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Effect of Thermal Shocking and Quenching on the Degradation Behaviour of a Thin PZT Disc

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Abstract. Thin lead zirconate titanate discs were subjected to thirty five thermal shocks from two different temperatures in deionized water and their relative dielectric constant, coupling factor and impedance values were measured with a view to investigating the behaviour of thin piezoelectric (PZT) discs at frequency of maximum and minimum impedance. Noticeable differences were observed in the electrical properties of the material, probably due to the change in dipole lengths and their orientations during thermal shocking. The results can be useful in modeling and designing of smart components for predicting their behaviour during such expected shocking conditions prior to fabrication.

Keywords: piezoelectric material, thermal shock, deionized water, dielectric constant, impedance, PZT

Introduction

Piezoelectric materials are used in various electromechanical applications where they are influenced by various cyclic loadings. Thermal cycling or thermal fatigue in most electronics materials may cause degradation in their internal characteristics. Thermal fatigue test methods include quench method and repeated heating method for thermal shocks which have been earlier discussed (Lamon and Pherson, 1991; Lamon, 1981). Influence of temperature on the electromechanical and fatigue behaviour of piezoelectric ceramics has been studied by Wang *et al*. (1998). Temperature gradient is developed due to sudden change in temperature in the ceramic materials and therefore, thermal stress is generated. Effect of thermal shocks has been studied by developing newly designed equipment. There are various popular thermal shock methods available in ascending and descending orders. Some of them popular for ascending thermal shocks, include hot jet gas method, high power radiation, melt immersion test, ribbon test method and high power laser heating method. Similarly, various test methods for descending thermal shocks are quenching in water, fluidized bed or a cold air jet impinging on hot discs; quenching in contact with huge brass rods and indentation method have been mentioned by Panda *et al*. (2002). Earlier, thermal shocks in a plate of finite thickness had been attempted. Thermal shock and thermal fatigue of ferroelectric thin films were investigated by Zheng *et al*. (2005). In all of the above methods, the water quenching method is mostly used for thermal shock tests in which samples are heated to a particular temperature and then quenched in water bath. Fatigue studies show that material degradation of PZT ceramics are strongly influenced by temperature. Lead zirconate titanate ceramics show decrease in the dielectric constant and the resonance frequency when subjected to thermal shocks. Importance of temperature stability for dielectric constants and resonance frequencies have been discussed by Lee and Kim (2005). Earlier thermal shock resistance of the materials was evaluated by water quenching. Degradation of various properties of the piezoelectric devices in the presence of water and AC voltage was investigated by Xiang *et al*. (2007). They concluded that water is an important cause of degradation of piezoelectric (PZT) ceramics. However, limited work has been published on the effect of thermal shocking, quenching and on the degradation behaviour of thin piezoelectric ceramic discs. In this study, the degradation phenomenon of thin PZT ceramic disc have been investigated when exposed to repeated heating and quenching cycles below its curie temperature.

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Materials and Methods

Lead zirconate titanate piezoelectric discs, nickel electroded on major faces, 0.191 mm thick and 12.7 mm in diameter, were used for the experimentation. The thin piezoelectric ceramic discs were heated at the heating rate of 9 °C/sec up to 100 °C, and 150 °C, using a thermal chamber and then quenched in deionized water at a temperature of about 20 °C. For all thermal cycling and quenching experiments, 2 PZT test samples were used and subjected to identical conditions. The temperature of the PZT samples was recorded using a spring loaded thermocouple and data acquisition system attached directly to the samples. In order to observe degradation phenomenon of the PZT ceramic, the capacitance, dissipation factor and impedance were measured at a frequency of 1 kHz at the start and after every five heating and quenching

cycles. Data was collected for a total of thirty-five thermal shocks and their relative frequencies of maximum and minimum impedance were observed between 100 kHz and 200 kHz. The capacitance and impedance at these frequencies were recorded using impedance analyzer and dielectric test fixture (model 1645 B). The fixture was attached to an LCR meter and impedance analyzer 4294 A which uses a 4 pair terminal measurement configuration. The values of capacitance measured with impedance analyzer were used to calculate dielectric constant (K_3^T and effective coupling factor (K_{eff}) by using the following equations from IEEE Standard 177 (IEEE Standard, 1976) and Moulson and Herbert (2005):

$$K_3^T = \frac{t_a \times C_p}{A \times \epsilon_0}$$

Effective and transverse coupling factors (K_{eff}) were determined by using the following relationships:

$$K_{eff} = \text{SQRT}\,(f_n^{\,2} - f_m^{\,2}) / f_n^{\,2}$$

$$K_{31} = \text{SQRT}\,(\Psi/(1+\Psi)),$$

where $\Psi = \pi/2\,(f_n/f_m) \times \tan |\, \pi/2 \times (f_n - f_m) / f_m \,|$

Abbreviations used are as follows:

f_m = frequency of maximum impedance [Hz]
f_n = frequency of minimum impedance [Hz]
C_p = equivalent parallel capacitance [F]
t_a = average thickness of testing material [m]
A = area of guarded electrode [m^2]
K_3^T = dielectric constant
K_{eff} = effective coupling factor
K_{31} = coupling factor with transverse excitation
ϵ_0 = permittivity at free space (8.854×10^{-12})
Ψ = phase angle

Results and Discussion

The changes in dielectric constant and coupling factor were measured as a function of frequency of maximum and minimum impedance (f_m and f_n, respectively). Increase in the value of the capacitance of the as-received PZT ceramic was observed to be 5.8×10^4 pF which gradually decreased with increasing thermal cycling (100 °C - 20 °C) to 1.72×10^4 pF. A corresponding change in the f_m was observed with a value of 160 kHz for the PZT sample at the start and then the value decreased to 116.5 kHz. This represented a 28% decrease in the f_m after the ceramic was thermal cycled. A similar change was observed for the f_n which decreased from 165.5 kHz for the as-received to 153.3 kHz after 35 thermal cycles. For the thermal cycling (150 °C - 20 °C) change in f_m was observed from 160.2 kHz to 141 kHz and from 165.175 kHz to 157.5 kHz in f_n. Change in dielectric constant and coupling factor for thirty five shocks in deionized water has been tabulated in Table 1.

Figure 1 indicates the value of capacitance directly measured by impedance analyzer for the unshocked discs at frequency of maximum impedance. Discs were shocked in deionized water from 100 °C to 20 °C for thirty five shocks when their capacitance value decreased from 58.041 nF to 17.237 nF (Fig. 1 and 2). Interestingly, PZT discs shocked from 150 °C to 20 °C showed a less decrease in capacitance value after having thirty five shocks. In this case capacitance value at frequency of maximum impedance decreased to 24.189 nF (Fig. 3).

A comparison of the graphical output for dielectric constant for the PZT samples before thermal cycling and then after exposing the ceramic to thirty five heating and quenching shocks is shown in Fig. 4. Dielectric constant remains independent when measured at 1kHz. The dielectric constant is an

Table 1. Change in dielectric constant and coupling factor for two different thermal shocking conditions

	Shocking from 100 °C to 20 °C				Shocking from 150 °C to 20 °C			
Shocks #	K_3^T at 1kHz	K_3^T at f_m	K_{eff}	K_{31}	K_3^T at 1kHz	K_3^T at f_m	K_{eff}	K_{31}
0	1853	9888	0.255	0.279	1869	10462	0.23	0.26
5	1891	9481	0.313	0.34	1915	8532	0.27	0.29
10	1911	7726	0.324	0.352	1923	7005	0.3	0.32
15	1930	6392	0.306	0.333	1932	4366	0.32	0.35
20	1918	5291	0.364	0.393	1954	3926	0.34	0.37
25	1922	4790	0.427	0.457	1976	3504	0.36	0.38
30	1928	3450	0.621	0.644	1976	3912	0.41	0.41
35	1928	2935	0.651	0.671	1976	4121	0.44	0.47

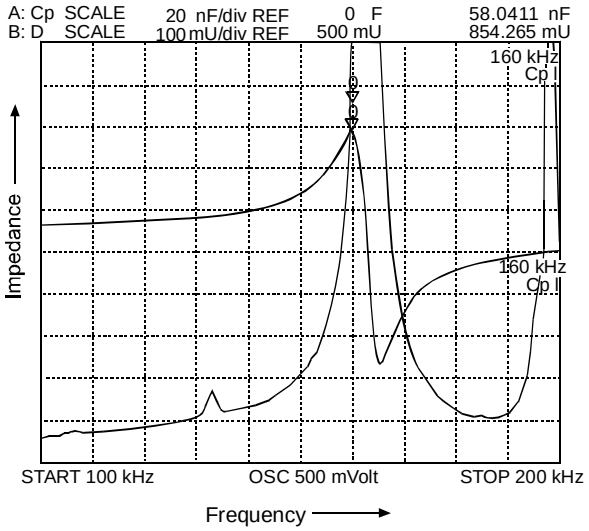

Fig. 1. Value of capacitance for un-shocked disc w.r.t. frequency (100 kHz-200 kHz); capacitance in nF at frequency of maximum impedance.

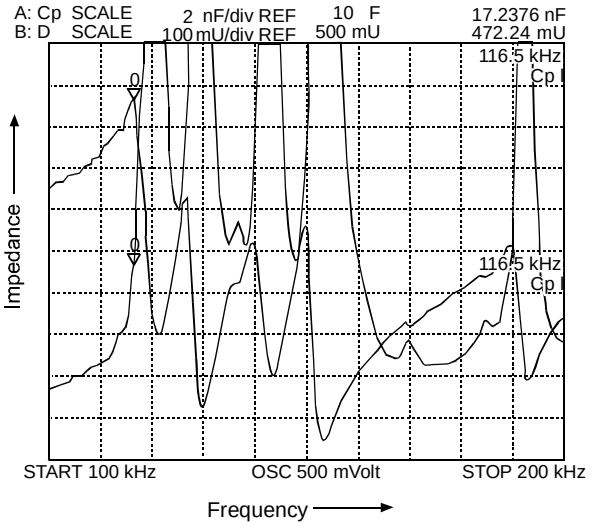

Fig. 2. Value of capacitance after thirty five shocks w.r.t. frequency (100 kHz-200 kHz); capacitance in nF at frequency of maximum impedance when shocked from 100 °C - 20 °C.

intrinsic property of the ceramic material and the results show that this value decreased with increasing thermal cycles at f_m and *vice versa*. The relative difference in the frequencies of the maximum and minimum impedance values depends on the material coupling factor and the resonator geometry (i.e., dimensions of the ceramic PZT sample). For

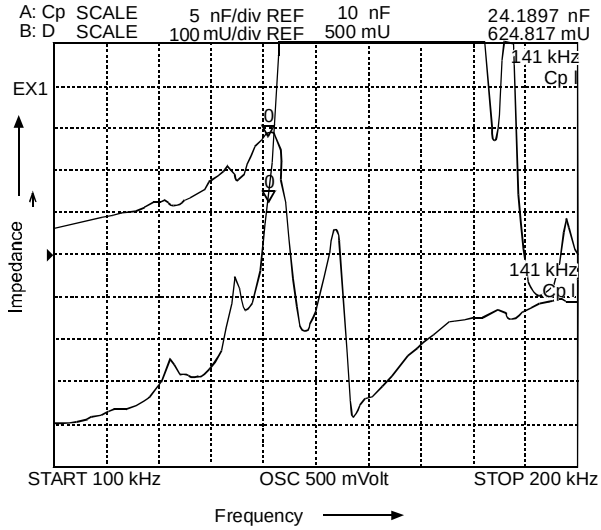

Fig. 3. Value of capacitance after thirty five shocked w.r.t. frequency (100 kHz-200 kHz); capacitance in nF at frequency of maximum impedance when shocked from 150 °C - 20 °C.

this reason, quantities known as the effective coupling factor (K_{eff}) and the transverse excitation factor (K_{31}) were calculated and compared as a function of the number of thermal shocks (Fig. 5). It was found that both the values of K_{31} and K_{eff} increased with increasing thermal cycles to which the PZT ceramic was exposed.

The change in modulus of impedance for two different shocking conditions were evaluated (Fig. 6). It can be seen that the modulus of impedance for both f_m and f_n, when shocked from 100 °C to 20 °C, increased whereas for the other conditions, it started decreasing after twenty five shocks. Another interesting result is that when impedance at f_m and f_n increased, the difference became larger at later shocks. Decrease in dielectric constant in thermal shocking is the expected normal behaviour. Various coupling factors were close to each other but a noticeable change was observed due to shocking and quenching effect. All changes may have occurred due to change in dipole moments and their expected random orientations. This reorientation may change the length of dipoles, due to which specimen undergoes a change in its piezoelectric properties.

The change in f_m and f_n causes the change of mechanical quality factor. This response of the material can be utilized in designing of oscillators. It is observed that the difference in these two stated frequencies (f_m and f_n) is small as compared to their impedance peaks during thermal shocking. The

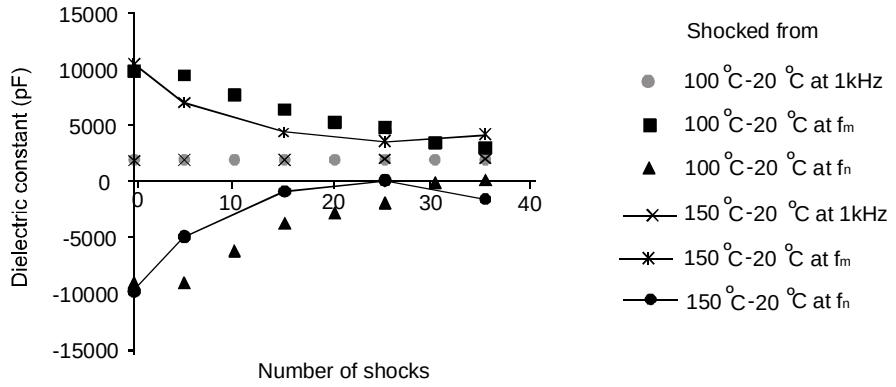

Fig. 4. Change in dielectric constant against number of shocks, at frequency 1kHz and frequencies of maximum and minimimum impedance.

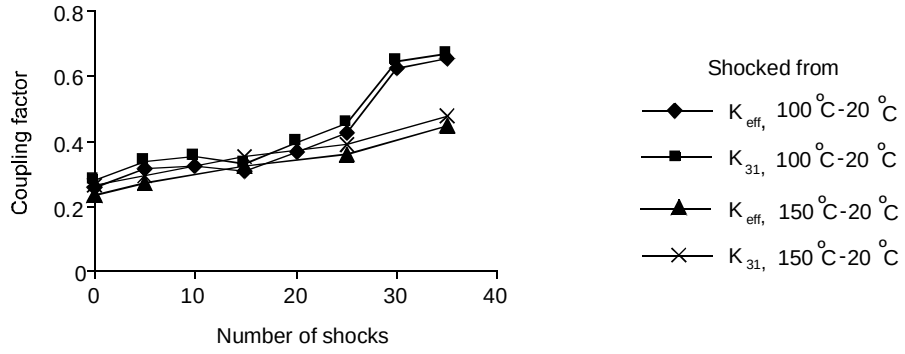

Fig. 5. Change in coupling factor (K_{31}, K_{eff}) against number of shocks from 100 °C - 20 °C and from 150 °C - 20 °C in deionized water.

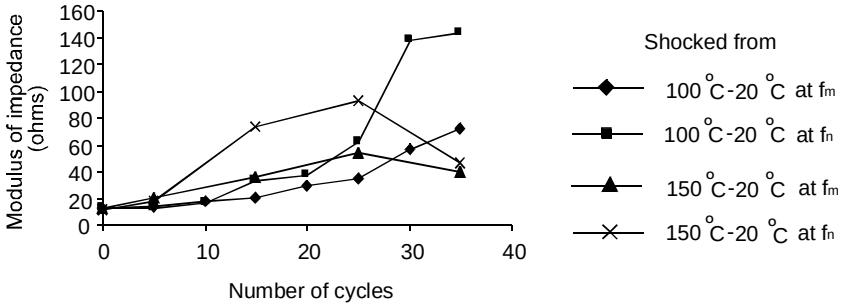

Fig. 6. Change in modulus of impedance (|Z|) against number of shocks from 100 °C - 20 °C and from 150 °C - 20 °C in deionized water.

results suggest that the PZT ceramics suffer a noticeable change in polarization when exposed to repeated heating and quenching cycles, well below the curie temperature (350 °C) for the PZT ceramic. It is thought that significant depolarization of the PZT ceramic occurs due to the disorientation of the ferroelectric domains and this reorientation is affecting the critical piezoelectric properties by thermal shocking and quenching. The behaviour is normal but the number of peaks increases due to expected change in length and reorientation of dipoles. Development of dipole moments

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Comparison of Ion Chromatography with Ion Selective Electrodes for the Determination of Inorganic Anions in Drinking Water Samples

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Abstract. Fluoride, chloride and nitrate anions were determined in drinking water samples using techniques of ion selective electrodes (ISE) and non-suppressed/suppressed ion chromatography (IC). Detection limit, percentage recovery and run time were evaluated for the two methods. Detection limits for ISE [0.02, 0.20 and 1.7 ppm (μg/mL) for fluoride, chloride and nitrate, respectively], were better than those for non suppressed IC (2.0, 1.0 and 2.0 ppm for fluoride, chloride and nitrate, respectively). Suppressed IC was used to measure fluoride. Statistical analysis of the data revealed no evidence of systematic difference between ISE and non suppressed IC for chloride and nitrate. Fluoride concentrations in all water samples were lower, while chloride and nitrate concentrations in some samples were higher than the maximum contaminant levels established by the United States Environmental Protection Agency.

