as compared to that of other areas.

The concentration of lead along the Lahore canal ranged from 105 ± 0.82 to 220 ± 0.82 mg/kg (Table 4). This may be due to the location of the site, being relatively open with wide roads, covered on both the sides by large trees and vegetation. Although deposition of lead depends on traffic density, but it may be influenced by many factors such as wind velocity and direction, precipitation and humidity (Faiz *et al.*, 1996). It was

Table 4. Lead concentration in dust samples of Canal Road area

S. no.	Pb concentration (mg/kg)	Sampling area
S-1	210.0 ± 0.82	Fatah Garh
S-2	220.0 ± 0.82	Mughalpura
S-3	130.0 ± 0.94	Dharampura
S-4	195.0 ± 0.82	Herbanspura
S-5	114.0 ± 0.94	Taj Pura
S-6	150.0 ± 1.24	Lal Pul
S-7	200.0 ± 0.82	Punjab University
S-8	143.0 ± 0.82	Shah the Khoi
S-9	201.0 ± 1.25	Jallo Road
S-10	< 1 ± 0.02	Blank sample
S-11	105.0 ± 0.82	Jallo Park
S-12	142.0 ± 0.82	Mall Road

Table 5. Lead concentration in vegetable samples collected from rural and urban areas of Lahore

S. no.	Vegetables	Pb concentration (mg/kg)
1.	Onion (U)	0.32 ± 0.02
2.	Spanich (U)	4.2 ± 0.12
3.	Mint (U)	0.3 ± 0.03
4.	Mustard (U)	5.0 ± 0.21
5.	Potato (U)	0.56 ± 0.12
6.	Radish (U)	0.13 ± 0.02
7.	Turnip (U)	0.35 ± 0.02
8.	Cauli flower (U)	1.3 ± 0.16
9.	Carrot (U)	0.52 ± 0.04
10.	Pea (U)	--
11.	Lettuce (U)	--
12.	Coriander (U)	2.99 ± 0.07
13.	Maize (U)	0.20 ± 0.03
14.	Wheat (R)*	0.15 ± 0.03
15.	Rice (R)*	0.13 ± 0.02
16.	Ginger (U)	0.20 ± 0.03
17.	Brinjal (U)	0.50 ± 0.04
18.	Cucumber (U)	0.30 ± 0.04
19.	Tomato (U)	0.25 ± 0.02
20.	Lady finger (U)	0.20 ± 0.03
21.	Cabbage (PU)	0.15 ± 0.02

U = urban area; R = rural area; all samples were taken along the Lahore Motor Way to Kalashah Kaku and Defence rural area to Badian Road, High Way and Bund Road; * = wheat and rice samples were collected from the rural areas, whereas all the rest of vegetables, from the urban areas.

observed that the average concentration of lead in vegetables ranged from 0.13 ± 0.02 to 5.0 ± 0.21 mg/kg (Table 5) and in some vegetables, lead was below the detection limit. The accumulation of lead in green vegetables may be due to the use of polluted water for irrigation, use of lead arsenate as insecticide/fungicide or due to the fertilizer impurities or atmospheric pollution. However, lead concentration was higher in leafy vegetables as compared to the root tubers and vegetables (Duong and Lee, 2011; Farmer, 1993).

Faiz *et al.* (2009) conducted a study on road pollution with respect to heavy metals in Islamabad Pakistan, in which average lead concentration was shown as 29 ± 104 mg/kg while in the present study, samples from the residential areas (Table 1) have lower concentration of lead (minimum 25 ± 0.17 to maximum 165 ± 0.20 mg/kg). The minimum and maximum lead concentrations in the rural areas (Table 2) were 9.5 ± 0.20 mg/kg and 13 ± 0.12 mg/kg, respectively, while there were very high concentrations of lead in commercial areas of Lahore (Table 3) as compared to the residential and rural areas; these results are also much higher than the results of Faiz *et al.* (2009) reported in his study. The results of commercial areas in our study are much higher conforming to the reports of Faiz *et al.* (2009). The reason for higher concentration of lead in Lahore is the heavy transport load and dense population of the city (Faiz *et al.*, 2009; Han *et al.*, 2008).

Conclusion

It is concluded from the data presented here that high level of lead is present in the road side dust and leafy vegetables of Lahore. Lead is directly/indirectly entering and continuously polluting the environment. The hazards of lead pollution especially in growing children require particular attention, because children absorb and retain about 50% of the ingested lead as compared to 8% absorbed and retained by adults.

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HPLC Determination of Betamethasone and Prednisolone in Urine Samples Using Monolithic Column

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Abstract. A fast and reliable HPLC method is reported for the separation and quantification of betamethasone and prednisolone in urine samples using Chromolith® Performance RP-18e (100 mm × 4.6 mm) column. The separation and detection was achieved using an isocratic mobile phase composed of methanol:water (44:56 v/v) at 2.0 mL/min and wavelength of 254 nm. After successful optimisation of method parameters, it was applied to the urine samples. Solid phase extraction technique was used to clean the sample before analysis. The developed method was validated for the system suitability, precision and accuracy. The limits of detection for the prednisolone and betamethasone are 0.11 ng and 0.075 ng/10 µL injection, respectively allowing their determination in human urine samples. Recovery for spiked urine samples was in the range of 97-103%. The method offers a valuable alternative to the methodologies currently employed for separation and quantification of prednisolone and betamethasone in urine samples.

Keywords: monolithic column, HPLC, betamethasone, prednisolone, urine, solid phase extraction

Introduction

Corticosteroids are a family of drugs which include cortisol (hydrocortisone), an adrenal hormone found naturally in the body, as well as synthetic drugs. Though natural and synthetic corticosteroids, both are potent anti-inflammatory compounds, the synthetics exert a stronger effect. Corticosteroids derivatives, betamethasone, dexamethazone, prednisolone, triamcinilone including cortisone, are used to treat numerous autoimmune and inflammatory conditions, including asthma, bursitis, Crohn's disease, skin disorders, tendinitis, ulcerative colitis and others (Nozaki, 2001). Assay of steroids is important in pharmaceutical formulations (Hashem and Jira, 2005) and in biological fluids for disease diagnosis (Lin *et al.*, 1997), in pharmacokinetics (Glówka *et al.*, 2006), in study of metabolism of selected steroids (Kartsova *et al.*, 2004; Gallego and Arroyo, 2002) and as a test for doping and veterinary control (Touber *et al.*, 2007; Baiocchi *et al.*, 2003). Separation techniques like HPLC (Frerichs and Tomatore, 2004), GC (Vanluchene and Vandekerckhove, 1985) and CE (Jumppanen *et al.*, 1994) are reported to separate and determine multiple steroids and single analyte of interest from its interfering components. For separation of analytes from endogenous materials, use of coupled column chromatography with mass spectrometric (Polettini *et al.*, 1998) or tandem MS have been reported (Tamvakopoulos *et al.*, 2002).