Keywords: drinking water, nitrate, chloride, fluoride, ion selective electrode, ion chromatography

Introduction

Due to increase in population, urbanization and continued industrial growth, per capita water availability in Pakistan has decreased from 5000 m^3/annum in 1951 to 1100 m^3/annum in 2007 (WWF, 2007). The increasing gap between water demand and supply has led to severe water shortage in almost all sectors and has adversely affected the quality of drinking water; consequently, water pollution has become a serious problem in the country and most of the reported health problems are directly or indirectly related to water (PCRWR, 2008).

There are various sources of contaminants in drinking water which, when exceeding certain levels, are harmful to man. These contaminants are microorganisms, inorganic and organic chemicals and certain radioisotopes. Inorganic anions may affect the quality of water. Fluoride, chloride and nitrate have considerable importance in the quality of drinking water. Specially, the excess of nitrate and fluoride in drinking water has intense effects on human health (Meenakshi and Maheshwari, 2006; Fraser and Chilvers, 1981). Excess nitrate in drinking water could cause serious illness in infants below the age of six months. Fluoride might be the reason for different bone diseases and tenderness of bones in children (US EPA, 2009).

Fluoride, chloride and nitrate in groundwater and surface water originate from natural sources, sewage, industrial effluents, different food additives and as a result of leaching or runoff from agricultural land (WHO, 2004).

Various analytical methods have been proposed for the determination of fluoride in aqueous solutions, such as colorimetric, conductometric, complexometric and potentiometric methods (APHA, 1985). Some methods are rapid, sensitive, precise and relatively free of interferences. Traditional methods used for determination of fluoride, chloride and nitrate anions are based on colorimetric method, which due to interference by various ions, require special treatment of the samples like distillation or reduction and special analytical skills. Ion chromatography is becoming more popular for the analysis of water samples and is also recognized by the US Environmental Protection Agency (US EPA, 2009) as a method of choice for the determination of anions in water samples (Bosch *et al*., 1995; Cheam, 1992; Pereira, 1992; Frankenberger *et al*., 1990). Potentiometry has been widely used for quite some time due to its simplicity and prompt results. However, the selectivity is rather limited, especially if chemically similar ions are present in the sample. Recent developments in separation techniques have led to an improvement especially in the determination of fluoride in terms of selectivity and sensitivity (Weiss *et al*., 1995; Vasconcelos *et al*., 1994). Results of determination of bromide, chloride, fluoride, nitrate and sulphate using ion chromatography (IC) had been compared with those obtained by colorimetry for rainfall, cloud water and stream waters. According to that, there was no significant difference in chloride and nitrate measurements between the

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two methods. For fluoride, the IC method gave lower values than the colorimetry, especially for the stream waters. Since, the colorimetric method determines total fluorine, differences in the values might be expected, for example fluoride forms complexes with the available aluminium, especially in the stream water (Neal *et al*., 2007). Statistical analysis of fluoride concentrations in rain water samples as obtained by capillary electrophoresis (CE), IC and ISE indicated that there were no systematic differences between CE and ISE, but the fluoride concentrations obtained by IC were significantly higher. The observed differences are most likely due to presence of aluminium cations (Van den Hoop *et al*., 1996). A fully validated dual ion chromatographic method, complying with ISO 17025, has been developed at the chemical laboratory of the Athens Water Supply and Sewerage Company (EYDAP SA) for the concurrent determination of ten ions (F^-, Cl^-, NO_3^-, Br^-, PO_4^{3-}, SO_4^{2-}, Na^+, K^+, Ca^{2+} and Mg^{2+}) in surface, ground and potable water samples (Miskaki *et al*., 2007).

The aim of the present study was to optimize a simple, selective and efficient method for simultaneous determination of chloride, fluoride and nitrate ions in drinking water samples collected from various sources by using ion chromatography and ion selective electrodes.

Materials and Methods

Reagents. High purity distilled deionized water was used throughout the work. Anion standards solutions were prepared using sodium salt of fluoride (Merck, Germany), chloride (Merck, Germany) and nitrate (BDH Chemical, England). Other chemicals were analytically pure reagents from RDH Chemicals, Germany.

Ion selective electrode. Cole-Parmer ion selective electrode chloride model EW-27502-13 (USA), Cole-Parmer combination ion selective electrode fluoride model EW-27504-14 (USA) and Cole-Parmer combination ion selective electrode nitrate model EW-27504-22 (USA) were used. The response was in mV given by OAKTON pH/mV/Ion Meter (pH 2100 series USA). Glacial acetic acid and sodium chloride were used as low level total ionic strength adjuster buffer (TISAB-2) for low level fluoride measurement by ion selective electrodes. Sodium nitrate and ammonium sulphate were used as ionic strength adjuster for chloride and nitrate, respectively.

By serial dilution, 10 ppm fluoride standard was prepared by diluting 1000 ppm standard solution. 50 mL low level TISAB-2 was added to 50 mL of the above standard solution. In a 150 mL beaker, 50 mL of distilled water and 50 mL low level TISAB-2 were added. The volume of real water samples and TISAB-2 were same as that of the standard. This solution was stirred at constant rate. The electrode tip was dipped in solution while the meter was in mV mode. Increments of 10 ppm standard solution were made after 90 second intervals to get 0.01, 0.02, 0.04, 0.06, 0.10, 0.29, 0.48 and 1.10 ppm concentrations. For nitrate and chloride, 1, 10, 50, 100, 500 ppm standard solutions were used and 2 mL of ISA (ion strength adjuster) was added to 100 mL of standard solution. Same amount of ISA was added to 100 mL of water samples. Rest of the procedure was same. Calibration curve was obtained by plotting a graph between electrode potential and concentrations from which the unknown concentrations of F^-, Cl^- and NO_3^- in water samples were calculated.

Ion chromatography. Ion chromatograph consisted of Kanauer HPLC quaternary pump Model K-1001 (Germany) with maximum operating pressure of 400 bars and flow range of 0.001-9.999 ml/min. HAMILTON PRP-X-100 polymer base reverse phase No. R-79439 (USA) anion exchange column PRP X-100 (150 mm × 4.1 mm) having 10 µm particle size with comparative guard column was used. A comparative guard column was also used. Alltech model 650 conductivity detector (USA) was used as detector. Alltech model 640 suppressor (USA) and Metrosep A supp 3 (Metrohm, Switzerland) anion exchange column (250 mm × 4.6 mm) having particle size 9 µm, packed with polystyrene/divinylbenzene copolymer were used with comparative guard column in suppressed ion chromatography. The volume of sample loop used for injection was 20 µL. 4 mM solution of *p*-hydroxy benzoic acid was used as mobile phase for non suppressed ion chromatography while 1.8 mM Na_2CO_3/ 1.7 mM $NaHCO_3$ solution was used as mobile phase for suppressed ion chromatography.

Optimal mobile phase and its flow rate were used for separation of F^-, Cl^- and NO_3^-, using the standard solutions. Standard solutions of varying concentrations of fluoride, chloride and nitrate were prepared from standard stock solutions. These solutions were injected into ion chromatograph. Peak areas and heights of all these solutions were measured and calibration curves for fluoride, chloride and nitrate were obtained. All water samples were filtered through 0.45 µm pore diameter membrane syringe filters and injected. The concentration of these anions in samples was determined using these calibration curves.

ICP-OES instrument. The ICP-OES instrument used in the present work is ARL 3580 model, made by Applied Research Laboratories, Switzerland. The instrument is equipped with a monochromator, a polychromator and a spark excitation source besides ICP source. Both the monochromator and the

polychromator are 1 meter focal length Paschen-Runge spectrometers having 1080 groves/mm concave grating mounted in Rowland circles. The operating conditions of the ICP-OES used are given in Table 1. Metal ions were investigated in some water samples and their emission wavelengths were as follows: Al (309.271 nm), Ca (393.366 nm), Fe (261.187 nm), K (766.490 nm), Mg (279.553 nm), Mn (257.610 nm), Na (588.995 nm), Ni (221.647 nm), Pb (220.353 nm), Si (251.611 nm), Sr (407.771 nm) and Zn (213.856 nm).

Table 1. Operating conditions of ICP-OES

Generator frequency	27MHz
Incident power	1.25 kW
Out gas flow	12 L/min.
Intermediate gas flow	0.8 L/min
Carrier gas flow	1 L/min
Observation height	16 mm above the coil
Sample uptake	1-3 mL/min

Samples. Surface water and groundwater are the main water sources available to the residents of Islamabad. Drinking water samples were collected from water sources in the month of October 2008, from different sectors and nearby villages of Islamabad. The water was allowed to flow from the source for about 1-2 min in order to stabilize different parameters i.e., conductivity and pH. The collected samples were stored in pre-cleaned, sterilized polyethylene bottles of one litre capacity. The samples were cooled to 4 °C in clean and dust free environment.

Statistical analysis. A paired t-test was performed to check the validity of two methods (Miller and Miller, 1997). The formula of paired t-test is:

$$t_{cal} = \frac{\overline{x}_d \sqrt{n}}{S_d}$$

where:

S_d = standard deviation
$\overline{x}_d$ = mean of group one minus group two
n = the number of values
If t_{cal} is less than t_{tab} at a specific confidence limit then there is no significant difference between the two methods.

Results and Discussion

Optimization of mobile phase for non-suppressed IC. In order to obtain optimal separation, pH and flow rate of mobile phase was optimized. Standards containing 10 ppm, 20 ppm and 40 ppm of fluoride, chloride and nitrate, respectively, were injected at 1.0 mL/min flow rate at various pH of mobile phase. The retention was decreased by increasing the pH of the mobile phase. Variation of pH of the mobile phase led to shift in the dissociation equilibrium and thus to change the retention time. The peak height and peak area decreased, by increasing the pH of mobile phase. This might be due to the decrease in retention time. There was a shift in the base line showing incomplete separation of anions. So optimum pH was determined which was based on good resolution. The clearer picture is given in Fig. 1. Consequently optimal pH necessary for complete and in-time separation was 8.5.

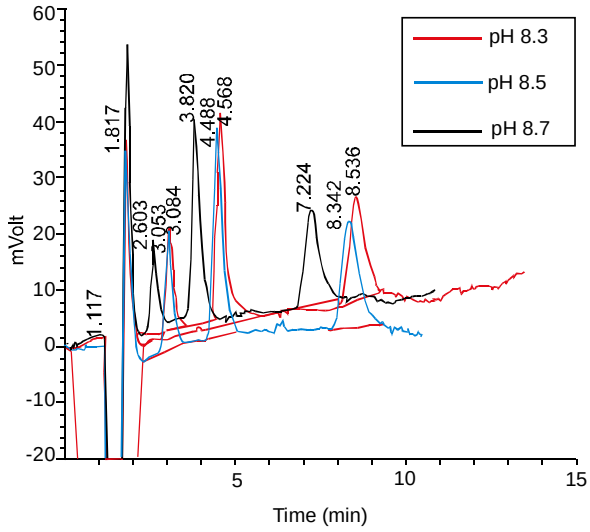

Fig. 1. Chromatogram of F^- 10 ppm, Cl^- 20 ppm and NO_3^- 40 ppm at different pH at flow rate of 1.0 mL/min by non-suppressed IC.

To see the effect of flow rate on retention time, a single standard containing 10 ppm, 20 ppm and 40 ppm of fluoride, chloride and nitrate, respectively, was injected by using mobile phase of optimal pH 8.5. The effect of flow rate was studied in the range of 0.8-1.2 mL/min. The results are shown in Fig. 2. By increasing the flow rate, the retention time decreased. The peak height and area also decreased with retention time. This was due to faster separation of anions resulting in incomplete separation of ions. The optimum flow rate was 1 mL/min.

Performance characteristics. Performance characteristics in terms of detection limit, percent recovery and total run time of the analytical response were calculated from reproducibility experiments which are shown in Table 2. The detection limits for ISE were estimated based on three times standard deviation of response plus mean response from determination of

Table 2. Performance characteristics of the applied techniques

Anions	Ion selective electrode			Non-suppressed IC			Suppressed IC		
	Detection limit (ppm)	Run time (min)	Recovery (%)	Detection limit (ppm)	Run time (min)	Recovery (%)	Detection limit (ppm)	Run time (min)	Recovery (%)
Fluoride	0.02	3	98.6	2	20	102.7	0.05	25	99.3
Chloride	0.2	3	101.5	1	20	103.2	0.05	25	102.6
Nitrate	1.7	3	109.4	2	20	98.5	0.1	25	106.5

six blank samples. The detection limit is thus the corresponding concentration of the response from calibration curve of each anion (Skoog *et al.*, 2005). The detection limit for IC is three times signal-to-noise ratio. Hence the detection limits were found by using the standard whose response was three times signal to noise. In order to evaluate the accuracy of method, percentage recovery was calculated by adding known amount of fluoride, chloride and nitrate to drinking water samples according to the following equation:

$$\text{Recovery (\%)} = \left(\frac{\text{spiked conc.} - \text{actual conc.}}{\text{conc. of standard added}}\right) \times 100$$

The total run time includes sample introduction, purging/washing time and run time, whereas, the time needed for pretreatment of the sample and to calculate the corresponding concentration were not taken into account.

Analysis of water samples. ***Measurement of pH.*** pH of all the collected samples was measured which was in the range of 6.85-8.65 (Table 3). pH of most samples was in good agreement with US EPA which is 6.5-8.5 except that of sample no.11 which was slightly higher. This sample was from Malal stream in periphery of the village of Islamabad. People living nearby this stream wash their clothes in the stream so pH may be higher due to mixing of soapy water.

Determination of anions. Determination of concentrations of fluoride, chloride and nitrate was carried out using ISE and suppressed/non-suppressed ion chromatography. The results are given in Table 3.

For the analysis of water samples, optimized non-suppressed IC conditions were used. The chromatograms obtained by injecting the samples were compared to standard chromatogram; peaks of these chromatograms were quite sharp and resolution was also very good. Some of the chromatograms of a standard and a sample are shown in Fig. 3 and 4, respectively.

Samples were analyzed by non suppressed/suppressed IC as described above. Results obtained for chloride and nitrate concentration by non suppressed IC are shown in Table 3.

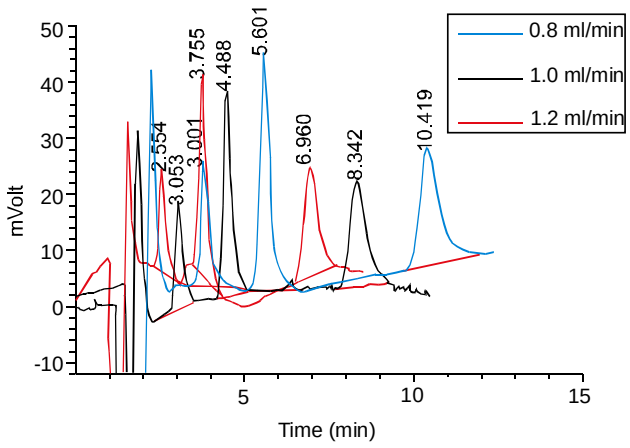

Fig. 2. Chromatogram of F^- 10 ppm, Cl^- 20 ppm and NO_3^- 40 ppm at different flow rates at pH 8.5 by non-suppressed IC.

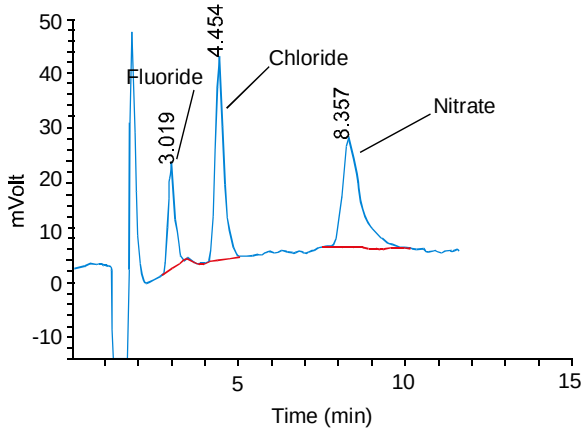

Fig. 3. Chromatogram of F^- 10 ppm, Cl^- 20 ppm and NO_3^- 40 ppm by non-suppressed IC at flow rate of 1.0 mL/min.