Monolith columns are new generation in HPLC stationary phases. Silica-based monoliths have small-size skeletons and a bimodal pore size distribution with µm-sized throughpores and nm-size mesopores, which impart silica-based monoliths favourable properties of high-efficiency, fast separation through low-pressure drop across the column, fast mass transfer kinetics and high binding capacity (Rieux *et al.*, 2005). Many successful applications have been reported in pharmaceutical and biological analysis (Satínský *et al.*, 2006; Hashem and Jira, 2005; Zarghi *et al.*, 2005). Monolithics have found applications in assay of corticosteroids as well (Valencia *et al.*, 2008; 2007).

Here, monolithic column was used for separation of betamethasone – a corticosteroid known for its effects on nervous system, carbohydrate metabolism and cardiovascular system (attractive for drug doping) – from predinisolone and urinary endogenous compounds (Polettini *et al.*, 1998).

Materials and Methods

Chemicals. Prednisolone, betamethasone and cortisone were obtained from Pfizer Laboratories Ltd., Pakistan, GSK Pakistan (Ltd.) and Sigma-Aldrich, St. Louis, MO, USA, respectively. HPLC grade methanol was

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*Author for correspondence; E-mail: abrokamran@gmail.com purchased from Sigma-Aldrich Chemi GmbH, Germany. Water was doubly distilled and deionized. Mobile phase components were degassed before use.

Instrumentation. Spectra SYSTEM P-2000 pump with a UV6000LP diode array detector and SCM1000 degasser (Thermo Finigan).

Separation was achieved with the analytical column Chromolith® Performance RP-18e (100 mm × 4.6 mm) by Merck KGaA (Darmstadt, Germany) with an isocratic mobile phase of MeOH/H_2O (44 : 56 v/v) at a flow rate of 2.0 mL/min. Detection was achieved at 254 nm. ChromQuest software was used for data analysis. Solid-phase extraction was carried out using Supelco Discovery DSC-18 (PA, USA) SPE cartridges.

Standard solutions of the corticosteroids. Stock solutions of 1000 µg/mL (1 mg/mL) of each corticosteroid were prepared in methanol. Working standard solutions were prepared by diluting aliquots of each stock solution to obtain concentrations ranging from 1 to 10 µg/mL. Calibration graphs were constructed by plotting the peak areas obtained at wavelength 254 nm *vs* the corresponding injected amounts (ng).

Urine sample preparation. Urine samples were collected from five volunteers. Steroids were determined by spiking urine samples of healthy persons to get final concentration of 5 µg/mL and extracted using solid phase extraction technique. SPE cartridge was washed with 10 mL methanol followed by 10 mL water, then 10 mL of urine sample was passed through the cartridge. After that, 10 mL of 10% methanol was passed through the cartridge to remove weakly bound components. Then 3 mL of methanol was passed through the cartridge at the flow rate of 3 mL/min. Finally, eluate was collected and filtered with 0.45 µm filter paper and then 10 µL of the filtrate was injected into HPLC for the analysis.

Results and Discussion

Urine samples from healthy volunteers were cleaned-up using solid-phase extraction procedure and run as blank. Chromatogram shows the retention of some endogenous compounds which may be urinary free steroid as inferred by adding cortisone to urine sample and comparing the UV spectra (Fig. 1). No further attempt was made to separate or identify endogenous steroids due to non-availability of standards. Keeping in view the retention of endogenous compounds, mobile phase was modified to achieve the separation of betamethasone and prednisolone and found to be methanol-water 44:56 (v:v), respectively, with a flow rate of 2.00 mL/min (Fig. 2). As the proposed activity was intended to develop a method that can be used in routine analysis, the system was validated systematically, the parameters used being system performance, linearity and calibration, reproducibility and intra-day precision. Analysis of corticosteroids in tablets and urine samples and the robustness of method were validated by checking the slight variations in flow rate, methanol content, injection volume and the wavelength of detection.

The system performance was calculated by the reproducibility tests of the retention time, number of theoretical plates, capacity factor, resolution and the relative retention of corticosteroids (Table 1). The linearity and calibration of corticosteroids was determined in the range of 1 to 10 µg/mL with detection limit,

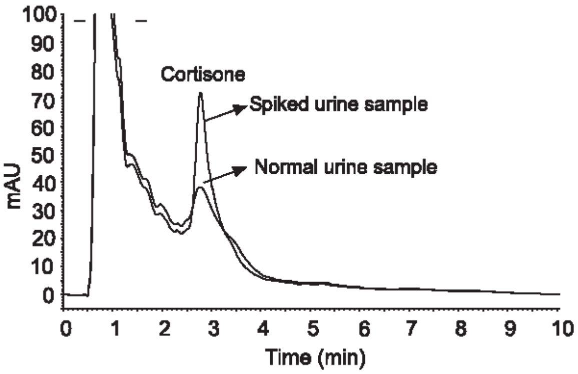

Fig. 1. Chromatogram of urine showing both normal and spiked urine samples.

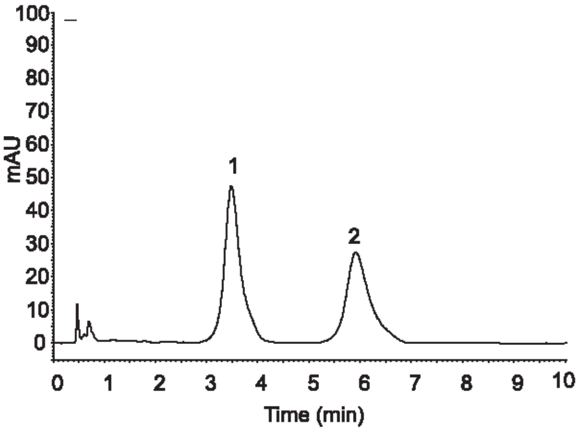

Fig. 2. Separation of prednisolone 5 µg/mL and betamethasone 5 µg/mL, using monolithic column methanol-water (44:56) as mobile phase @ 254 nm with flow rate 2.0 mL/min.

calculated by the classical method of 3σ and it was found to be 1.1 ng and 0.75 ng with equation of straight line, $y = 2.175\chi + 0.12$ and $y = 1.683\chi - 0.13$, for the prednisolone and betamethasone, respectively. The reproducibility test of the method was determined by running 5 samples of known concentration daily and for consecutive five days and it was found reproducible in both intra-and inter-day analysis precision analysis. The coefficient of variance was 1.16 and 1.09 for intra-day and 1.52 and 3.10 for inter-day precision of prednisolone and beta-methasone, respectively (Table 2).

The robustness of the method was determined by calculating slight variations in analytical conditions (Table 3). Flow rate of mobile phase did not show any significant change in the resolution of the peaks. Only variations were noted in the retention time but slight variations of methanol content in the mobile phase were determined to be very sensitive for both retention time and resolution for two corticosteroids, so the methanol content in the mobile phase is to be controlled carefully to attain the separation. The amount of sample injected into the HPLC was also determined to be very sensitive because slight variation in the sample amount injected had significant effect on the percent recovery though no change in the separation was observed. This shows high capacity of monolith column and could be helpful in enhancing sensitivity by using higher sample volumes. Wavelength was not found much sensitive as all the recoveries are in the range of ± 5%.

Table 1. System performance for corticosteroids ($n = 5$)

Compound	${}^{t}R$ ± SD (min)	N	k	RS	α
Prednisolone	3.47 ± 0.03	1229	3.448	2.594	1.302
Betamethasone	5.77 ± 0.04	2317	6.397	6.852	1.623

Table 2. Reproducibility, inter-day and intra-day precision of predinisolone and betamethasone

Compound	Inter-day analysis			
	Used concentration (µg/mL)	Observed concentration (µg/mL)	C.V (%)*	Accuracy (%)**
Prednisolone	4.5	4.69 ± 0.05	1.16	104.24
Betamethasone	4.5	4.59 ± 0.05	1.09	102.21
	Intra-day analysis			
Prednisolone	4.5	4.67 ± 0.07	1.52	103.86
Betamethasone	4.5	4.67 ± 0.14	3.10	103.83

* = coefficient of variance (%) = SD × 100/mean; ** = accuracy (%) = observed concentration × 100/used concentration.

Urine samples. The spiked urine samples were analyzed by the above mentioned procedure. Fig. 3 shows clean chromatogram with baseline separation from endogenous compounds and the two steroids are completely resolved. Percent recovery ranges from 97%-103% for the five samples assayed.

Conclusion and future work. With the proposed method, satisfactory separation of the analytes, extended linear range and rapid analysis time was achieved. The

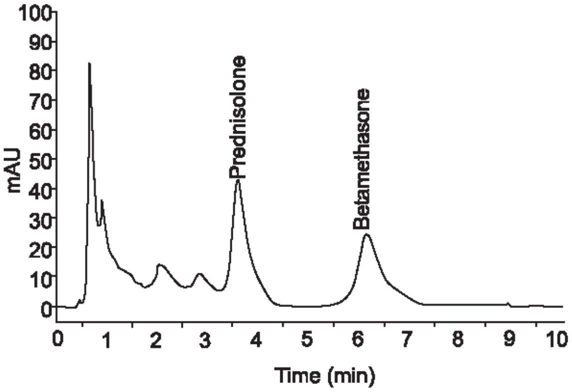

Fig. 3. Urine samples spiked with prednisolone and betamethasone after clean up by solid phase extraction.

Table 3. Robustness parameters for corticosteroids

Compound	Flow rate (mL/min)			Methanol content (%)			Wavelength (nm)			Sample amount (µL)		
	1.9	2.0	2.1	43	44	45	252	254	256	16	18	20
	Retention time (min)						Recovery %					
Prednisolone	3.79	3.62	3.43	4.04	3.62	3.35	99.58	102.78	97.15	87.50	96.70	102.50
Betamethasone	6.46	6.16	5.83	7.07	6.16	5.59	104.90	103.21	100.00	88.00	97.50	103.70

corticosteroids were separated in less than 7 min. Good recovery of each steroid was achieved using monolithic column, which indicated good agreement with corticosteroid amount spiked in samples. The proposed HPLC method ensures precise and accurate determination of prednisolone and betamethasone in urine samples. Work is in progress to resolve other corticosteroids along with prednisolone and betamethasone. Application will be extended to determine corticosteroids in blood samples for screening and diagnostic purposes.

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Technology for Treatment and Recycling of Wastewater of Automobile Service Stations

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Abstract. A prototype treatment plant was fabricated and tested at varied hydraulic retention times for settling the suspended/settleable matter and skimming out oil and grease at laboratory plants of 40 L and 56 L. The results showed that 99.7% of oil and grease and almost all total suspended solids were removed and sparkling clear water was obtained, reusable for washing vehicles.

Keywords: water treatment technology, water recycling, wastewater treatment, automobile service stations

Introduction

Water resources of Pakistan are not enough to meet the present and the future needs. Greater increase in population during the last two decades is putting strain on meager water resources for the three main consumers i.e. agriculture, industry and human beings. About 47% of the population is without safe drinking water, and nearly 84% of rural population is without sanitation facilities (Afzal, 2003). Like other countries, Pakistan is also facing problems of environmental pollution, threat of depletion of ozone layer, global warming and degradation of natural resources. According to Pakistan Council of Scientific and Industrial Research (PCSIR), only 3% industries treat their wastewater (JICA, 1999), while the rest discharge untreated effluents into rivers, lakes and seas. Dumping of untreated municipal and industrial wastes has contaminated surface and ground water resources and threatened the aquatic life.

On the other hand, rapid urbanization is changing patterns of consumption causing severe misuse of water resources (GOP, 2002). The city of Peshawar has over 350,000 registered vehicles (Mullick, 1987), out of which the share of the main city is 60% (Miller, 1999). Service stations or car wash stations use more than 16,000 L potable water per day. Thus, thousands of service stations in the country can waste billion litres water per day in Pakistan. This water contains oil/grease, suspended solids and even toxic metals. Oil and grease form high oxygen demanding waste which depletes the dissolved oxygen in water bodies and thus contributes to bacterial growth and may also contaminate underground water reservoirs (APHA/AWWA/WEF, 1998). It is estimated that one gallon of used oil can pollute more than one million gallons of drinking water (NEQS, 1993).