Table 3. Results obtained by ion selective electrodes/pH electrode and non-suppressed ion chromatography (suppressed ion chromatography for fluoride only)

Sample	pH	Ion selective electrodes/pH electrode			Non-suppressed ion chromatography			Suppressed ion chromatography
		Fluoride (ppm)	Chloride (ppm)	Nitrate (ppm)	Fluoride (ppm)	Nitrate (ppm)	Chloride (ppm)	Fluoride (ppm)
1	7.45	0.34	92.11	90.72	nd	80.61	90.10	0.31
2	7.45	0.77	114.10	287.69	nd	222.70	118.25	0.73
3	7.30	0.15	53.93	42.31	nd	38.20	50.23	0.15
4	7.20	0.43	67.10	42.73	nd	39.10	65.53	0.41
5	7.30	0.64	128.09	226.11	nd	205.23	122.50	0.60
6	6.85	0.33	1062.07	1321.69	nd	1080.60	855.50	0.41
7	7.50	0.13	39.79	19.05	nd	15.50	37.90	0.10
8	7.35	0.79	96.14	70.59	nd	63.54	95.69	0.81
9	7.30	0.96	67.96	39.24	nd	35.42	65.21	0.92
10	7.35	0.59	30.13	38.27	nd	23.45	20.28	0.62
11	8.65	0.38	29.11	6.01	nd	5.24	22.50	0.36
12	7.05	0.23	13.02	16.39	nd	11.34	9.52	0.22
13	7.85	0.19	13.53	15.90	nd	10.59	10.15	0.20
14	8.00	0.23	12.79	19.15	nd	14.69	9.24	0.24
15	7.80	0.89	19.97	22.48	nd	16.22	19.12	0.90
16	7.25	1.08	55.81	31.94	nd	26.45	18.20	1.12
17	7.30	0.25	10.92	23.75	nd	18.65	9.10	0.24
18	7.55	0.21	8.79	28.03	nd	23.57	6.24	0.26
19	8.10	0.20	9.63	16.88	nd	13.82	8.13	0.22
20	7.55	0.07	2.92	6.82	nd	4.56	2.25	0.05
21	8.00	0.08	3.20	6.91	nd	4.89	3.10	0.06
22	7.65	0.08	251.34	379.91	nd	251.32	240.53	0.09
23	7.05	0.25	80.24	2.88	nd	2.13	78.20	0.27
24	7.15	0.08	12.34	23.52	nd	21.52	10.88	0.07

nd = not detected.

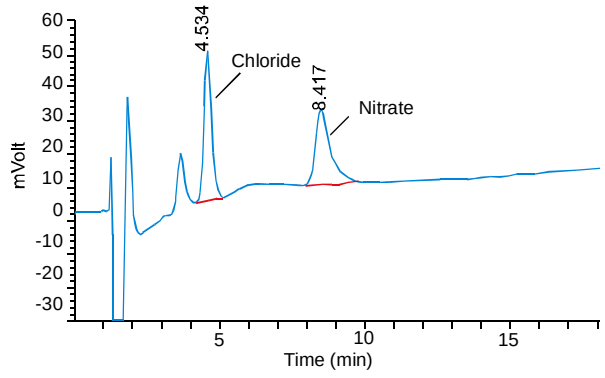

Fig. 4. Chromatogram of sample # 2 by non-suppressed IC.

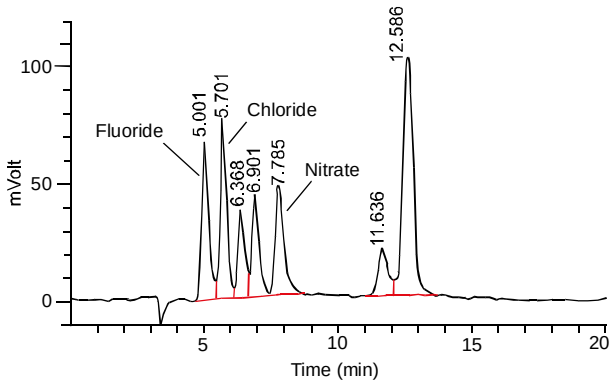

Fig. 5. Chromatogram of F^- 3 ppm, Cl^- 3 ppm, NO_2^- 4 ppm, Br^- 4 ppm, NO_3^- 4 ppm, PO_4^{3-} 8 ppm and SO_4^{2-} 8 ppm at flow rate of 1.0 mL/min by suppressed IC.

Fluoride was not measured by non-suppressed ion chromatography due to its low concentration in the samples. So, suppressed IC was used. Seven anion standards were injected at the flow rate of 1 mL/min. The chromatogram is shown in Fig. 5. Quantitative determination of fluoride in the water samples was made by comparison of peak areas in the chromatograms of the samples and that of the standard; chromatogram of a sample is shown in Fig. 6. The results for fluoride analysis in water samples by suppressed IC are given in Table 3.

The concentration of fluoride in all the water samples was within the limits established by USEPA (4.0 ppm). The chloride level was also within the permissible range i.e.,

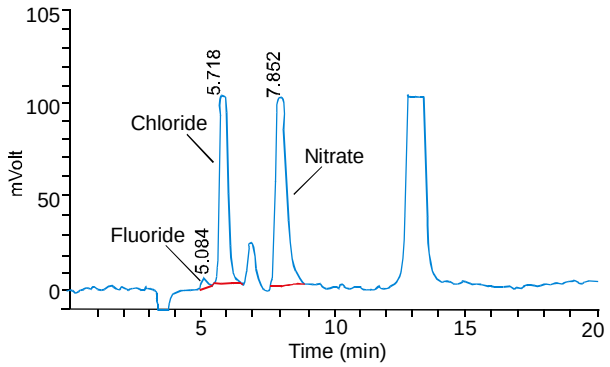

Fig. 6. Chromatogram of sample # 10 by suppressed IC at flow rate of 1.0 mL/min.

Table 4. Concentration of cations by ICP-OES

Cations	Sample 6 (ppm)	Sample 7 (ppm)	Sample 16 (ppm)
Al	nd	nd	nd
Ca	176.39	32.77	30.64
Fe	nd	nd	nd
K	5.50	8.94	1.70
Mg	328.42	17.79	37.41
Mn	nd	nd	nd
Na	481.06	61.51	185.66
Ni	nd	nd	nd
Pb	nd	nd	nd
Si	6.30	5.58	4.86
Sr	7.40	0.22	1.20
Zn	nd	nd	nd

nd = not detected.

250 ppm in all the samples except sample no. 6. In most of the samples, nitrate level was higher than US EPA standard for safe drinking water i.e. 10 ppm. Sample # 6 has the maximum level of chloride and nitrate exceeding 1000 ppm. This is the water obtained from house pump installed by boring in Nilore colony situated in the surrounding area of Islamabad. The underground water in these areas is in the narrow channels rather than in large reservoirs. So the water may be in contact with some rocks containing salts of nitrates and chlorides. Thus, metal ion analysis especially of samples 6, 7 and 17 were performed using Inductive Coupled Plasma Optical Emission Spectroscopy by conditions given in the experimental section. The results are given in the Table 4. It is clear from the results that sample 6 contained a high concentration of sodium, magnesium and calcium ions. So most probably the nitrate and chloride of these cations may exist.

The results obtained by the two methods are compared in Fig. 7-9. The correlation coefficient in each case shows good linearity in the results of the two methods from low concentration to the higher concentration.

A paired t-test was also performed to check the validity of two methods. According to this test, if t_{cal} is less than t_{tab} at a specific confidence limit then there is no significant difference between the two methods. The results are given in Table 5. The results of statistical analysis, according to student's t-test, shows that there is no significant difference between the results obtained with non-suppressed ion chromatography and ion selective electrodes for chloride and nitrate determination in water samples.

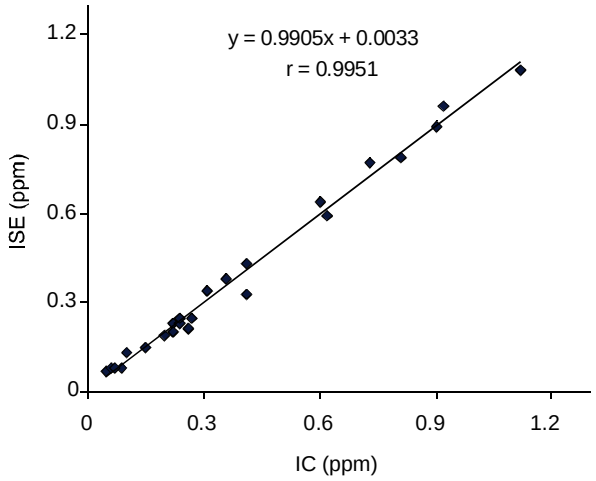

Fig. 7. Comparison of the results for the determination of fluoride in drinking water samples (n = 23) using IC (suppressed) and ISE.

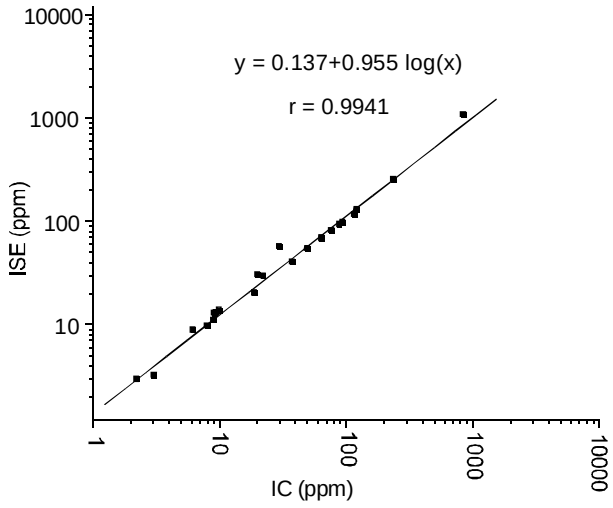

Fig. 8. Comparison of the results for the determination of chloride in drinking water samples (n = 23) using IC (non-suppressed) and ISE.

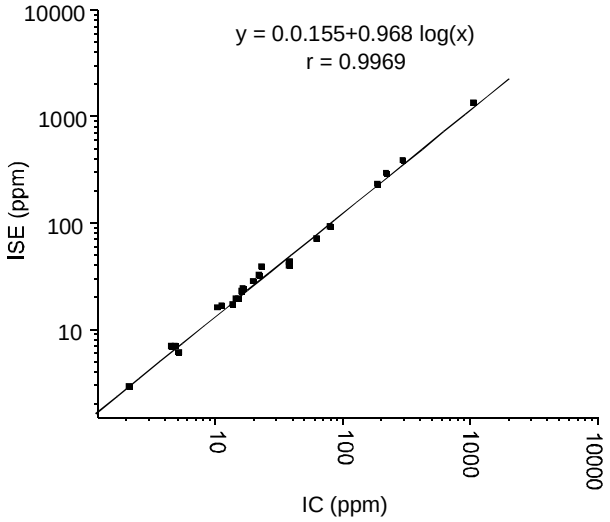

Fig. 9. Comparison of the results for the determination of nitrate in drinking water samples (n = 23) using IC (non-suppressed) and ISE.

Table 5. Statistical analysis of the correlation between chloride and nitrate concentrations by suppressed/non-suppressed IC and ISE

Anions	Intercept	Slope	r^2	t_{cal}	t_{tab}
Fluoride	0.0033 ±0.0005	0.9905 ±0.01201	0.99022	- 0.70200	2.074
Chloride	0.16042 ±0.02570	0.9637 ±0.01636	0.99398	-1.46362	2.074
Nitrate	0.15521 ±0.02604	0.9682 ±0.0166	0.99386	-2.06386	2.074

Confidence limits = ±95; two tail; n=23.

Conclusion

Both ion chromatography and ion selective electrode were employed for the determination of three anions (fluoride, chloride and nitrate) in drinking water samples. ISE is a preferred technique due to shorter analysis time and less operational cost of the equipment. Ion chromatography is sophisticated and reliable for simultaneous determination of anions in routine water analysis. This technique can be used for comparison and validation of methods.

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References

APHA, 1985. *Standard Methods for the Examination of Water and Wastewater*, 16th edition, American Public Health Association, Washington DC., USA.

Bosch, N.B., Mata, M.G., Penuela, M.J., Galan, T.R., Ruiz, B.L. 1995. Determination of nitrite levels in refrigerated and frozen spinach by ion chromatography. *Journal of Chromatography A*, **706:** 221-228.

Cheam, V. 1992. Comparison of ion chromatographic methods for the determination of organic and inorganic acids in precipitation samples. *Analyst*, **117:** 1137-1144.

Frankenberger Jr., W.T., Mehra, H.C., Gjerde, D.T. 1990. Environmental application of ion chromatography. *Journal of Chromatography A*, **504:** 211-245.

Fraser, P., Chilvers, C. 1981. Health aspects of nitrate in drinking water. *Science of The Total Environment*, **18:** 103-116.

Meenakshi, Maheshwari, R.C. 2006. Fluoride in drinking water and its removal. *Journal of Hazardous Materials*, **137:** 456-463.

Miller, J.C., Miller, J.N. 1997. *Statistics for Analytical Chemistry*, 3rd edition, Ellis Horwood Limited, UK.

Miskaki, P., Lytras, E., Kousouris, L., Tzoumerkas, P. 2007. Data quality in water analysis: validation of ion chromatographic method for the determination of routine ions in potable water. *Desalination*, **213:** 182-188.

Neal, M., Neal, C., Wickham, H., Harman, S. 2007. Determination of bromide, chloride, fluoride, nitrate and sulphate by ion chromatography: Comparison of methodologies for rainfall, cloud water and river waters at the Plynlimon catchments of mid-Wales. *Hydrology and Earth System Sciences*, **11:** 294-300.

PCRWR, 2008. Water Quality Report. National Water Quality Monitoring Programme. Pakistan Council of Research in Water Resources, Ministry of Science & Technology, Islamabad, Pakistan.

Pereira, C.F. 1992. Application of ion chromatography to determination of inorganic anions in foodstuffs. *Journal of Chromatography A*, **624:** 451-470.

Skoog, D.A., Holler, F.J., Nieman, T.A. 2005. *Principles of Instrumental Analysis*, 5th edition, Thomson Asia Pvt., Ltd., Singapore.

US EPA, 2009. *Drinking Water Contaminants*, United States Environmental Protection Agency, Office of Ground Water and Drinking Water, Washington DC., USA.

Van den Hoop, M.A.G.T., Cleven, R.F.M.J., Van Staden, J.J.,

Neele, J. 1996. Analysis of flouride in rain water: Comparison of capillary electrophoresis with ion chromatography and ion selective electrode potentiometry. *Journal of Chromatography A*, **739:** 241-248.

Vasconcelos, M.T.S.D., Gomes, C.A.R., Machado, A.A.S.C. 1994. Ion chromatographic determination of fluoride in welding fumes with elimination of high contents of iron by solid-phase extraction. *Journal of Chromatography A*, **685:** 53-60.

Weiss, J., Reinhard, S., Pohl, C., Saini, C., Narayaran, L. 1995. Stationary phase for the determination of fluoride and other inorganic anions. *Journal of Chromatography A*, **706:** 81-92.

WHO, 2004. *Guidelines for Drinking-Water Quality Recommendations*, vol. **1,** 3rd edition, World Health Organization, Geneva, Swizerland.

WWF, 2007. Pakistan Water at Risk. Special Report, WWF-Pakistan, Ferozepur Road, Lahore-54600, Pakistan.

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Physical and Chemical Evaluation of Oils of Two Varieties of *Carthamus tinctorius* Grown in Pakistan

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Abstract. On evaluation of oils of two spineless varieties of *Carthamus tinctorius*, Thori-78 and Pawari-95 growing in Sindh, Pakistan, the quality of the oil was found to be similar, only the oil content differed. The hexane-extracted oil content of Thori-78 and Pawari-95 was 28.33±1.15 and 33.07±1.12, respectively. The oils contained 90.97% and 89.55% unsaturated fatty acids and 8.44% and 9.69%, saturated fatty acids, respectively. Linoleic acid was 75.42±0.59% and 76.40±1.0% and oleic acid was 15.55±0.30% and 13.15±0.49% by weight, respectively, and were the predominant fatty acids present in the oil.

Keywords: safflower oil, Thori-78, Pawari-95, linoleic acid, oleic acid

Introduction

Safflower (*Carthamus tinctorius*) is an annual herb belonging to the family Compositae. It is widely distributed throughout the world such as in Pakistan, India, Bangladesh, Afghanistan, Middle East, Thailand, China, Japan, Ethiopia, Sudan, Tanzania, Keneya, Tunisia, Europe, Argintina, USA, Canada and Australia (Knights *et al.*, 2001). *C. tinctorius* flowers, seeds and oil have wide range of medicinal uses in different countries. Flowers are used for the preparation of dyes and drugs which are used for treating a number of disorders such as for dilation of arteries, reduction of hypertension, increasing blood flow, decreasing blood cholesterol, in treatment of rheumotoid arthritis, menstrual problems, skin diseases, urinary problems and jaundice etc. (Kaffka *et al.*, 2001; Sastri, 1950). Seed decoction is used as laxative in Pakistan. The oil is used in Iran to treat liver and heart ailments and in charred state, used in India in treatment of sores and rheumatism. In Northern America, it is cultivated for using as bird feed, animal meal and for industrial applications (Oyen *et al.*, 2007; Mündel *et al.*, 2004; Oplinger *et al.*, 1992).

Safflower is used as a substitute for saffron; its flowers are commonly mixed with rice, pickles and other foods to give an attractive colour (Sastri, 1950). America, India and Africa are the main producers of safflower oil. Its seeds are edible and are eaten after roasting. The seed oil content varies from 24 to 36%, depending on the variety of safflower, soil texture, climate and other conditions (Pritchard, 1991; Swern, 1964a). There are two types of safflower oil: high oleic (high in mono-unsaturated fatty acids) and high linoleic (high in polyunsaturated fatty acids). Gas chromatography has been an indispensable analytical technique ever since its first use in the fatty acid determination of plant seed oil (Echard *et al.*, 2007; Peris-Vicente *et al.*, 2006; Seppänen-Laakso *et al.*, 2002). High performance liquid chromatography (HPLC) with ultraviolet and fluorescence detectors are the alternative methods for separation of volatile short chain and long chain fatty acids (Peris-Vicente *et al.*, 2005; 2004; Chen and Chuang, 2002).

Safflower oil can be used in cosmetics, foods, nutritional supplements, personal care products, soaps and shampoos. Cold press oil is golden yellow and is used for culinary purposes. The oil obtained by dry hot distillation is dark and sticky and is used only for greasing ropes and leather goods which are exposed to water. Developed countries have created the most significant market for safflower oil for use as salad oil and cooking oil and in making margarine; being non-allergenic, it is considered to be one of the healthiest oils for human consumption because it has a high ratio of polyunsaturated/saturated fatty acids.