The National Water Research Institute of Canada in a study on highway runoff toxicity found that the car wash wastewater contains TSS, salts, particulate matter, oil, grease, organic matter, chlorinated solvents, detergents, lubricants, additives, heavy metals, antifreeze agents and acids/alkalies (Metcalf and Eddy, 1974).

In this background, devising technologies for treatment and recycling of wastewater could save the natural resources of potable water within the country. Studies have been carried out in the recent past on the development of treatment technologies for the wastewater of different industries. Efforts have been made to detoxify the tannery effluents employing physicochemical methods followed by biological methods (Khan *et al.*, 2005). In another study a treatment technology was developed for the treatment of wastewater of sugar industry through which biological oxygen demand (BOD) and chemical oxygen demand (COD) of the wastewater was reduced by 96% and 95%, respectively (Khan *et al.*, 2003). Studies on COD reduction of effluent of cotton textile industry (Babu *et al.*, 2000) showed achievement of an overall COD reduction of 80% using biological treatment technique. Similarly, a physicochemical process was developed for the reduction of excessive fluoride concentration in potable water using indigenous materials (Khan *et al.*,

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2006). Treatment of municipal wastewater in a Korean city was carried out by the city government and the private sector with the establishment of wastewater treatment plants which played significant role in the reduction of BOD and COD (Mullick, 1987). Studies have also been conducted on the treatment of industrial effluents from aminoplast industry for COD reduction (Khan *et al.*, 2008).

The present study is aimed at characterizing and developing an economically feasible and technically viable method for the treatment of wastewater of automobile service stations and reducing the harmful effects on the nearby water bodies.

Materials and Methods

Representative samples of wastewater from different service stations in Peshawar were collected and analyzed for the parameters like pH, total hardness, chloride, sulphate, total suspended solids, total dissolved solids, biological oxygen demand, chemical oxygen demand and oil/grease employing standard methods (APHA/AWWA/WEF, 1998). Heavy metals like lead, copper, cadmium, zinc, iron and nickel were determined by atomic absorption spectrophoto-meter (Z-8000, Hitachi-Japan).

Treatment plant design. Based on trials of different systems and chemical analysis of wastewater, the most economical design amongst all was found to be that of 42 L capacity treatment plant, as shown in Fig. 1, having four compartments. Wastewater was passed through this system continuously for 7-8 h and was then approved to be a better option for recycling of wastewater for skimming out oil/grease and removal of settleable matter.

The system was further facilitated with a side valve for the collection of oil/grease from compartment 1. A 0.75 cm diameter hole on the side was made with a stop valve in the first compartment for removal of floating material. A baffle was made between the 2nd and the 3rd compartment with 1″ gap at the base. This all arrangement was made from 4 mm thick plastic sheet. Last compartment had an under-drainage system of 0.5″ diameter and layers of filtration material as mentioned below:

First layer (gravel)	2.5-3.0 cm
Second layer (gravel)	1.5-2.0 cm
Third layer (gravel)	0.5-0.7 cm
Fourth top layer (sand)	0.03-0.05 cm

The under-drainage was placed 5 cm above the base and was given a slope of 1:100 for easy flow of water. Both the materials i.e. sand and gravel used were washed and cleaned before using as filtering media.

Results and Discussion

Critically evaluating the data in Table 1, it becomes apparent that the pH of wastewater was in the range of 7.55-8.25, with an average of 7.161. The total

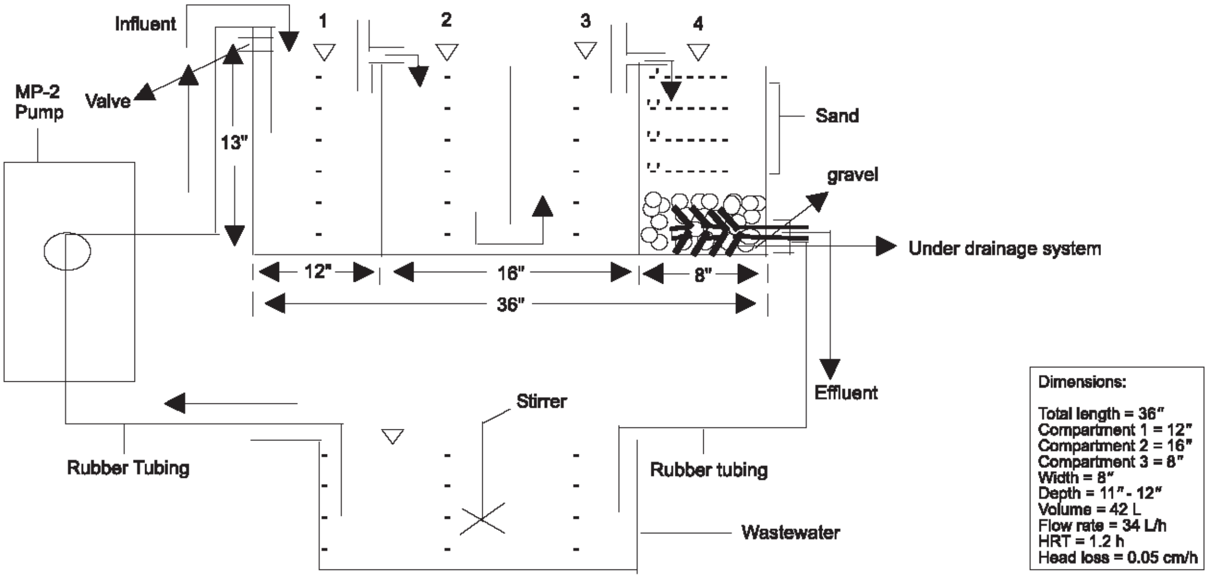

Fig. 1. Schematic diagram of a 42 L capacity treatment plant.

hardness as $CaCO_3$ was in the range of 380-632 mg/L (average 502.4 mg/L), whereas, calcium and magnesium hardness were, respectively, in the range 144-248 mg/L (average 207.2 mg/L) and 164-416 mg/L (average 255.8 mg/L). Chloride was in the range of 56-204 mg/L (average 110.2 mg/L) and sulphate concentration, in the range of 72.9-291.84 mg/L (average value being 165.5 mg/L). Service station wastewater contained soluble as well as dissolved solids. Total suspended solids (TSS) varied widely, in the range of 128-8640 mg/L (average 4397.7 mg/L).