Safflower was introduced as oilseed crop in Pakistan in 1960. It is mainly cultivated in Sindh and Baluchistan provinces. Being a drought-tolerant crop, it is recommended for planting in rainfed areas. In Sindh it is cultivated after the rice crop on residual moisture. Due to the increasing interest in the safflower oil for edible purposes based on its high content of linoleic acid, our studies are mainly focused on the content and physical and chemical evaluation of the oils of two spineless varieties of safflower, Thori-78 and Pawari-95, grown in

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Sindh, Pakistan. Proximate analysis of oils were carried out for the content, glycerides composition and physical and chemical parameters such as free fatty acid, acid value, peroxide value, iodine value, refractive index, saponification value, unsaponifiable matter, specific gravity and colour; the fatty acid composition of both the varieties of oils were investigated as methyl esters by gas chromatography.

Materials and Methods

Plant material. Two varieties of *Carthamus tictorius* seeds, Thori-78 and Pawari-95, were collected from Tandojam, Sindh, Pakistan. The fruit is an achene (dry, one seeded with a thin hull) and resembles sunflower seed but is smaller in size and creamish in colour. It is irregularly pear-shaped, smooth and shiny up to 10 mm long.

Reagents. Solvents and chemicals such as *n*-hexane (95%), *n*-heptane (99%), ethanol (95%), carbon tetrachloride (95.5%), chloroform (99.5%), methanol (98.8%), sulphuric acid (95.98%), hydrochloric acid (37%), acetic acid (100%), glacial acetic acid (99.5%); sodium hydroxide (98%), potassium hydroxide (98%), sodium thiosulphate pentahydrate (R.G), oxalic acid (extra pure), potassium dichromate (extra pure), potassium iodide (extra pure), iodine monochloride (R.G) and anhydrous sodium sulphate were purchased from E. Merck (Damstadt, Germany) and Labscan (Bankok, Thialand). Standards of fatty acid methyl esters were purchased from Supelco (Bellefonte, PA, USA) and Sigma Aldrich Co. (St. Louis, MO, USA).

Apparatus. The apparatus used included gas chromatograph with flame ionization detector, model Clarus 500, from Perkin Elmer Instruments LLC, (Shelton, CT, USA), capillary column Rtx-2330 (60 × 0.25 mm × 0.20 ìm, film thickness) from Supelco (Bellefonte, PA, USA), Lovibond model E tintometer (Salisbury, UK), Abbé refractometer model 2W (Shijiazhuang, China), Gallen Kamp air oven (West Mildlands, UK) and vacuum oven (Melrose Park, IL, USA).

Oil extraction. Safflower, Thori-78 and Pawari-95, seeds (500 g each) were crushed and finely ground to flour and then subjected to extraction with *n*-hexane (0.5 litre) in a one litre Soxhlet extractor for 8 h (AOCS, 2004). The fat was recovered using a rotary evaporator. The extracted fat was placed in an oven at 60 °C for 1 h, transferred to a capped reagent bottle and stored at 4 °C until required.

Quantitative separation of tri-, di- and mono-acylglycerols of oil. The lipid class composition, comprising of TAGs, DAGs, and MAGs mixture in *C. tinctorius* seed oil was determined by solid-liquid adsorption chromatography (SLAC), using silica gel as the adsorbent and eluted with different solvent systems by following the AOCS method (AOCS, 2004) with little modification. The effectiveness of separation was verified by thin layer chromatography, using solvent system (petroleum-ether and acetone: 9:1).

Fatty acid composition. Methyl esters of fatty acids were prepared according to standard IUPAC method 2.301 (IUPAC, 1987). The chemical composition of fatty acid methyl esters was accomplished with a Perkin Elmer gas chromatograph model Clarus 500 fitted with a polar capillary column Rtx-2330 (60×0.25 mm×0.20 µm, film thickness) and a flame ionization detector. Nitrogen was used as carrier gas at a flow rate of 3 mL/min. Other conditions were as follows: initial oven temperature, 70 °C was maintained for 5 min then ramped at 10 °C/min to 180 °C, followed by 3 °C/min to final temperature of 220 °C, where it was held for 15 mins; injector temperature and detector temperature was 270 °C. A sample volume of 0.3 µL was injected (splitless). Fatty acid methyl esters were identified by comparing their relative and absolute retention times to those of authentic standards of fatty acid methyl esters purchased from Supelco Sigma-Aldrich Co. Quantification was done by a built-in data-handling programme, provided by the manufacturer of the gas chromatograph. Analyses were performed in triplicate.

Physical and chemical analysis of the extracted oils. The following tests for refractive index, specific gravity, colour, free fatty acid, acid value, peroxide value, iodine value, saponification value and unsaponifiable matter of the extracted oils were performed by the standard methods of AOCS (2004). Colour of the oils was determined by a Lovi bond tintometer (Tintometer Ltd., Salisbury, UK) using a one inch cell.

Results and Discussion

Hexane extracted oil content of the two varieties of *C. tinctorius*, Thori-78 and Pawari-95, seeds was found to be 28.33±1.15 and 33.07±1.12%, respectively; the high percentage of oil gives these varieties distinct potential for the oil industry, because the average oil content of the seeds exceeds those of conventional oil seeds i.e., cotton (15.0-24.0%), canola (17-21%), soyabean (17-21%), olive (20-25%) which are grown in the USA, Brazil and Asia (Pritchard, 1991) but oil content is slightly lower than that of sunflower (25-35%).

Physical and chemical parameters of the oils are depicted in Table 1. At room temperature, both varieties of seed oil were present in a liquid state. The refractive index and specific gravity of Thori-78 and Pawari-95 oils were determined at 40 °C, which were concordant with the reported value and comparable with other vegetable oils (Rossell, 1991a; Swern, 1964a; 1964b). The values determined for free fatty acids as

OA and acid values are comparable with the reported values of the crude oil (Dhellot *et al.,* 2006; El-Adawy and Taha, 2001). Very low value of free fatty acid and acid value in the present analysis is an indication of the good quality of crude oil. Peroxide values (Table 1), indicating the presence of hydroperoxides in oils, were high, thus showing low resistance to oxidation (Onyeike and Acheru *et al.,* 2002); thus oils could be used after slight refining. The analyzed crude oils were high in colour index 2.13R + 45.4Y + 0.71N (Thori-78) and 2.48R + 46.16Y + 0.81N (Pawari-95). Intense colour of vegetable oils depend mainly on the presence of various colouring pigments of plants such as carotenoids, chlorophyll etc., which are effectively removed during refining and bleaching steps of oil processing. Vegetable oils with minimum values of colour index are good for edible purpose.

Table 1. Proximate and physicochemical characteristics of *C. tinctorius* oils

Parameters	Thori-78	Pawari-95
Oil content	28.33±1.15 (27.11-29.88)	33.07±1.12 (31.5-34.0)
Free fatty acid (% as OA)	0.52±0.012 (0.51-0.54)	0.53±0.004 (0.53-0.54)
Acid value (mg/kg)	1.12±0.15 (0.99-1.34)	1.06±0.02 (1.04-1.09)
Peroxide value (Meq/kg)	17.2±0.25 (16.94-17.54)	20.75±0.38 (20.2-21.1)
Iodine value (g of I/100 g of oil)	134.82±0.68 (133.99-135.67)	136.16±0.96 (134.89-137.21)
Saponification value (mg of KOH/ g of oil)	187.56±2.34 (184.89-190.60)	188.96±2.18 (186.5-191.8)
Unsaponifiable matter (%)	0.41±0.06 (0.32-0.46)	0.57±0.05 (0.50-0.62)
Refractive index at 40 °C	1.4734±0.0005 (1.4730-1.4742)	1.4679±0.0007 (1.4672-1.4689)
Specific gravity at 40 °C	0.9064±0.002 (0.9031-0.9085)	0.9240±0.0004 (0.9234-0.9245)
Colour (Red unit)	2.13±0.04 (2.1-2.2)	2.48±0.08 (2.4-2.6)
(Yellow unit)	45.4±0.29 (45.0-45.7)	46.16±0.62 (45.5-47.0)
(Blue unit)	0	0
(Neutral unit)	0.71±0.06 (0.65-0.80)	0.81±0.01 (0.79-0.83)

Iodine values were comparatively high due to the presence of high content of unsaturated fatty acids and are comparable with the values of poppy, soybean and sunflower oils (Rossell, 1991a). High iodine value shows that both the varieties of seed oils have good qualities of oils, required for edible and drying purposes (Eromosele *et al*., 1994). Values of saponification and unsaponifiable matter of Thori-78 and Pawari-95 oils are concordant with the values of sunflower, poppy seed and soybean oils (Rossell, 1991a; Swern, 1964a; 1964b), indicating them to be good source of industrial oil which can be used in the manufacture of soap and liquid soap.

Table 2 shows glyceride composition of oils of both the varieties of *C. tinctorius*. The content of triacylglycerides is over 83%. Comparison of free fatty acid value, acid value, saponification value, unsaponifiable matter, iodine value, refractive index, specific gravity and colour of the studied oils with those of the known edible oils reveal that the quality of both the varieties of oil have great potential for edible usage.

Table 2: Glyceride composition of *C. tinctorius* seed oils (wt. %)

C. tinctorius variety	Monoglyceride	Diglyceride	Triglyceride
Thori-78	5.70±0.55	7.62±0.87	85.61±0.61
Pawari-95	5.77±0.50	8.72±0.53	83.93±0.88

Values are means ± SD, analyzed in triplicate.

Fatty acid composition of the oils of the two varieties was determined using gas chromatography (Fig. 1 and 2 and Table 3). The principal fatty acid components in Thori-78 and Pawari-95 were palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids. Linoleic acid is predominantly present in both the varieties as compared to other varieties, U.S.-10, S.-208 and V. F.-stp (53-1) grown in Pakistan (Table 3) (Raie, 2008). Fatty acid composition was more or less similar to that of sunflower, soybean, corn, and cotton seed oils and safflower oil originating from different geographic regions (Cosge *et al.*, 2007; Rossell, 1991b). These results suggest that these varieties of *C. tinctorius* can serve as potential dietary sources of mono unsaturated fatty acid (MUFA) and poly unsaturated fatty acid (PUFA).

Present studies revealed that seed oils of *C. tinctorius* varieties, Thori-78 and Pawari-95, indigenous to Pakistan have very good potential for edible and industrial usage and also for use in developing nutritionally balanced formulations blended with other high stearic or high oleic oils. These Oils are used in the food and pharmaceutical industries to produce cooking oils,

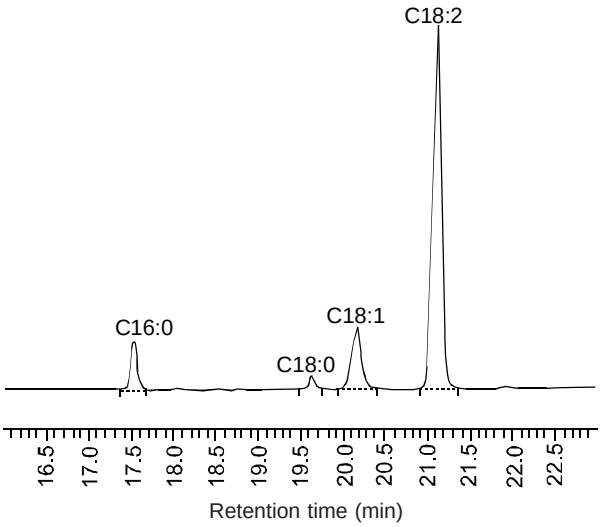

Fig. 1: Gas chromatogram of fatty acids of safflower (Thori-78) seed oil; major components are labelled.

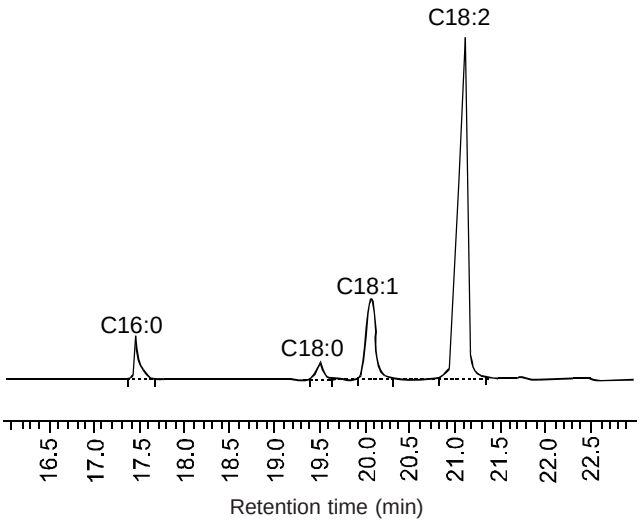

Fig. 2: Gas chromatogram of fatty acids of safflower (Pawari-95) seed oil; major components are labelled.

food supplements and skin care products. The good drying property and high content of linoleic acid and absence of linolenic acid and wax and low content of free fatty acid, colour and unsaponifiable compounds make them suitable for use in the production of high quality paints, alkyd resins, coatings, varnishes and linoleum. They can also be used in the production of biodiesel.

Pakistan imports huge amount of palm oil and soybean from foreign countries to fulfil the increasing demand of oil in the country. Moreover, Pakistan has suitable atmosphere for cultivating all the conventional and non-conventional oilseed crops. Cultivation of safflower varieties at larger scale could fulfil the requirements of the country and save enormous amount of foreign exchange spent otherwise.

Table 3. Fatty acid composition of high linoleic *C. tinctorius* varieties grown in Pakistan (wt. %)

Fatty acids	Thori-78	Pawari-95	US-10	S.-208	V.F.-stp (53-1)
Myristic Acid ($C_{14:0}$)	-	-	3.1	0.9	2.8
Palmitic Acid ($C_{16:0}$)	6.45±0.57 (5.66-7.02)	6.92±0.37 (6.41-7.28)	10.2	9.4	12.0
Stearic Acid ($C_{18:0}$)	1.99±0.09 (1.89-2.12)	2.77±0.49 (2.24-3.42)	5.5	2.3	3.6
Oleic Acid ($C_{18:1}$)	15.55±0.30 (15.31-15.98)	13.15±0.49 (12.67-13.81)	14.4	14.0	15.7
Linoleic Acid ($C_{18:2}$)	75.42±0.59 (74.65-76.11)	76.40±1.0 (75.01-77.32)	66.8	73.4	65.9
Others	0.59±0.04 (0.54-0.64)	0.76±0.06 (0.68-0.83)	-	-	-
Total saturated fatty acids	8.44	9.69	18.8	12.6	18.4
Total unsaturated fatty acids	90.97	89.55	81.2	87.4	81.6

Values are mean ± SD, analyzed in triplicate.

Conclusion

These studies were focussed on the yield and physical and chemical evaluation of seed oils of *C. tinctorius* varieties, Thori-78 and Pawari-95, cultivated in the region of Sindh, Pakistan. It was revealed that oils of both the varieties have very good potential for developing nutritionally blended formulations balanced with other high saturated fatty oils, as well as for different industrial usage due to the presence of high percentage of polyunsaturated fatty acids. These can also be used in the production of biodiesel and thus in different industries in Pakistan.

References

AOCS, 2004. *Official Methods and Recommended Practices of American Oil Chemists Society*, 5th edition, American Oil Chemists Society, Champaign, Illinoise, USA.

Chen, S.H., Chuang, Y.J. 2002. Analysis of fatty acids by column liquid chromatography. *Analytica Chimica Acta*, **465:** 145-155.

Cosge, B., Gûrbûz, B., Kiralan, M. 2007. Oil content and fatty acid composition of some safflower (*Carthamus tinctorius* L.) varieties sown in spring and winter. *International Journal of Natural and Engineering Sciences*, **1:** 11-15.

Dhellot, J.R., Matouba, E., Maloumbi, M.G., Nzikou, J.M., Safou Ngoma, D.G., Linder, M., Desobry, S., Parmentier, M. 2006. Extraction, chemical composition and nutritional characterization of vegetable oils: Case of *Amaranthus hybridus* (var1 and 2) of Congo Brazzaville. *African Journal of Biotechnology*, **5:** 1095-1101.

Echard, J.P., Benoit, C., Peris-Vicente, J., Malecki, V., Gimeno-Adelantado, J.V., Vaiedelich, S. 2007. Gas chromatography/ mass spectrometry characterization of historical varnishes of ancient Italian lutes and violin. *Analytica Chimica Acta,* **584:** 172-180.

El-Adawy, T.A., Taha, K.M. 2001.Characteristics and composition of different seed oils and flours. *Food Chemistry,* **74:** 47-54.

Eromosele, I.C., Eromosele C.O., Akintoye, A.O., Komolafe, T.O. 1994. Characterization of oils and chemical analyses of the seeds of wild plants. *Plant Foods for Human Nutrition,* **46:** 361-365.

IUPAC, 1987. *Standard Methods for the Analysis of Oils, Fats and Derivativs*, C. Paquot and A. Hautfenne (eds.), 96 pp., 7th revised and enlarged edition, Blackwell Scientific Publications, London, UK.

Kaffka, S.R., Kearny, T.E., Knowles, P.D., Miller, M.D. 2001. Safflower production in California. [http://agric.ucdavis.edu/agronomy/crops/oilseed/safflower.html].