The organic strength of wastewater which was determined in the form of BOD and COD was present in the effluent due to the presence of organics originating from oil/grease in the wastewater. The BOD varied in the range of 52.33-280 mg O_2/L, with average concentration being 158.24 mg O_2/L. COD of wastewater varied from 160.0 mg O_2/L to 470.6 mg O_2/L (average 267.2 mg O_2/L).

Although most of the oil/grease was removed during oil change, a small quantity found its way into the wastewater, which ultimately became a cause of oxygen depletion upon its discharge into the water bodies. Hence, oil/grease is not welcomed in water bodies, due to the hazardous effects and long-term consequences. The concentration of oil/grease in the service station wastewater was higher than the threshold limit of 10 mg/L (NEQS, 1993), the actual range being 13.6-20.3 mg/L, averaging 16.32 mg/L.

Oil/grease is regarded as one of the most unacceptable substance in potable water. It has been reported that one gallon of oil can spoil one million gallons of fresh water. Similarly 1.5 hectares of water surface can have a film of oil 0.3 micron thick due to just 10 litres of oil (Khan *et al.*, 2005). In the present study, the oxygen demanding waste (BOD and COD) was mostly higher than the threshold limit of NEQS (1993). BOD values in some of the samples were although less than the limits set by NEQS, yet their corresponding COD values made them unfit for discharge into water bodies.

As indicated in Table 1, sample No 2 and 4 had BOD less than 80 mg/L of NEQS; the corresponding COD values of these samples were 235.3 and 470.6 mg O_2/L. Similarly the COD of sample No. 9 and 10 was less than the threshold limit of NEQS (150 mg O_2/L). Despite higher concentration of TSS in the most wastewater samples, the materials of high oxygen demanding wastes were sufficient to make service station wastewater unfit for its safe disposal.

The service station wastewater samples also contained heavy metals (Pb^{++}, Cu^{++}, Zn^{++}, Fe^{++} and Ni^{++}) in less than 0.5 mg/L limit of NEQS (Table 1). As such, service station wastewater with regards to heavy metals was safe as the concentration of the latter lied below the

Table 1. Chemical analysis of wastewater of automobiles service station

Parameters*	Wastewater sample number										Average value	NEQS
	1	2	3	4	5	6	7	8	9	10		
pH 7.87	7.55	7.62	7.89	7.83	8.21	8.15	8.24	8.24	8.25	7.161	6-10	
Total hardness as $CaCO_3$	*380	632	348	468	464	500	420	408	484	436	502.4	-
Calcium as $CaCO_3$	204	216	184	144	220	240	208	200	208	248	207.2	-
Magnesium as $CaCO_3$	176	416	164	324	244	260	212	298	276	188	255.8	-
Chloride as Cl'	88	204	56	200	70	73	56	90	155	110	110.2	1000
Sulphate as SO_4''	291.8	245.7	161.3	238.1	176.6	96	80.6	72.9	134.0	157.5	165.5	600
TSS	128	2047	585	6241	3975	6104	5420	5642	8640	5195	4397.7	200
TDS	457	251	286	435	307	1478	697	708	958	836	641.3	3500
BOD	101.79	73.9	216.73	52.33	157.63	270	280	150	120	100	158.2	80
COD	164.71	235.3	288.3	470.6	188.6	160.0	323.5	270.5	260.3	310.5	267.2	150
Oil/grease	18.0	19.1	14.1	15.1	15.6	13.5	14.1	13.6	19.9	20.3	16.3	10
Heavy metals												
Lead as Pb^{++}	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL		0.5
Copper as Cu^{++}	0.06	BDL	0.02	BDL	0.03	BDL	BDL	BDL	BDL	BDL		1.0
Cadmium as Cd^{++}	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL		0.1
Zinc as Zn^{++}	BDL	0.01	0.04	0.03	0.01	BDL	BDL	BDL	BDL	0.02		5.0
Iron as Fe^{++}	BDL	0.15	0.11	BDL	BDL	BDL	0.09	BDL	0.18	0.05		2.0
Nickel as Ni^{++}	0.06	0.01	0.01	0.02	0.05	0.02	BDL	0.03	0.04	BDL		1.0

BDL = below limit of detection; * = except pH, all values are in mg/L.

detection limit of the instrument. (i.e. 0.001 mg), being unlikely to pose a serious threat to water bodies.

The data in Table 2 are related to the average discharge of total dissolved solids (TDS), total suspended solids (TSS), BOD, COD and oil/grease in service station wastewater. TSS discharge was estimated to be 8.79 tons/day. This tonnage is quite significant being able to choke water channels during the course of water flow. TDS was only 1.28 tons/day. BOD and COD discharge were 0.355 and 0.498 ton/day, respectively, whereas oil and grease discharge was 0.034 ton/day.

Table 2. Analysis of actual service station wastewater (average of 10 service stations)

Parameters	Concentration	Quantity
TDS*	641.0 mg/L	1.28 tons/day
TSS*	4397.7 mg/L	8.794 tons/day
BOD*	158.24 mg O_2/L	0.355 tons O_2/day
COD*	249.46 mg O_2/day	0.498 tons O_2/day
Oil and grease*	16.79 mg/L	0.034 tons/day

* = on the basis of 5 million vehicles in Pakistan @ 600 liter/month (if washed once a month) for one year.

Treatment of wastewater. Several options of laboratory scale treatment plants were tried. Variation was made in the number of compartments, size, flow rate, hydraulic retention time etc. Studies were also conducted in the presence and absence of sand and gravel bed and each time chemical analysis was performed and efficiency of the plant was examined. It was found that none of the options were effective in reducing the concentration of oil/grease and settleable matter and above all, the effluent was not sparkling clear especially in the absence of sand/gravel bed. Finally an option with sand/gravel bed was adopted since filtration is an important process for solid-liquid separation in many industries, and especially for final removal of suspended particles in water and wastewater treatment (Babu *et al.*, 2000). Four compartments system with 56 L capacity as shown in Fig. 2 was selected owing to the efficiency and the quality of effluent; the results are presented in Table 3. The Fig. 3 represents the settling characteristics of different waste water samples collected from various service stations with respect to time.

The data of Fig. 3 clearly suggest that most of the matter settled within 10 min. After 30 min, very small percentage of settleable matter was left in the liquid portion.

The data in Table 3 suggest serial reduction of different pollutants from the service station wastewater. Compartment 1 contains service station wastewater (influent) supplemented with additional 15-20 times higher oil and grease to assess the capability of the treatment plant at a higher concentration of these pollutants. Generally oil/grease in service station wastewater lied in the range of 13.60 -20.27 mg/L

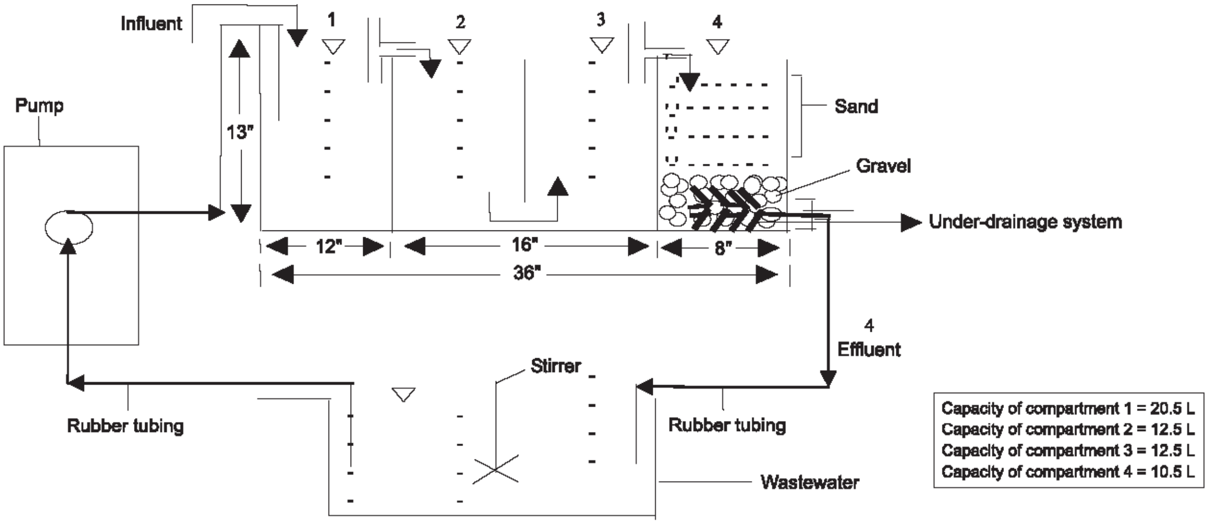

Fig. 2. Schematic diagram of a 56 L capacity laboratory scale treatment plant.

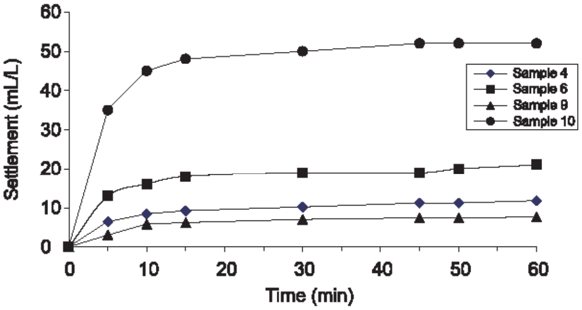

Fig. 3. Settling characteristics of service station wastewater.

Table 3. Chemical analysis of wastewater in 56 L laboratory scale treatment plant

Parameters	Concentration (mg/L) in compartment no.				NEQS (mg/L)
	1	2	3	4	
	Influent			Effluent	
pH	7.38	7.33	7.50	7.20	6-10
Total alkalinity (mg/L)	250	224	160	140	-
Total hardness as $CaCO_3$ (mg/L)	290	260	180	140	250
Calcium as $CaCO_3$ (mg/L)	94	84	80	78	-
Magnesium as $CaCO_3$ (mg/L)	196	176	100	62	-
Chloride (mg/L)	13.7	12.8	8	3.6	1000
Sulphate (mg/L)	290.72	240.56	220.18	176.78	600
TSS (mg/L)	5503	4323	585	5	150
Settlable matter (mL/L)	17.5	5.5	5.5	0	-
BOD (mg O_2/L)	250.00	140.35	60.28	3.00	80
Oil and grease (mg/L)	323.6	15.6	10.7	2.8	10

averaging 16.79 mg/L (Table 1). The oil/grease concentration in influent, in this case, was nearly 323.6 mg/L.

Table 3 also indicates that the quality of wastewater improved in compartment 2 with slight reduction in almost all parameters. Significant decrease is apparent in oil/grease, BOD, settleable matter and TSS. Oil/grease quantity decreased from 323.6 mg/L to 15.6 mg/L in compartment 2, the reduction being 95.18%. Settleable matter markedly decreased from 17.5 to 5.5 ml/L, the decrease being 68.57%. BOD decreased by 43.86% from 250.0 mg O_2/L to 140.35 mg O_2/L. TSS lowered from 5503 to 4323 mg/L in compartment 2; the reduction was 21.44%.

There was no marked decrease in the concentrations of different parameters in compartments 2 and 3 except BOD, oil/grease and TSS. The concentration of the settleable matter remained unchanged in the compartment 3 compared to that in compartment 2, whereas BOD decreased by 57.05% due to removal of oil/grease. BOD decreased from 140.35 mg O_2/L (compartment 2) to 60.28 mg O_2/L (compartment 3), and oil/grease, from 15.6 mg/L (compartment 2) to 10.70 mg/L (compartment 3), the decrease in oil and grease being 31.41%.

The effluent sample (from sand and gravel bed) of compartment 4 was markedly improved and the concentrations of almost all parameters decreased. The effluent was sparkling clear with very low concentration of TSS (5 mg/L), oil/grease, (2.8 mg/L) and BOD (3 mg O_2/L). Settelable matter of the effluent was nil in compartment 4 as compared to the influent in compartment 1, indicating almost 100% removal. The suspended solids decreased to as low as 5 mg/L, i.e. by 99.91%. Likewise, the BOD decreased from 250 mg O_2/L in the influent to as low as 3 mg O_2/L with a total removal of 98.8%. The quality of the effluent as mentioned in Table 3 is fit enough to be successively used for washing purposes.

Conclusion

Chemical analysis of wastewater of service stations of Peshawar city indicated that the pollutants like suspended solids, oil/grease and BOD exceeded the NEQS (1993) limit set by the Government of Pakistan. A huge quantity of potable water of Peshawar i.e. 1.75 to 2 million litres/day is wasted in just washing of automobiles. The system devised for treatment of wastewater of automobile service stations successfully removed almost 100% turbidity, 99.18% BOD, 99.74% oil/grease and 99.95% suspended solids. Filter media i.e. sand and gravel of the plant must be cleaned/washed before using, requiring back washing after a week or ten days and complete cleaning after 2-2½ months. The accumulated oil/grease may be removed manually on daily/weekly basis as deemed necessary. The accumulated sludge may also be removed after 2-2½ months or at the time of cleaning of plant. These items should then be disposed off properly. The treated wastewater (effluent) may be used for other purposes like washing floors, irrigation, gardening and sprinkling except drinking. Employing the developed technology, huge volumes of potable water could be saved for human consumption.