Knights, S.E., Wachsman, N.G. and Norton, R.M. 2001. Safflower (*Carthamus tinctorius* L.) Production and research in southern Australia. (Abstract: Fifth International Safflower Conference).[http://www.sidney.ars. usda. gov/state/saffcon/abstracts/ProductionManagement/knights.html].

Mündel, H.H., Blackshaw, R.E., Byers, J.R., Huang, H.C., Johnson, D.L., Keon, R., Kubik, J., Mckenzie, R., Otto, B., Roth, B., Standford, K. 2004. Safflower production on the Canadian prairies: revisited in 2004. In: *Agriculture and Agri-Food Canada*, pp. 4-5, Graphcom Printers Ltd., Lethbridge, Alberta, Canada. [http://Safflower.wsu.edu/SafflowerProduction_Canada.pdf]or//res2.agr.ca/lethbridge/safflo/part 1- e.htm.].

Onyeike, E.N., Acheru, G.N. 2002. Chemical composition of selected Nigerian oil seeds and physicochemical properties of the oil extracts. *Food Chemistry*, **77:** 431-437.

Oplinger, E.S., Oelke, E.A., Kaminski, A. R., Putnam, D. T., Teynor, T.M., Doll, D.J., Kelling, K.A., Durgan, B. R., Noetzel, D.M. 1991. Safflower. In: *Alternative Field Crops Manual*.[http://www. hort.purdue.edu/NEWCROP/AFCM/Safflower.html].

Oyen, L.P.A., Umali, B.E. 2007. *Carthamus tinctorius* L. In: PROTA 14: *Vegetable Oils/ Oléagineux*, H.A.M. van der Vossen, and G.S. Mkamilo (eds.), PROTA, Wageningen, Netherlands.[http://database.prota.org/PROTAhtml/Carthamus%20tinctorius_En.html]

Peris-Vicente, J., Gimeno Adelantado, J.V., Doménech-Carbó, M.T., Mateo-Castro, R., Bosch-Reig, F. 2006. Characterization of waxes used in pictorial art works according to their relative amount of fatty acids and hydrocarbons by gas chromatography. *J. Chromatography A*, **1101:** 254-260.

Peris-Vicente, J., Gimeno Adelantado, J.V., Doménech-Carbó, M.T., Mateo-Castro, R., Bosch-Reig, F. 2005. Identification of lipid binders in old oil paintings by separation of 4-bromomethyl-7-methoxycoumarin derivatives of fatty acids by liquid chromatography with fluorescence detection. *Journal of Chromatography A*, **1076:** 44-50.

Peris-Vicente, J. Gimeno Adelantado, J.V., Doménech-Carbó, M.T., Mateo-Castro, R., Bosch-Reig, F. 2004. Identification of drying oil used in pictorial works of art by liquid chromatography of the 2-nitro phenyl hydrazides derivatives of fatty acids. *Talanta*, **64:** 326-333.

Pritchard, J.L.R. 1991. Analysis and properties of oilseeds. In: *Analysis of Oilseeds, Fats and Fatty Foods*, J.B. Rossell and J.L.R. Pritchard (eds.), pp. 82-93, Elsevier Applied Science, New York, USA.

Raie, M.Y. 2008. Nutritional aspects. In: *Oils, Fats and Waxes*, pp. 123, National Book Foundation, Combine Printers,

Lahore, Pakistan.

Rossell, J.B. 1991a. Vegetable Oils and Fats. In: *Analysis of Oilseeds, Fats and Fatty Foods*, J.B. Rossell, and J.L.R. Pritchard (eds.), pp. 266-268, Elsevier Applied Science, New York, USA.

Rossell, J.B. 1991b. Vegetable oils and fats. In: *Analysis of Oilseeds, Fats and Fatty Foods*, J.B. Rossell and J.L.R. Pritchard (eds.), pp. 290-296, Elsevier Applied Science, New York, USA.

Sastri, B.N. (ed.) 1950. *The Wealth of India*, A *Dictionary of Indian Raw Materials and Industrial Product*. vol. **II**, pp. 84-88, Publication and Information Directorate, CSIR New Dehli, India.

Seppänen-Laakso, T., Laakso, I., Hiltunen, R. 2002. Analysis of fatty acids by gas chromatography and its relevance to research on health and nutrition. *Analytica Chimica Acta*, **465:** 39-62.

Swern, D. 1964a. Structure and composition of fats and oils. In: *Bailey's Industrial Oil and Fat Products*, D. Swern (ed.), pp. 211-212, John Wiley & Sons, New York, USA.

Swern, D. 1964b. Structure and composition of fats and oils. In: *Bailey's Industrial Oil and Fat Products*, D. Swern (ed.), pp. 207, John Wiley & Sons, New York, USA.

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Analysis of Caffeine and Heavy Metal Contents in Branded and Unbranded Tea Available in Pakistan

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Abstract. In the investigation of caffeine and heavy metal contents in four branded and six unbranded tea samples collected from local markets of Lahore, Faisalabad and Peshawar, the amount of caffeine and heavy metals in all the branded tea samples were in agreement with the international standards. In unbranded tea samples, though the amount of caffeine was within standard limits but two of the samples collected from Peshawar had high concentrations of lead being, 13.69 and 15.78 mg/kg, consumption of which can lead to serious problems.

Keywords: tea, caffeine, heavy metals

Introduction

Tea (*Camellia sinensis*) is one of the most popular beverages all over the world. According to an estimate, 2.5 million metric tonnes of dried tea is manufactured annually, 75% of which is processed as black tea and consumed in different countries. In UK, on an average, one litre of tea is consumed per person per day (Al-Oud, 2003). Different brands of tea are manufactured to meet the increasing demands of consumers worldwide. Positive and negative effects of tea on the health have been investigated by many researchers, recently (Yao *et al*., 2006a).

Caffeine ascribes quality characteristics to tea, such as briskness, taste etc., and has been considered an important quality parameter in the evaluation of tea quality (Yao *et al*., 2006b). Caffeine is a pharmacologically active substance and, depending on the dose, can be a mild nervous system stimulant. Caffeine does not accumulate in the body and is normally excreted within several hours of consumption (Mumin *et al*., 2006; Obanda *et al*., 1999). Human body requires both metallic and non-metallic elements for healthy growth and development within certain permissible limits. The optimum concentration needed for this purpose varies widely from one element to another, from infant to childhood to adult and from male to female (Atta, 1995). Determination of these elements in beverages, water, food, plant and soil is thus of utmost important. Tremendous research has been rendered on finding tolerance limits for daily intake of nearly all essential elements needed for healthy growth and sound physiological changes in human body. There is a fairly narrow gap between the essential and the toxic levels of metals and essential trace elements that can otherwise accumulate in bone, hair and soft tissues such as liver, kidney, brain or lungs (Tautkus *et al*., 2004).

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Materials and Methods

Caffeine. ***Preparation of tea solution.*** Two grams of tea were added to boiling water, (200 mL) in a 250 mL conical flask placed on a hot plate at 90 °C while stirring for 10 min by a magnetic bar. Then the tea solution was filtered through cotton wool and the residue was washed thrice with distilled water (10 mL). The tea solution was cooled to room temperature and washings were diluted to 250 mL with distilled water. The sample was analyzed in duplicate.

Measurement. To 10 mL of tea solution, 5 mL HCl (0.9 mL of 36% HCl was diluted to 1000 mL with distilled water) and 1 mL lead acetate solution (100 g of lead acetate was dissolved in small quantity of water and diluted to 200 mL with distilled water) were added and diluted upto 100 mL with distilled water. The solution was then filtered through Whatman filter paper # 42. Filtrate 25 mL and 0.3 mL sulphuric acid (167 mL of 98% H_2SO_4 was diluted to 1000 mL with distilled water) were placed in a volumetric flask and diluted to 50 mL with distilled water. The solution was filtered using the same type of filter paper. Absorbance of the filtrate was measured using spectrophotometer (Spectronic Unicam) at 274 nm. Readings were taken in duplicate.

Standard curve. Caffeine stock solution (20 mg caffeine/10 mL, w/v distilled water) was diluted to 200 mL with distilled water. Next, 0, 10, 20, 30, 40 and 50 mL of the diluted caffeine solution were separately mixed, each with 4 mL HCl in a volumetric flask and diluted to 100 mL with distilled water. Thereafter, the

measuring steps were repeated as described above. Readings of the absorption of the standard solution against its concentration were used to prepare the standard curve. Following formula was used to calculate the caffeine contents:

$$\text{Caffeine (\%)} = (E/1000) \times V_o \times (100 \times V_1) \times (50/25) / W$$

or

$$= 0.2\, EV_o / V_1 / W$$

where:

E is caffeine (mg) from the standard curve against the reading of the spectrophotometer

E/1000 is used to convert 'mg' into 'g',

V_o is the total volume of tea solution (250 mL),

V_1 is the volume used for the measurement (10 mL),

$100/V_1$ indicates 10 mL tea solution that were diluted to 1000 mL,

50/25 shows another dilution from 25 mL filtrate made to 50 mL in the measurement and

W is the dry weight of the tea sample.

Heavy metals. Double distilled water and analytical grade reagents were used in all the experiments. Standard solution (10 ppm) of heavy metals i.e., Zn, Cu, Cd, Co, Pb, Mn and Ni were prepared by taking 1 mL of each heavy metal stock solution (1000 ppm) in a 100 mL volumetric flask and diluted upto the mark with double distilled water. Heavy metal working standard solutions i.e., 0.5, 1.0, 1.5 and 2.0 ppm were prepared by diluting 5, 10, 15 and 20 mL, respectively, standard solution (10 ppm) of each metal made upto 100 mL with double distilled water.

The calibration curve used for the determination of heavy metals in tea samples by flame atomic absorption spectrometry (FAAS) was established using the working standard solutions. For the determination of metals in tea, 3 g of the oven dried (105 °C) samples were preheated for 30 min at 250 °C and burned for one hour at 800-850 °C. The resulting ash was wetted with double distilled water and mixed with 10 mL of diluted HCl (1:1). The mixture was mildly boiled, cooled to room temperature, filtered (if necessary), transferred to 100 mL volumetric flask and diluted with double distilled water.

A Varian (AA 240) flame atomic absorption spectrophotometer equipped with hollow cathode lamps was used for the analysis. The instrumental setup was adjusted according to the manufacturer's instructions.

Results and Discussion

The spectrophotometric method is the most common method used for the determination of caffeine. Flame atomic absorption spectrometry (FAAS) is one of the techniques most extensively used for determining various elements with a significant precision and accuracy. This analytical technique is remarkable for its selectivity, speed and fairly low operation cost. However, in some cases it is rather difficult to determine traces of heavy metals in environmental samples due to unsufficient sensitivity or matrix interferences. Thus, a pre-concentration or/and separation step is necessary.

Caffeine. Caffeine content in different tea samples ranged from 3.41 to 3.74% with a mean of 3.56% of the dry mass (Table 1). Earlier studies showed that caffeine content in black tea was affected by clone of plant, season and stage of plucking of leaves (Obanda and Owuor, 1995). Caffeine content can vary in tea leaves from 24% to 40% depending on the maturity i.e., the young leaves may contain more caffeine than the older tea leaves (Owuor and Orchard, 1992; Owuor *et al*., 1987). The results (Table 1) of the current study showed that the mean caffeine content in tea varieties marketed in Pakistan was

Table 1. Concentrations of caffeine and heavy metals in branded and unbranded tea samples

Tea sample (code)	Caffeine (%)	Heavy metals (mg/kg)						
		Cu	Cd	Mn	Co	Ni	Zn	Pb
Branded (S 1)	3.41	1.20	nd	2.75	0.69	4.58	nd	4.64
Branded (S 2)	3.45	1.05	nd	40.50	0.60	4.58	nd	3.28
Branded (S 3)	3.42	0.75	nd	0.25	0.65	4.59	0.25	4.75
Branded (S 4)	3.46	0.75	nd	39.75	0.61	4.58	1.00	4.28
Unbranded (LHR 1) (S 5)	3.64	0.50	0.25	19.50	0.10	9.71	nd	4.64
Unbranded (LHR 2) (S 6)	3.72	1.0	0.28	15.70	1.29	9.27	0.76	4.59
Unbranded (FSD 1) (S 7)	3.53	0.50	0.25	16.10	1.20	9.98	0.84	4.20
Unbranded (FSD 2) (S 8)	3.49	0.75	0.50	25.75	1.14	8.66	0.25	3.75
Unbranded (PWR 1) (S 9)	3.74	1.45	0.34	25.50	1.02	10.01	nd	13.69
Unbranded (PWR 2) (S 10)	3.73	0.75	0.25	16.50	0.89	9.69	0.75	15.78

nd = not detected.

similar to the values reported in literature, 3.32 to 31.81%, and also that the tea varieties originating from different sources, with differences in clones, seasons and maturity, differ in caffein content (Gulati *et al.*, 1999).

Heavy metals. Results of the study show that different tea samples contain the elements Cu, Cd, Mn, Co, Ni, Zn and Pb in various proportions (Table 1 and Fig. 1). Variations in elemental contents from sample to sample can be attributed to the differences in botanical profile as well as the mineral composition of the soil in which plants are cultivated. Other factors responsible for variation in elemental contents may be pre-ferential absorbability of the plant, fertilizers, irrigation water, climatic conditions and, most of all, differences in the methods of processing and packing of tea leaves. Deposition of various metals from the vehicular emission and other sources on the open tea leaves could contribute to differences in analytical results.

Lead. Lead was found in variable amounts in all tea samples (Table 1). For example high concentration of lead i.e., 13.69 and 15.78 mg/kg were found in two tea samples (S-9 and 10, respectively), while in other samples the amount of Pb varied from 3.28 to 4.75 mg/kg which is lower than the prescribed limit of WHO (1998) for Pb content in herbs i.e. 10 mg/kg. It is believed that 95% of Pb present in plants is due to foliar uptake from the surroundings. As majority of the tea varieties are imported from other countries, so source plants with different origins have different air, water and soil environment. The high concentration of Pb in plants growing above the ground is due to air borne Pb and the surrounding polluted environment.

Cadmium. Cd is another toxic metal not required by any living being. In the four branded tea samples (1, 2, 3 and 4) Cd was not detected while in other samples its value ranged from 0.20 to 0.50 mg/kg (Table 1). It may have been introduced during packaging or the tea plants might have absorbed this pollutant from the surrounding air and the soil. Thus consumption of such tea for long period of time is dangerous due to Cd contamination. Cd damages nerve cells; it inhibits the release of acetylcholine and activates cholinesterase enzyme, resulting in hyper activity of the nervous system. By altering calcium and phosphorus metabolism, toxic level of Cd can contribute to arthritis, osteoporosis and neuromuscular diseases. In cardio-vascular system, Cd replaces Zn in the arteries making them brittle and inflexible. Cd accumulates in the kidneys and once accumulated, it stays there and is not removed through excretion, resulting in high blood pressure and kidney diseases.

Copper. In all the tea samples, high Cu concentration was found. In sample-9, concentration of Cu was 1.45 mg/kg (Table 1), while in all other samples, its concentration varied from 0.50 to 1.20 mg/kg. Although Cu is an essential enzymatic element for normal plant growth and development but at excessive levels, it can be toxic. Phytotoxicity can occur if its concentration in plants is higher than 20 mg/kg. Copper accumulation in body can result in a tendency for hyperactivity in autistic children. An excess of Cu can cause stuttering, insomnia and hypertension as well as oily skin, loss of skin tone (due to blocking of vitamin C absorption) and dark pigmentation of the skin, usually around the face. Cu can also make nails brittle and thin and may contribute to hair loss especially in women.

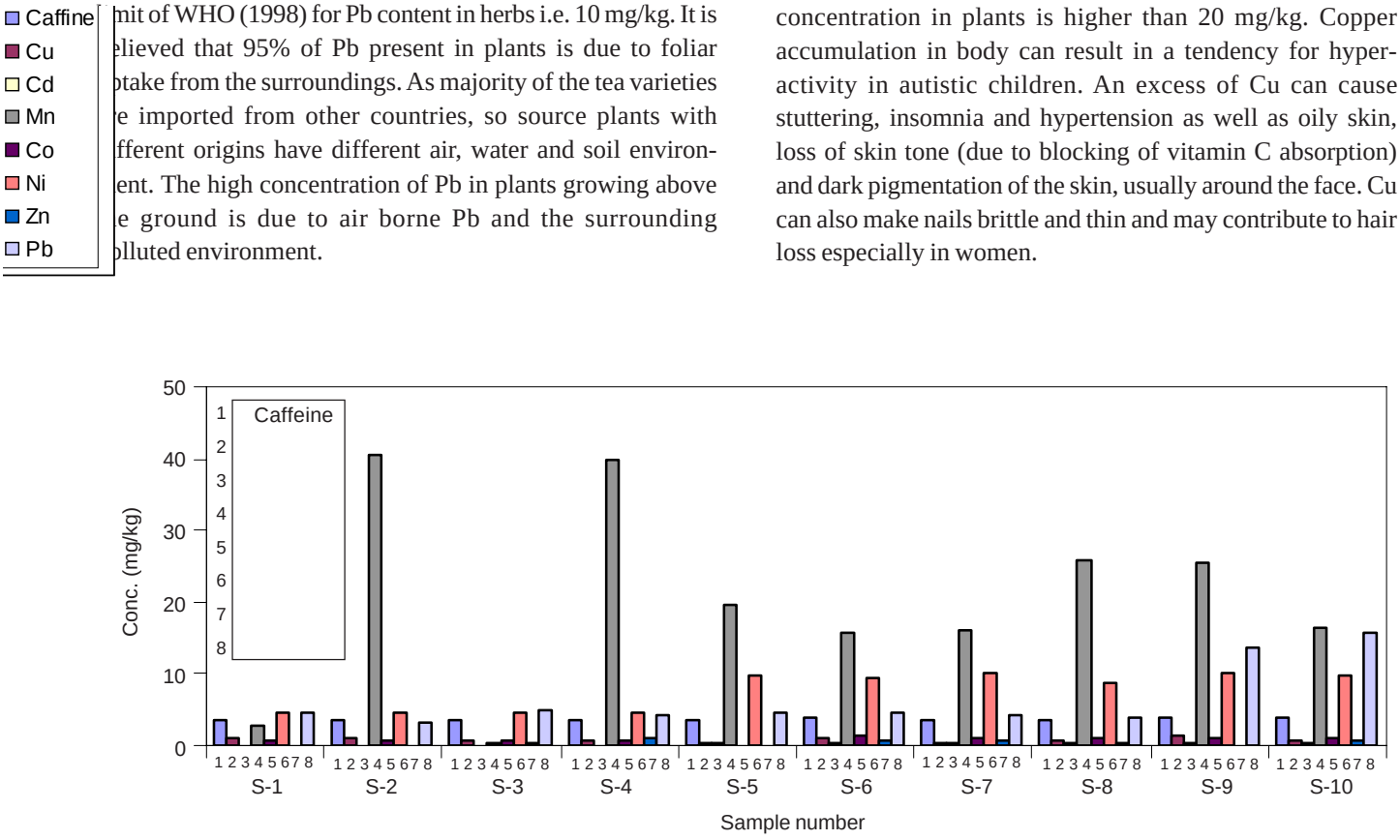

Fig. 1. Concentration of caffeine and heavy metals in different tea samples.

Zinc. Zn is an important metal required by both plants and animals. The highest concentrations of Zn (1.0 mg/kg) was found in one branded sample (sample-4), while in other samples, its amount varied from 0.25 to 0.84 mg/kg (Table 1). In few tea samples, its concentration was very low. Zn is very important for plant and human life. In the blood about 85% of Zn combines with proteins for the transport of the latter after its absorption and its turnover is rapid in the pancreas. Deficiency of Zn causes diabetic hyposmia, hypogensia or coma.

Nickel. In all the studied unbranded tea samples, higher amount of Ni was recorded: 10.01 mg/kg in sample-9, followed by 8.66-9.98 mg/kg in five samples (samples 5, 6, 7, 8 and 10), while equal amounts of Ni (4.58 mg/kg) was found in all the branded samples. Ni plays an important role in the production of insulin in the pancreas. Its deficiency results in disorder of the liver. However, higher concentration, of Ni have adverse effects on health. For example Ni tends to accumulate in kidneys causing kidney damage. Being a common ingredient in fashion jewellery, Ni can cause allergic reactions in some wearers; eczema and even asthma attacks may develop. A steady exposure ot Ni can cause cancer of lungs and nasal sinus.

Cobalt. As shown in Table 1, the highest concentration of Co was found in one sample (sample 6, 1.29 mg/kg), followed by 1.20 mg/kg in sample-7. Other tea samples had a concentration of 0.60 mg/kg to 1.14 mg/kg. Thus as a whole, considerable variations were recorded in all the tea samples which may be due to the differences in the air, soil and water environment of the source plants as well as local activities in their nearby surroundings. Human body needs Co in trace amounts and it is toxic in higher concentrations. Co in the form of vitamin B_{12} is in its physiologically active form.

Manganese. Mn was the most abundant metal found in tea samples in variable amounts (Table 1). For example high Mn concentration was found in two branded samples No.2 and 4, being 40.50 and 39.75 mg/kg, respectively, and 25.75 and 25.50 mg/kg in two unbranded samples, samples 8 and 9, respectively. The least amount of Mn was found in one branded sample (sample 3) being 0.25 mg/kg. The presence of Mn in tea samples may be due to its use as a colouring material for tea leaves.

Daily intake of heavy metals. Due to lack of information about the maximum allowable levels of heavy metals in tea leaves the discussion will be extended to the acceptable daily intake that can be taken into account through foods and drinking water. For intance, the expected calculated intake of Mn in the present study was 121.38 mg/day for tea (Table 2). This value is much higher than the intake through food (0.008) and at the same time nearly equal to 3.8 mg/day through drinking water according to the standards of US Environmental Protection Agency (MAFF, 1997). The calculated overall mean intake of Ni through tea was 49.39 mg/day. This value is in agreement with the human requirements (50 mg/day). However, this value is higher than the average intake in the UK (0.13 mg/day) recorded for the total diet studied (MAFF, 1999). According to MAFF (1998) tea beverage has considerable amount of Ni that could significantly contribute to daily intake of metals. Calculated intake of Cu through tea was 5.22 µg daily or 0.005 mg daily. This value was much lower than that of 1.2 mg daily set by UK and WHO (1998).

Table 2. Daily intake of different heavy metals

Heavy metal	Total (ppm)	Intake (mg/day)
Cu	0.87	5.22
Cd	0.18	1.12
Mn	20.23	121.38
Co	0.91	5.46
Ni	7.56	49.39
Zn	0.38	2.31
Pb	6.36	38.16

The mentioned values were calculated based on the assumption that the average consumption of tea beverage for a single person is three cups a day with one packet of 2 g in a cup, i.e., 6 g of tea particles/day.

The results show (Table 2) that only small part of heavy metal content through tea leaves may be introduced into beverage preparation. All branded and unbranded tea samples were found to contain heavey metal contents within safe levels except for only two unbranded samples collected from Peshawar, which had high concentrations of Pb.

References

Al-Oud, S.S. 2003. Heavy metal contents in tea and herb leaves. *Pakistan Journal of Biological Sciences*, **6:** 208-212.

Atta, M.B. 1995. Aluminium content in dust black tea leaves and its beverages. Menofiya. *Journal of Agricultural Research*, **20:** 137-150.

Gulati, A., Ravindranath, S.D. 1999. Seasonal variations in quality of kangra tea *Amellia sinensis* (L.) O kuntze) in Himachal Pradesh. *Journal of the Science of Food and Agriculture*, **71:** 231-236.

MAFF, 1999. Total diet study: Aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. *Food Surveillance Information Sheet No. 191*. Ministry of Agriculture Fisheries and Food (1997), UK.

MAFF 1998. Lead, Arsenic and other metals in food. *Food Surveillance Paper No.52*. London, UK.

MAFF 1997. Total diet study : metals and other elements. *Food Surveillance Information Sheet No. 131*, Ministry of Agriculture Fisheries and Food, UK.

Mumin, M.A., Akhter, K.F., Abedin, M.Z., Hossain, M.Z. 2006. Determination and characterization of caffeine in tea, coffee and soft drinks by solid phase extraction and high performance liquid chromatography (SPE-HPLC). *Malaysian Journal of Chemistry*, **8:** 45-51.

Obanda, M., Owuor P.O., Taylor, S.J. 1999. Flavanol composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas. *Journal of the Science of Food and Agriculture*, **74:** 209-215.

Obanda, M., Owuor, P.O. 1995. Changes in black tea quality chemical parameter due to storage, duration and packaging method. *Tea*, **16:** 34-40.

Owuor, P.O., Orchard, J.E. 1992. Effects of storage time in a two stage withering process on the quality of seedling black tea. *Food Chemistry*, **45:** 45-49.

Owuor, P.O., Obanda, A.M., Tsushida, T., Horita, H., Murai, T. 1987. Geographical variation of theaflavin, thearubigins and caffeine in Kenyan clonal black teas. *Food Chemistry*, **26:** 223-230.

Tautkus, S., Kazlauskas, R., Kareiva, A. 2004. Determination of copper in tea leaves by flame atomic absorption spectrometry. *Chemistry*, **15:** 49-52.

WHO, 1998. *Guidelines for Drinking Water Quality,* 2nd edition, Addendum to volume **2:** *Health Criteria and other Supporting Information*, pp. 46-61, World Health Organization, Geneva, Switzerland.

Yao, L.H., Jiang, Y.M., Caffin, N., Arcy, B.D., Datta, N., Liu, X., Singanusong, R. Xu, Y. 2006a. Phenol compounds in tea from Australian supermarkets. *Food Chemistry*, **96:** 614-620.

Yao, L., Liu, X., Jiang, Y., Caffin, N., Arcy, B.D., Singanusong, R., Datta, N., Xu, Y. 2006b. Compositional analysis of teas from Australian supermarkets. *Food Chemistry*, **94:** 115-122.

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Measurement of Atmospheric Concentrations of CO, SO_2, NO and NOx in Urban Areas of Karachi City, Pakistan

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Abstract. In the assessment of variation trends in ambient air quality at five selected regions of Karachi city, four air pollutants namely carbon monoxide, sulphur dioxide, nitrogen oxide and nitrogen dioxide were monitored, along with metrological parameters, for eight consecutive days. The results suggested that all the pollutants were mainly due to the emissions from motor vehicles and industries, owing to the absence of regulatory laws/standards about ambient air quality in Pakistan. The results have been discussed with reference to recommendations of the World Health Organization for the same.

Keywords: air pollution, industrial emission, vehicular emission, atmosphere

Introduction

Karachi is the largest metropolitan city of Pakistan having an estimated population of above 10 million. Total amount and complexity of toxic pollutants in the environment of Karachi are increasing day by day with the rapid increase of population and proportional increase of industries, vehicular traffic and open air garbage burning. Rate of atmospheric pollution is 40 percent higher in Karachi than the other cities of Pakistan (Qureshi, 1997).

Typical major ambient air pollutants in the urban environment include CO, SO_2, NO, NOx, HC and PM10. CO is formed during combustion of carbon containing compounds. It is a toxic gas and its prolonged exposure, even at very low levels, may adversely affect central nervous system. When inhaled, it reacts with the haemoglobin of the blood stream to form carboxy-haemoglobin. CO attaches to haemoglobin roughly about 210 times more than the oxygen (All Refer.com, 2005). SO_2 is also generated by the combustion of high sulphur fuels. SO_2 is toxic to human body especially for persons having previous history of respiratory diseases, such as emphysema; besides, it also causes pneumonia. Nitrogen oxides are generated at high temperatures during combustion. Their ultimate effect on human beings is still not clearly understood, but they act as irritants to breathing and create discomfort to eyes and also destroy the celia in the respiratory system.

Present study was carried out in various industrial, residential/commercial and down-town regions of Karachi city to generate base-line data on these localities by air pollution monitoring analyzers, to identify major sources of air pollution and suggest their remedial measures. The data so generated may assist in the formulation of the country air quality standards. Information about the industries was obtained from different civic agencies and the Department of Industries.

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Materials and Methods

The subtropical city of Karachi is located in a semiarid zone. It is the largest industrial and commercial centre in Pakistan and declared as one of the twenty mega cities of the world (Mage *et al.,* 1996). Growing urban population, industrialization and traffic congestion are the main causes of air pollution in Karachi city. In order to assess the load of air pollutants in the environments of the city, monitoring of different air pollutants was carried out at five different locations (as shown in location map) of the city, categorized as follows:

1- Region A: the site with urban background, moderately populated, having low vehicular traffic density. It is one km distant from the main super highway. The area around the sampling site is sparsely populated.
2- Region B: a commercial site, densely populated, having high vehicular traffic density. This site is the busiest intersection of Karachi, surrounded by multistoried commercial as well as residential buildings. The population around this site mostly belongs to high income group.
3- Region C: an industrial area in district South of Karachi, with nearly 2000 different types of industries, approximately 60 percent comprising of textile mills, while

Location map: sample collection points

other industries are related to pharmaceuticals, chemicals, detergents, iron and steel, sulphur refining, vegetable oils, beverages and food products.

4- Region D: an industrial area in district East of Karachi with approximately 2000 various types of industries including tanneries (more than 100 units), pharmaceutical, textile and chemical units and refineries etc.

5- Region E: also an industrial area in district East of Karachi with 300 industrial units of different categories including textile, food, chemical, pharmaceutical and engineering units.

Measurement of major ambient air pollution components such as CO, SO_2, NO and NO_2 was carried out in summer season, for eight consecutive days at each of the five stations. Average variations of the pollutants were recorded for 11 hours at hourly intervals, at the selected regions (A-E).

Air quality measurements were performed, using air analyzer, designed and fabricated by Environmental SA, France. Average values of CO, SO_2, NO, NO_2 and NOx concentrations for 15 min were used for determining daily average hourly concentrations. The daily hourly average concentration values were further averaged for determining values for 8 days and for time weighted average (TWA) values for 1 h, 8 h and 24 h for each region.

UV fluorescent SO_2 Analyzer Model AF21 M consisted of zinc ray UV lamp with stabilized power supply, continuous energy monitor and compensation for measurement at constant energy level and integrated carbon kicker for continuous removal of interfering hydrocarbons.

The chemiluminescent NO-NOx Analyzer Model AC 31M was of two channel type coupled with serial R232 output signal processing and continuous zero control by the microprocessor. The air sampled by a pump placed at the circuit end, is carried, on the one hand *via* a converter oven towards the NOx chamber and on the other hand, directly into NO chamber. The radiation emitted in the NOx chamber is proportional to $NO+NO_2$ (reduced to NO).

Concentration of carbon monoxide was measured by Snift CO Analyzer (Model 50). The meter was kept at about 1.2 m

above the ground level and readings were taken at intervals of 15 minutes.

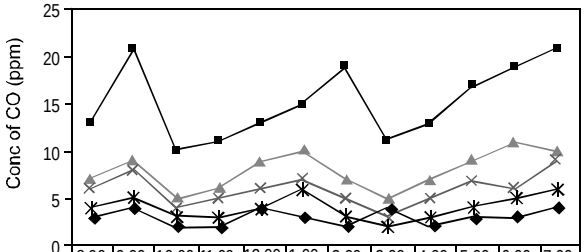
Results and Discussion

Hourly average variations of the pollutants, recorded at five selected regions A, B, C, D and E are graphically presented in Fig. 1 to 5. Table 1 gives the time weighted average (TWA) values for 1, 8, and 24 h, along with permissible ambient air quality limits, recommended by WHO.

Maximum average concentration of CO was found to be 4, 21, 11, 9, and 6 ppm in regions A, B, C, D and E, respectively (Fig. 1). The main source of CO at regions A and B may be motor vehicles plying on nearby main super highway and University Road, where traffic density is high, whereas, at regions C, D and E, the combustion of fuels in nearby industries and power generation plants.

Maximum hourly average concentrations of SO_2 were 5.7, 349.1, 74.5, 42.2 and 21.2 ppb at the regions A, B, C, D, and E, respectively, the highest concentration of SO_2 being 349.1, recorded at region B (Fig. 2). The main cause of this high concentration of SO_2 at this region may be very high traffic density due to narrow and congested roads, surrounded by high rising buildings. At region A, the main source of SO_2 is considered to be the vehicular traffic, whereas, at regions C, D, and E, the combustion of fuels in the nearby industries.

The highest average concentrations of NO were found to be 17.2, 231.3, 193.5, 157.4 and 132.5 ppb (Fig. 3), and those of NO_2, 9.8, 127.3, 122.2, 103.2 and 79.2 ppb (Fig. 4), whereas the highest average concentrations of NOx were found to be 27.0, 358.6, 315.7, 260.6 and 211.7 ppb (Fig. 5) in the selected regions A, B, C, D and E, respectively.



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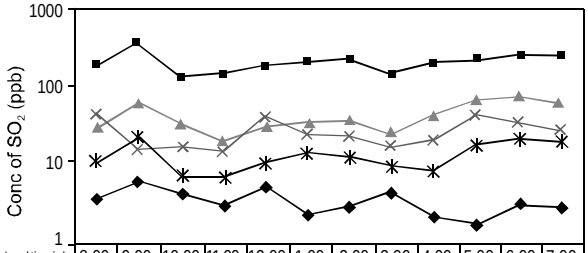
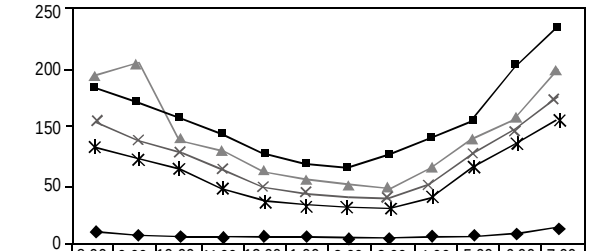
B	13	21	10	11	13	15	19	11	13	17	19	21
C	7	9	5	6	9	10	7	5	7	9	11	10
D	6	8	4	5	6	7	5	3	5	7	6	9
E	4	5	3	3	4	6	3	2	3	4	5	6

Fig. 1. Hourly average CO concentrations in regions A-E.



Local time in hr	8:00	9:00	10:00	11:00	12:00	1:00	2:00	3:00	4:00	5:00	6:00	7:00
A	3.4	5.7	3.9	2.9	5.1	2.1	2.9	4.3	2.1	1.7	3.1	2.8
B	175.2	349.1	123.6	142.1	179.6	202.4	221.3	140.6	197.2	213.7	250.9	249.1
C	29.1	58.9	31.1	19.3	29.6	34.2	36.7	23.6	41.9	67.3	74.5	61.3
D	41.3	15.2	15.7	13.9	39.6	24.1	21.5	16.3	20.1	42.2	34.4	27.1
E	10.2	21.1	6.7	6.7	10.2	13.4	11.9	9.2	8.1	17.6	21.2	19.3

Fig. 2. Hourly average SO_2 concentrations in regions A-E.