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Short Communication

Influence of Expeller Design Parameters on Free Fatty Acid Content and Colour of Palm Kernel (*Elaeis guineensis*) Oil

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Abstract. In the study of the influence of compressive stress (10, 20, 30 Mpa), feeding rate (50, 100, 150 kg/h) and rotational speed (50, 80, 110 rpm), of the expeller on the quality of expressed palm kernel oil, compressive stress and feeding rate were found to significantly affect palm kernel oil colour at $P < 0.05$. The lowest value of FFA content (1.09%) was produced at 10 Mpa compressive stress; 50 kg/h feeding rate and 110 rpm revolving worm speed. The highest colour intensity recorded was 87%. Optimum processing condition was achieved at compressive stress of 18.3 Mpa, 61.1 kg/h feeding rate and 76.7 rpm revolving worm speed.

Keywords: palm kernel oil, oil expeller design, free fatty acid, colour

Palm kernel oil is used for edible and non-edible purposes. The edible palm kernel oil must satisfy certain quality parameters including non-rancid flavour, low peroxide value, no contaminant, low free fatty acid (FFA) and attractive colour (NIS, 2000). FFA is formed as a result of lipid hydrolysis and has been identified as a major quality parameter since it influences other quality characteristics (Weiss, 2000). FFA results from splitting of the glyceride molecule at the ester linkage with the formation of fatty acids that contributes objectionable odour, flavour and other characteristics. It is an important quality indicator during each stage of fat and oil processing. FFA content, colour and appearance of the crude oil affect the cost of processing, and the quality of the finished product.

Moisture content, roasting duration and roasting temperature significantly influence yield and quality of palm kernel oil (Akinoso and Igbeka, 2007). These parameters are independent of oil expeller. Compressive stress, feeding rate and revolving worm speed are essential in design and production of expeller. These have been reported to be significant on palm kernel oil yield (Akinoso *et al.,* 2009). The objective of this study is to investigate the influence of compressive stress (10, 20, 30 Mpa), feeding rate (50, 100, 150 kg/h) and revolving worm speed (50, 80, 110 rpm) of oil expeller on free fatty acid and colour of palm kernel oil.

For the study, a 3 x 3 factorial experimental design was used. A *tenera* variety of palm kernel was procured from Nigeria Institute for Oil Palm Research (NIFOR), Benin City, Nigeria. Using ASABE (2008) standard for oil seed, moisture content of the palm kernel was determined to be 5.5% (wb). The oil seed was cleaned manually to remove contaminants such as shell, pebbles and broken kernel. Samples of 5 kg weight each were used for the experiment.

The expeller used was Tite 002 manufactured by Tiny Tech Plant, India, of a rated capacity of 180 kg/h, powered by a 30 kW electric motor with interchangeable speed. Rate of feeding used was 50, 100, and 150 kg/h and compressive stress was established at 10, 20, 30 Mpa. The transducer sensor was placed within the lining bars of the barrel, which relay corresponding internal pressure. Variation of the expeller worm shaft speed of 50, 80 and 110 rpm was achieved by changing the gear switch.

The expressed oil was clarified and analysed for free fatty acid (FFA) and colour change using standard methods (Ca 5a-40) for the FFA content and (Cc 13c-50) for the colour of oils (AOCS, 1994).

For FFA determination, 5 g of oil sample was mixed with 50 mL of hot neutral alcohol and phenolphthalein and titrated with 0.5 N NaOH. Reaction followed as under:

$$C_{17}H_{33}COOH + NaOH = C_{17}H_{35} + COONa + H_2O.... (1)$$

The results were analysed statistically by regression and

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ANOVA using SPSS 13.0 package to obtain the relationship between the independent and dependent variables. Mathematical models were developed. The optimum process parameters for oil expression were determined using simplex method of linear programming as reported by Belegundu and Chandrupatla (2003). A computer programme was employed to optimise interaction between the developed model equations. In optimising free fatty acid was minimised while colour was kept at acceptable level. To validate the optimal parameters, palm kernels were expressed at these conditions. The results were compared with predicted values.

The highest value of FFA (2.7 %) was recorded at 10 MPa compressive stress, 100 kg/h feeding rate and 50 rpm rotational speed while the lowest value (1.09 %) was obtained at 10 MPa compressive stress, 50 kg/h feeding and 110 rpm speed (Table 1). According to Nigeria Industrial Standard, the maximum permissible FFA content in virgin palm kernel oil is 2.0% (NIS, 2000). Out of the 27 treatments, 55.6 % satisfied the recommended quality standard.

The result of regression analysis and ANOVA revealed that the FFA content of the expressed oil didn't correlate well with the predictors: (constant), compressive stress (P, MPa), feeding rate (FR, kg/h), speed (S, rpm), although a relationship exists. This was evident in the very low value of R^2 (0.126). It was discovered that none of the predictors have significant effect on the FFA content at $P < 0.05$. This was also evident in the regression functional relationship with very low coefficients for all the predictors or the independent variables, which is given as equation 2.

$$FFA = 2.5 + 0.37 \times 10^{3} P + 1.17 \times 10^{3} FR - 1.82 \times 10^{2} S \ldots (2)$$

Where FFA is the free fatty acid, P is the compressive stress, FR is the feeding rate and S is the rotational speed. Non-significant effects of the predictors on FFA contents of expressed palm kernel oil were equally recorded from the analysis of the independent effect of the three variables. Correlation coefficients (R) of 0.04, -0.06 and -0.34 were obtained for compressive stress, feeding rate and rotational speed respectively. Values of coefficient of determination R^2 were very low, although relationships existed (equations 3-5).

$$FFA = 1.878 + 3.5\times10^{-3} P \cdots\cdots\cdots (3),\ R^2 = 0.002$$

$$FFA = 1.992 - 6.6\times10^{-4} FR \cdots\cdots (4),\ R^2 = 0.004$$

$$FFA = 2.68 - 9.2\times10^{-3} S \cdots\cdots\cdots (5),\ R^2 = 0.119$$

Contrary to high significant effects of pre-processing parameters (moisture content, roasting duration, roasting temperature and particles size) on palm kernel oil FFA content reported by Akinoso and Igbeka (2007), the studied design parameters (compressive stress, feeding rate and speed) influence was non significant at $P < 0.05$. However, Sivakumaran and Goodrum (1987) reported significant influence on internal pressure and feeding rate on oil expression efficiency using small screw expeller. Therefore, effects of the studied parameters on functionality of an expeller cannot be neglected. O'Brien (2008) attributed abnormal high FFA levels in crude vegetable oils to poor seed handling, field damage of seeds and improper storage. Seed enzyme lipase is activated by moisture and hydrolysis is initiated, which increases the FFA content. Palm kernel used for the experiment was properly cleaned, sorted and used within 24 h of harvest, this may account for the observed behaviour. Also, initial moisture content of the kernel was moderate.

The colour ratings of the expressed palm kernel oil are shown in Table 1. The highest colour intensity of 87 % was achieved at 30 MPa compressive stress, 150 kg/h feeding rate and 110 rpm speed while 20 MPa compressive stress, 50 kg/h feeding rate and 50 rpm speed produced the least colour intensity of50%. Recommended range as industrial standard for palm kernel oil colour is 45 – 80% (NIS, 1992). Out of the 27 treatments, 88.9 % fall within the range. Vegetable oil colour was due to the presence of carotene and chlorophyll pigments. It was known that crude oils could have unexpectedly high pigmentation caused by field damage, improper storage or faulty handling during crushing. Since the samples were subjected to same pre-treatment, none of the aforementioned factors might have influenced the observed colour variation. As reflected in the correlations analysis of the obtained data on colour and ANOVA, compressive stress (P, MPa), feeding rate (FR, kg/h), correlated positively to the colour while rotational speed (S, rpm) correlated negatively. From the analysis, it was discovered that only compressive stress and feeding rate are significant at $P<0.05$. This is also evident in the regression functional relationship given as equation 6. Coefficient of determination R_2 of the equation is 0.53, an indication that the model fits with the data.

$$Colour\ (CO) = 56.89 + 0.16\ P + 0.14FR - 0.03S \ldots (6)$$

Colour fixation in vegetable oil was reported by Akinoso *et al.* (2006) to be due to oxidation aided by high temperature. Thus, recorded significant effects of compressive stress and feeding rate on palm kernel colour may be traced to fluctuation in temperature of expeller barrel and worm which was noticed with the variation of these parameters. Equations 7-9 show

Table 1. Palm kernel FFA and colour as influenced by the technological parameters

Treatment	Compressive stress (Mpa)	Feeding rate (kg/h)	Speed (rpm)	*Free fatty acid (%)	*Colour (%)
1	10	50	50	2.13 ± 0.91	71 ± 16.3
2	10	100	50	2.70 ± 1.11	75 ± 14.7
3	10	150	50	2.01 ± 0.36	83 ± 18. 7
4	20	50	50	1.77 ± 0.98	50 ± 11.9
5	20	100	50	2.13 ± 0.85	76 ± 9.07
6	20	150	50	1.42 ± 0.11	80 ± 22.0
7	30	50	50	1.77 ± 0.09	60 ± 13.6
8	30	100	50	2.13 ± 1.64	76 ± 26.0
9	30	150	50	2.50 ± 0.17	84 ± 13.7
10	10	50	80	2.13 ± 0.84	65 ± 20.0
11	10	100	80	1.42 ± 1.02	75 ± 13.0
12	10	150	80	2.13 ± 1.47	75 ± 13.9
13	20	50	80	1.71 ± 1.17	75 ± 14.4
14	20	100	80	2.30 ± 0.81	64 ± 11.2
15	20	150	80	1.73 ± 0.77	79 ± 29.0
16	30	50	80	1.77 ± 1.13	67 ± 11.3
17	30	100	80	1.73 ± 0.75	67 ± 14.6
18	30	150	80	2.10 ± 1.93	79 ± 27.0
19	10	50	110	1.09 ± 0.07	59 ± 22.0
20	10	100	110	1.70 ± 1.14	68 ± 15.1
21	10	150	110	2.15 ± 2.01	67 ± 9.8
22	20	50	110	1.42 ± 1.13	61 ± 12.4
23	20	100	110	1.42 ± 1.01	72 ± 27.0
24	20	150	110	1.77 ± 0.99	77 ± 23.1
25	30	50	110	2.48 ± 1.21	73 ± 21.0
26	30	100	110	1.77 ± 0.68	73 ± 16.8
27	30	150	110	1.42 ± 1.07	87 ± 26.7

* = mean of three replicates.

independent effect of the three variables on palm kernel oil colour as obtained from the regression analysis. As revealed, only feeding rate is significant at $P < 0.05$ with correlation coefficient (R) of 0.71. Compressive stress and speed coefficients (R) are 0.15 and 0.1, respectively, which show that variation in compressive stress without accompany variation in feeding rate and speed will not significantly change the colour of palm kernel oil expressed using the expeller while the influence of feeding rate is independent of other factors. Similarly, Ajibola *et al.* (1993) also reported non-influence of pressure on colour of sesame seed oil expressed using hydraulic press.

$$CO = 68.67 + 0.16\ P \quad \text{.............} \quad (7),\ R^2 = 0.02$$

$$CO = 57.33 + 0.14\ FR \quad \text{..............} \quad (8),\ R^2 = 0.5$$

$$CO = 74.44 - 3.33\times10^{-2}\ S \quad \text{...........} \quad (9),\ R^2 = 0.01$$

Optimum processing condition was achieved at compressive stress of 18.3 Mpa, 61.1 kg/h feeding rate and 76.7 rpm revolving worm speed. This combination produced 1.24% FFA content and 76.9% colour rating which are within acceptable standard limits. The percentage errors recorded are 2.7 and 4.1% for FFA content and colour, respectively. At 5 % level of significance, all the optima parameters as predicted are permissible and thus appropriate to be applied.

Relationships existed between the compressive stress, feeding rate and rotation speed of the vegetable oil expeller FFA content and colour of the expressed palm kernel oil. Only colour was significantly influenced by compressive stress and feeding rate at $P < 0.05$. Quality of virgin palm kernel oil *viz:* FFA content and colour dependent on the studied parameters is marginal. The developed obtain model can be used to predict influence of studied technological parameters on FFA content and colour of expressed palm kernel oil.

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