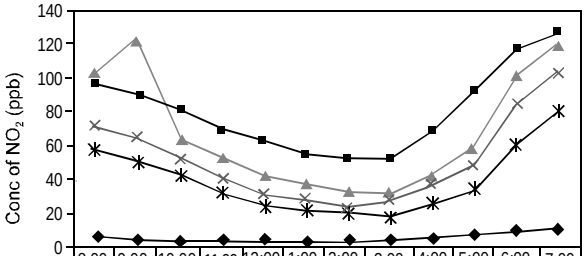


Local time in hr	8:00	9:00	10:00	11:00	12:00	1:00	2:00	3:00	4:00	5:00	6:00	7:00
A	13.5	10.1	9.6	9	8.6	8.1	7.5	6.7	7.9	9.2	12.3	17.2
B	167.5	152.9	135.3	117.6	96.2	84.8	80.5	95.1	112.7	130.5	119.3	231.3
C	182.2	193.5	112.3	100.4	79.1	68.8	63.7	60.4	81.3	112.3	134.4	186.5
D	131.4	112.2	98.6	80.5	61.3	53.5	51.2	47.8	64.5	97.2	123.3	157.4
E	105.6	91.3	80.5	61.4	46.7	41.6	40.2	38.1	51.1	82.6	107.9	132.5

Fig. 3. Hourly average NO concentrations in regions A-E.

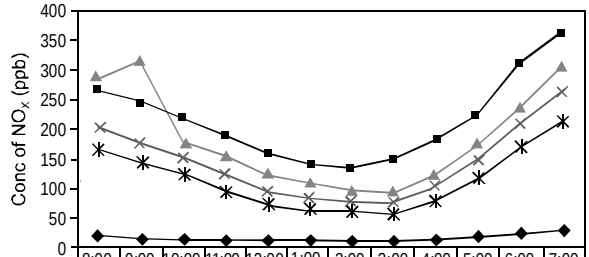
Table 1. Concentration of CO, SO_2 and NO_2 evaluated for 1, 8 and 24 h (TWA) and permissible limits of WHO.

Pollutants	Time weighted average (TWA) values							Averaging
	Region A	Region B	Region C	Region D	Region E	WHO	Unit	time
CO	3	12.25	6.7	3.7	3.5	30	mg/m^3	1 h
	2.1	15.75	8.8	4.1	3.8	10		8 h
SO_2	4	159.1	42.1	30.6	15.9	350	$\mu g/m^3$	1 h
						100-150		24 h
NO_2	5.6	79.4	47.7	35.9	32.9	400	$\mu g/m^3$	1 h
						150		24 h



Local time in hr	8:00	9:00	10:00	11:00	12:00	1:00	2:00	3:00	4:00	5:00	6:00	7:00
A	5.2	4.1	3.3	3.1	2.7	2.1	1.8	3.1	4.2	6.4	7.9	9.8
B	96.4	90.1	81.2	69.1	61.6	54.5	52.1	51.5	67.7	91.3	116.5	127.3
C	103.5	122.2	63.3	52.5	41.6	36.8	32.1	30.6	41.4	58.2	101.2	119.6
D	71.2	64.2	98.6	40.3	30.5	27.6	23.2	27.1	36.7	47.6	84.7	103.2
E	57.5	50.3	80.5	31.4	23.7	20.8	20.1	17.2	24.6	32.7	59.9	79.2

Fig. 4. Hourly average NO_2 concentrations in regions A-E.



Local time in hr	8:00	9:00	10:00	11:00	12:00	1:00	2:00	3:00	4:00	5:00	6:00	7:00
A	18.7	14.2	12.9	12.1	11.3	10.2	9.3	9.8	12.1	15.6	20.2	27
B	263.9	243	186.7	186.7	157.8	139.3	132.6	146.6	180.4	221.8	307.8	358.6
C	285.7	315.7	152.9	152.9	120.7	105.6	95.8	91	122.7	170.5	235.6	306.1
D	202.6	176.4	120.8	120.8	91.8	81.1	74.4	74.9	101.2	144.8	208	103.2
E	163.1	141.6	122.7	92.8	70.4	62.4	60.3	55.3	75.7	115.3	167.8	79.2

Fig. 5. Hourly average NOx concentrations in regions A-E.

Carbon monoxide is not easily detected by olfactory senses. High concentration of this pollutant in central parts of the cities due to traffic jams may creat serious problems. (ALA, 2000; WDNR, 2000). Exposure to carbon monoxide may lead to head ache, tiredness, dizziness, nausea, vomiting and drowsiness and in very acute situations, to unconsciousness and even death (Malakootian and Yaghmaeian, 2004). Exposure to elevated carbon monoxide level is associated with impairment of visual perception, work capacity, manual dexterity, learning ability and performance of complex tasks (Aziz and Qureshi, 2003).

In region A, the highest hourly average concentration of CO was recorded to be 4 ppm, from 7:00 to 9:00 a.m. and from 5:00 to 7:00 p.m. In the morning, the movement of traffic is down town and is reverse in the evening. Variations in the concentrations of carbon monoxide show that the concentration gradually increases till 9:00 a.m. and then comes down at 1:00 p.m. and again increases around 6:00 p.m. which are the rush hours. In region A, the air pollution was generated by vehicular traffic as the air currents were coming from main Super Highway that has quite high traffic density.

In region B, the pollution generation is mainly due to vehicular traffic. This site is the busiest intersection on M.A. Jinnah Road having high traffic density and traffic jams with high-rising buildings on both sides of the road creating tunnel effect. On the contrary, in regions C,D, and E, the pollution generation may be due to the emissions from nearby industries, power generation plants and boilers of the industries.

Sulphur dioxide originates mostly from the combustion of trace amounts of inorganic and organic sulphur, contained in the fuel. The estimated background concentration of SO_2 is 0.2 ppb and calculated atmospheric residence time is 4 days (Bhatia, 2005). Short term high level of SO_2 may enhance respiratory diseases, lung function disturbance and even mortality in adults and children (Nautiyal *et al.,* 2007). The maximum average concentrations of SO_2 at regions A and B were found to be 5.7 and 349.1 ppb, respectively, during 7.00 to 9.00 a.m. At these stations, the high concentration of SO_2 may be due to the fuel combustion by vehicular traffic. At regions C, D and E the highest values of SO_2 were 74.5, 42.2 and 21.2 ppb, respectively, at 6:00 p.m. The main source of SO_2 at station C may be power plants and boilers of the industries and at stations D and E, a large oil refinery, all located in SW directionof the areas. Relatively high concentration of SO_2 obtained at regions C, D, and E, during specified period may be due to emissions from the nearby industrial units.

Nitrogen oxides are of great concern, being precursors in ozone production in the presence of sun light. NO and NO_2 are emitted together from combustion sources and exist in equilibrium in the atmosphere; together, they are usually referred to as NOx. The diurnal pattern of NO and NOx has correlation with solar energy. A distinct photochemical relation between NO, NOx and solar energy has been established and as the solar energy increases during the day time, the level of NO, NOx decreases. The reaction of photochemical oxidants has a time scale of one to a few minutes (Clark, 1988). At region C, the highest concentrations of NO and NOx were found to be 193.5 ppb and 306.1 ppb, respectively, between 7.00 to 9.00 a.m. which may originate from the combustion of industries and power plants, about 45-60 meters away. The reaction is therefore, even more rapid here, having a time scale of only few seconds. The chemical reaction between the two mixing species was not completed due to time lag and shows high concentration of NO and NOx during the day time. However, at regions A, B, D and E, the highest values of hourly average concentration of NO and NOx were found before the sunrise which started decreasing as the ultra-violet radiations from the sun increased, and again increasing with the decrease of ultra-violet radiations from the sun. The main contributor of NO and NO_2 at regions A and B were the emissions from the vehicular traffic due to

combustion of fuel, whereas, at regions D and E, the combustion gases emitted by the industries.

A comparison of the time weighted average values of all the measured pollutants at the selected stations, with those of the WHO recommended air quality guidelines shows that the concentrations of ambient air pollutants found at these stations are well within the WHO limits.

Air pollution has become a world wide public health problem, particularly in large towns and cities of the developing countries where people are commonly exposed daily to very high levels of pollution for 3-7 hours for the last many years (Engel *et al.,* 1998). Effect of air pollution on human health varies according to the intensity, duration of exposure and health status of exposed population. Air pollution increases the risk of chronic obstructive pulmonary diseases and acute respiratory infections in childhood, lung and chest cancer, tuberculosis, prenatal outcomes including low birth weight and eye diseases. The worst affected age group had been between 50-60 years, followed by the lower age group of 45-55 years (Maddission, 1997).

Conclusion

The baseline data generated for major ambient air pollutants at different urban sites of Karachi show that the concentration of ambient air pollutants such as CO, SO_2, NO and NOx, are all within WHO threshold limits. The values recorded indicate that all the pollutants are emitted by the industries and motor vehicles. It is expected that the generated data will play a part in laying the foundation for developing appropriate ambient air quality standards for Pakistan and their implementation.

References

AllRefer.com 2005. Hemoglobin derivatives. Available at: http://health.allrefer.com/health/hemoglobinderatives-info.html.Accessed April 6, 2005.

ALA, 2000. *Carbon Monoxide and the Environment*, American Lung Association,USA, available at: http;// www.lunguas.org/air/carbon.

Aziz, J.A., Qureshi, T.A. 2003. Measurement of ambient particulate matter and carbon monoxide in Peshawar. *Science Technology and Development,* **22:** 1-4.

Bhatia, S.C. 2005. *Environmental Pollution and Control in Chemical Process Industry*, *Delhi*, pp. 104, published by Khanna Publishers, Nai Sarak, India.

Clark, .P.A. 1988. Mixing models for simulation of plume interaction with ambient air. *Atmospheric Environment*, **22:** 1097-1106.

Engel, P., Hortodo, E., Ruel, M. 1998. Smoke Exposure of women and young children in highland Guatemala, predictions recall Accuracy. *Human Organization,* **54:** 408-417.

Mage, D., Ozolins, G., Peterson, P., Webster, A., Orthofer, R., Vandeweerd, V., Gwynne, M. 1996. Urban air pollution in megacities of the world. *Atmospheric Environment,* **30:** 681-686.

Malakootian, Yaghmaeian, K. 2004. Investigation of carbon monoxide in heavy traffic intersections of Municipal districts. *International Journal of Environmental Science and Technology,* **1:** 227-231.

Maddission, D.A. 1997. *Meta Analysis of Air Pollution Epidemiological Studies,* London Centre for Social and Economic Research on the Global Environment, University College London, UK.

Nautiyal, J., Garg, M.I., Kumar, M.S., Khan, A.A., Thakur, J.S., Kumar, R. 2007. Air pollution and cardiovascular health in Mandi Gobindgarh, Punjab, India, -A pilot study. *International Journal of Environmental Research and Public Health,* **4:** 268-282. (doi10.3390/)

Qureshi, O.R. 1997. Atmospheric pollution in Karachi 40 percent higher than other cities. *Frontier Post,* June 07, Karachi.

WDNR, 2000. Carbon monoxide sources and health effects. Wisconsin Department of Natural Resources, USA, (http://www.dnr.state.wi.us).

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Seasonal and Year Wise Variations of Water Quality Parameters in the Dhanmondi Lake, Dhaka, Bangladesh

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Abstract. The quality of the surface water through 16 physicochemical variables was monitored at three sites of Dharmondi Lake of Dhaka, Bangladesh, over 5 years during 2002-2007. The concentration of heavy metals (Pb, Cd, Cr, Co, Ni, Cu) was below detection limits with few exceptions. No clear seasonal variation trend for Fe, Mn, Zn, PO_4^{3-}, SO_4^{2}, Cl^- and F^- was observed which differed from year to year. Slight increasing tendency in case of sulphate, phosphate, chloride concentrations and electrical conductivity was observed but it was not clear in other parameters. The levels of all parameters were found well below the standards for drinking water.

Keywords: lake water, seasonal variability, pollution trend, water quality, heavy metals

Introduction

Over the past 25 years, the quality of water bodies around Dhaka city has deteriorated a lot due to unplanned discharge of untreated effluents from factories and sewage. Dhanmondi Lake is one of the biggest lake and a great recreation place for the people of Dhaka city. But, this Lake is being contaminated due to the increase in human activities during the last few years. Lot of construction work had also been done during 1980 to 2006 along the valley of this Lake, which had directly influenced the water quality of the Lake. In addition, frequent floods during recent years have also contributed in polluting the lake water. The number of tourists has also increased in recent years, directly affecting the quality of water. Fishing activities around the lake are another source of contamination. Thus, a constant and systematic monitoring is essential to study long term pollution in the Lake environment especially when it is impacted by the increasing tourist population which disturbs normal activities in the area. Some short-term research work had been carried out in the past on water quality parameters of the river, and lake water in our laboratory (Quraishi *et al.*, 2006; Azim 2005; Chowdhury *et al.*, 2005; Hossain, 2005; Hadi *et al.*, 1996; 1991; Maroof *et al.*, 1985). But long-term monitoring is necessary to evaluate the pollution sources and to get a clear trend of pollution. Therefore, a long-term monitoring program was initiated in 2002 spread over a period of 5 years during 2002-2007, for a wide range of water quality parameters. The main objectives of this work were (i) to establish background levels for Mn, Fe, Zn, Cr, Ni, Co, Cu, Cd, and Pb in the lake water of Dhaka City and (ii) to examine the seasonal and year-wise variability of trace metals in lake water on seasonal basis.

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Trace toxic metals and physicochemical parameters e.g., Pb, Cd, Cr, Ni, Co, Cu, Fe, Mn, Zn, EC, pH, Cl, F, SO_4, PO_4, CN, NO_3 concentrations were monitored three times at three different locations over a period of five years between March 2002 and September 2007; data for the year 2003 is not available.

Materials and Methods

Reagents. All chemicals were of analytical reagent grade. HNO_3, HCl and H_2SO_4 were of analar grade from BDH Laboratories. Certified reference material was obtained from the National Institute of Standards and Technology, USA. Commercially available 1000 mg/L (ICP grade) single element standard solutions (Merck or SPEX Certiprep, Metuchen, NJ, USA) were used in preparation of the working standards. Standard solutions were freshly prepared from 1000 ppm stock by dilution with deionized water (DI).

Sample collection, preparation and analysis. Water samples were collected from three locations thrice in a year between March 2002 and September 2007. Sampling was done in March (pre-monsoon), July (monsoon) and September (post-monsoon). The locations of the three sampling sites are shown in Fig. 1.

Each sample was divided into two portions, one for the analysis of metals ions and another for that of anion. pH of the portion for the analysis of metals was adjusted below 1 by addition of nitric acid to prevent adsorption to the bottle and the portion of anions was filtered, using Whatman filter #41 to remove suspended matter and stored at 4 °C. Water sample (250 mL) was quantitatively transferred to 250 mL beaker and then heated on a hot plate with 2 mL of HNO_3 until the total volume was reduced to approximately 5 mL. The concentrate

obtained was transferred to a 10 mL volumetric flask and made up to volume.

Electrical Conductivity (EC) and pH were measured using Jenway Conductivity Meter, model No. 4070 and WTW Multiline P4 Universal Meter, respectively. Concentration of anions (Cl^-, F^-, NO_3^-) were determined by ion selective electrode (ISE). For fluoride determination, 1:1 (sample: TISAB) low level TISAB was used whereas for chloride and nitrate, 2% of 5M $NaNO_3$ and 2% of 2M $(NH_4)_2SO_4$ were used, respectively, as ionic strength adjuster (ISA) according to the users manuals (Quraishi *et al.*, 2006; Chowdhury *et al.*, 2005). The concentrations of chloride, fluoride and nitrate in samples were measured by the ISE method based on direct calibration. 50 mL of each of the calibration standard solutions (0.01, 0.10, 1.0, 10.0, 100 ppm) were taken in a 100 mL beaker and the required amount of ISA buffer was added to it. The electrode potentials (mV) of the standards were measured using the digital ion-selective electrode meter (Orion Ionalyzer/model 470 A). After measurement, a calibration curve was drawn by plotting electrode potentials of the standards against their respective concentrations. Then the target electrode was connected to the meter for determination of target anion in the real samples similarly treated as the standards. From the calibration curve constructed by the instrument as mentioned above, the slope was found to be -57±3 mV/decad. The computer code in the instrument provided the concentration of anion in the sample directly by carrying out the calculations based on the calibration factor (slope: -57±3 mV/decad) and the electrode potential value of the sample. SO_4^{2-} and PO_4^{3-} concentration were measured by Shimadzu 1201 uv-visible spectrophotometer. After digestion, CRM and samples were analyzed for metal content using a three point calibration by atomic absorption spectrophotometer (Perkin Elmer 3110 and 560, USA). Standard solutions were prepared from single element standards in acid media matching with sample solution. Calibration curve was constructed for all elements using at least three different concentrations. Very good R square value of 0.9995 was obtained for all the elements.

Quality assurance. Quality assurance measures included the calculation of method detection limit, recovery and analysis of standard reference materials. To determine the DL, a low concentration standard solution was analyzed several times and the standard deviation (δ) was calculated for the data. The detection limits of the method were calculated using 10 δ, recommended by IUPAC, including preconcentration factors for the elements Pb, Cd, Cr, Ni, Co, Cu, Fe, Mn, Zn, as 10.0, 4.46, 5.07, 5.30, 1.50, 3.38, 3.0, 4.0 and 12.0 µg/L, respectively. The accuracy of the method was checked by recovery assays of known amounts of analyte added to the samples. The recovery values obtained for spiked samples were 95-102 % in case of all elements. Procedural blanks were used throughout the sample preparation and analysis to evaluate contamination from reagents, containers etc. and the procedures were validated by triplicate analysis of water samples, reference material and blanks.

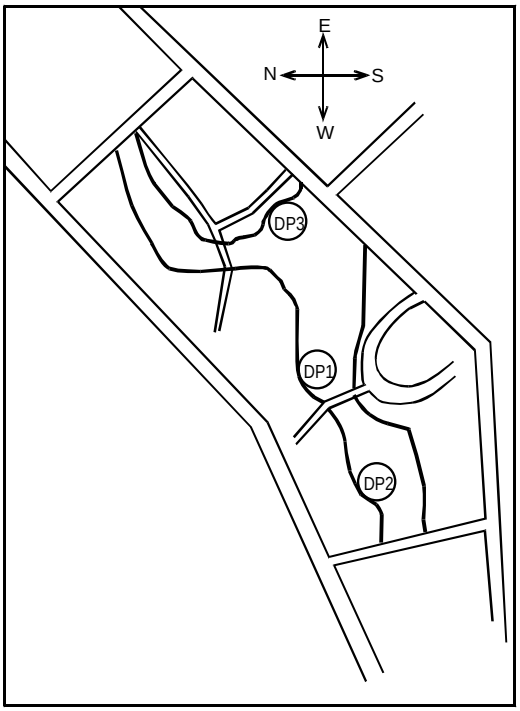

Fig. 1. Map of Dhanmondi lake

Results and Discussion

The baseline-monitoring programme was initiated in 2001 to monitor heavy metals in different lakes in Dhaka city by the

Table 1. Analytical results for analysis of NIST SRM 1643d and 1640

Elements	Method	NIST certified values for SRM 1643d, (µg/L)	Measured values (µg/L)	Trueness (%)
Fe	AAS	91.2 ± 3.9	91.5 ± 0.94	100
Mn	AAS	37.66 ± 0.83	35.55 ± 0.36	94.4
Zn	AAS	72.48 ± 0.65	69 ± 1.86	95.2
Cu	AAS	20.5 ± 3.8	21.77 ± 0.22	106
Cd	AAS	6.47 ± 0.37	7 ± 0.07	108
Cr	AAS	18.53 ± 0.20	18.75 ± 0.19	101
Ni	AAS	58.1 ± 2.7	54.54 ± 0.56	93.9
NIST SRM 1640, Trace elements in natural water (µg/L)				
Cu	AAS	85.2 ± 1.2	80.28 ± 0.81	94.2
Mn	AAS	121.5 ± 1.1	129.12 ± 1.31	106

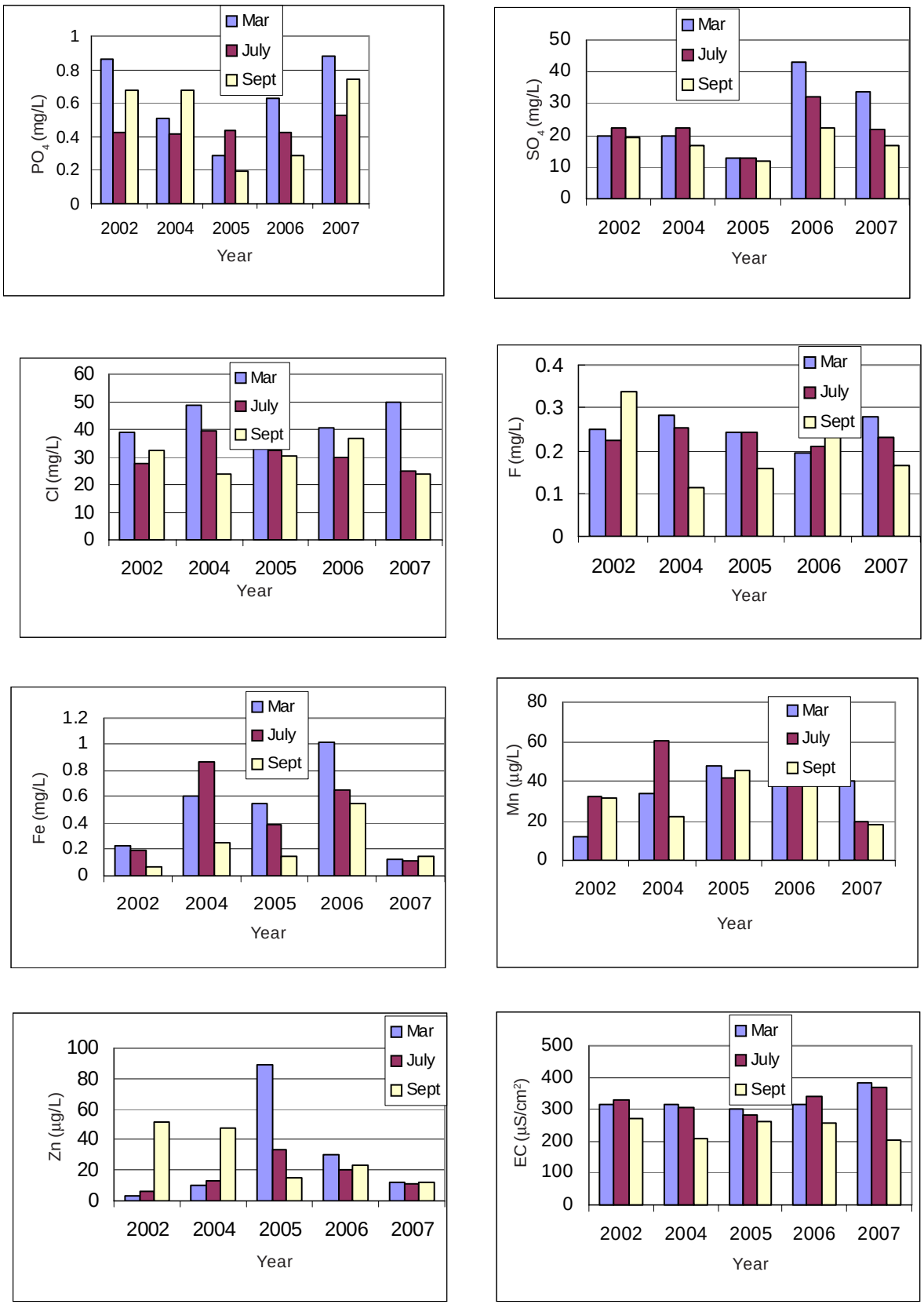

Fig. 2. Seasonal variations of different parameters of the Lake water during five years (2002-2007).

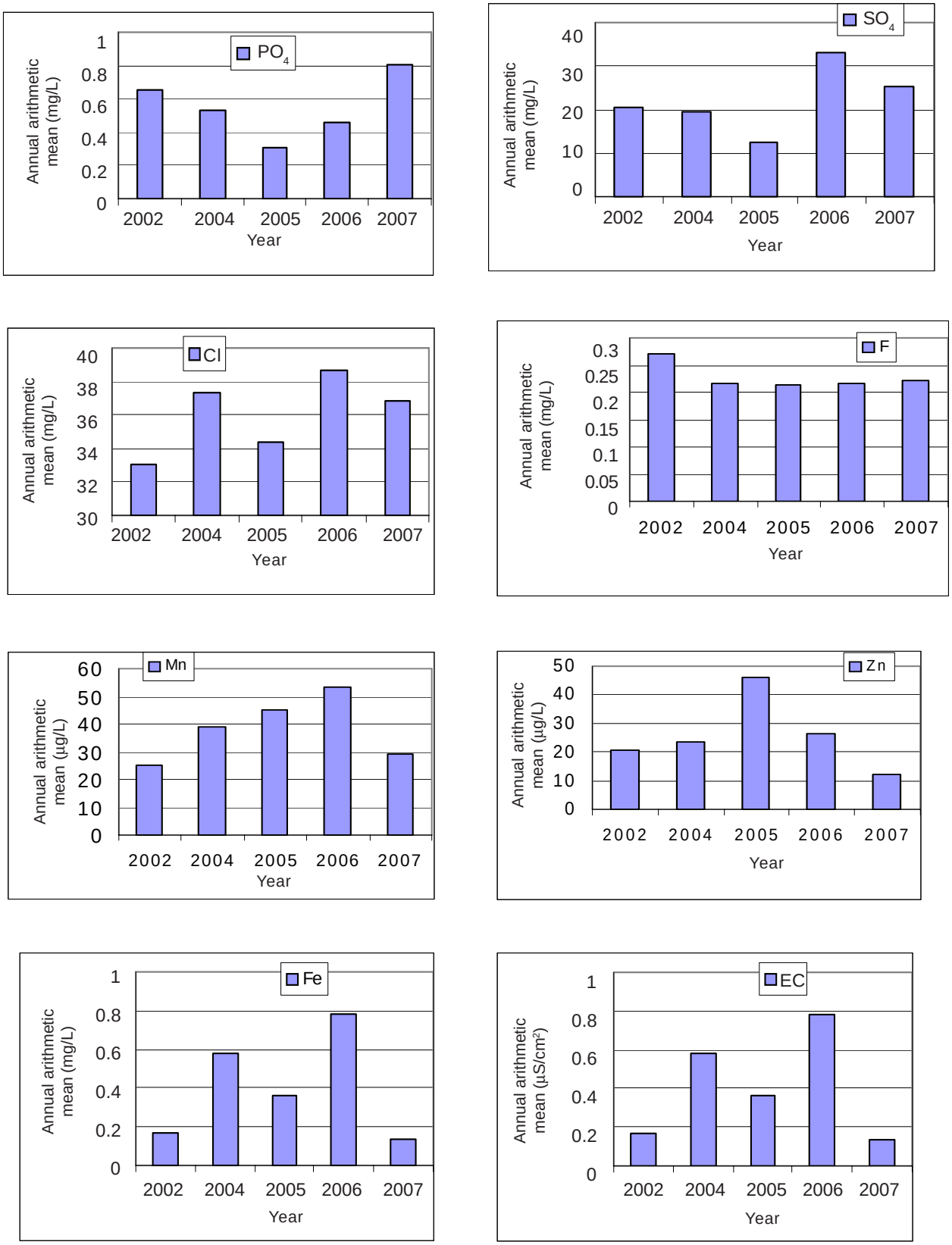

Fig. 3. Year wise variations of different parameters of Lake water during five years (2002, 2004-2007).

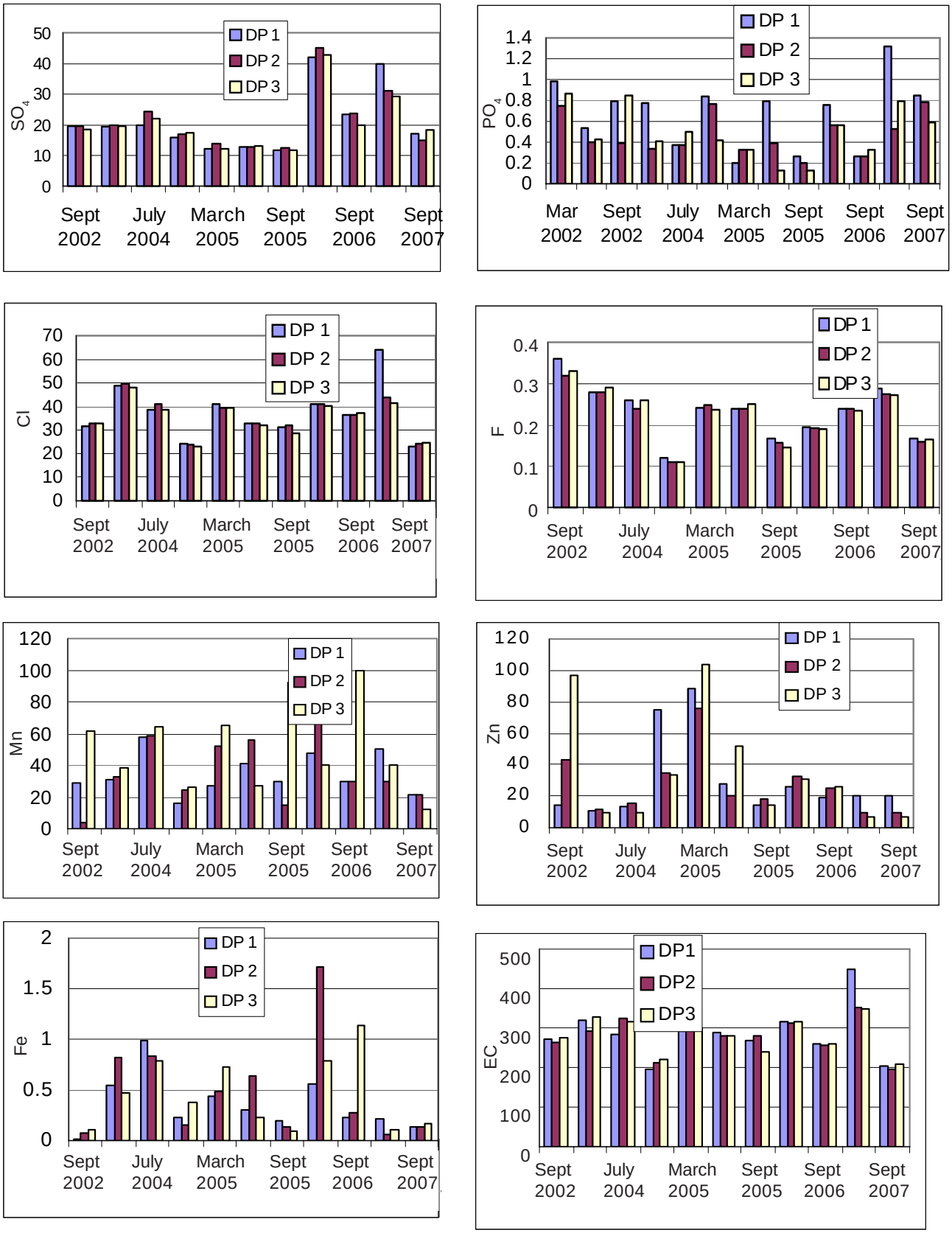

Fig. 4. Variations in different parameters of water between the sampling locations of the Lake (DP1, DP2 and DP3).

Chemistry Division of Atomic Energy Centre. Analytical results for analysis of NIST SRM 1643d and 1640 are summarized in Table 1. Good agreement was found between the measured and the certified values. The data of heavy metals and various physical parameter of water for 2002 and 2004-2007 (five years) are presented in Fig. 2-4. It was observed that during the study period (2002 and 2004-2007), the levels of various parameters including toxic metals were much lower than the drinking water standard of Bangladesh. Annual mean concentrations of phosphate in 2006 and 2007 were 0.45 and 0.81 mg/L, respectively, (Fig. 3) (MEF,1997) which were much lower than the standard set as 6 mg/L. The mean fluoride concentrations (0.20 mg/L) observed during the study period was also much lower than the standard, which is set at 1.0 mg/L. Mean sulphate concentration in Dhanmondi lake water was 17.5 mg/L which was much lower than the drinking water standard (400 mg/L). Concentrations of Pb, Cd, Cr, Co, Ni, known as toxic metals, were found to be below the method of detection limits. The levels of Fe, Mn and Zn, which are known to be the essential elements, were found to be in the range of 60-1000, 11.6-60 and 3.2-89 µg/L, respectively, the levels of Mn and Zn were much lower than the Bangladesh drinking water standards (Mn: 100 µg/L and Zn: 5000 µg/L) but were found much higher as compared to those of other lakes (Nojiri *et al.*, 1985).

Seasonal variations. Seasonal variations of phosphate, sulphate, chloride, fluoride, electrical conductivity, iron, manganese and zinc in the lake water are shown in Fig. 2 which were found to be inconsistent. Fluoride level in September 2002, was markedly high as compared to those in March and July. In the year 2004, a systematic decrease of fluoride was noticed during March to September. In case of phosphate, concentration was higher in March (pre-monsoon) than in the July (rainy season) in the year 2002. But in the year 2005, just reverse trend was observed and in the year 2006, the highest concentration was found in March and the lowest in September. Therefore, it could be concluded that seasonal variations of phosphate, sulphate, chloride, fluoride, electrical conductivity, iron, manganese and zinc were not consistent (Fig. 2). This inconsistency indicated that there is no particular or permanent source of contaminants but sudden contamination events appear which cannot be related to the local pollution events or other local anthropogenic origins but rather to the influence of tourism-related activities (Topalian *et al.*, 1999). Contamination by metals can be related to the metallic objects used in fishing activities, metallic containers and/or packing materials of food which are directly thrown into the lake by the tourists and thus contamination can increase with the increase in the number of tourists (Conde and Garcia - Montelongo, 2004).

Year wise variations. Time course changes in the concentrations of phosphate, sulphate, chloride, fluoride, electrical conductivity, iron, manganese and zinc in the lake water are shown in Fig. 3. Phosphate concentration decreased till 2005 and increased again in 2006 and 2007. Cl^- level increased from 2002 to 2004, decreased in 2005 and again increased in 2006 and fell in 2007. Sulphate concentration significantly decreased from 2002 to 2005 and again remarkably increased in 2006 and 2007. Fluoride concentration fell from 2002 to 2004 and remained almost constant until 2007. Zinc concentration increased from 2002 to 2005 and then sharply decreased in 2006 followed by 2007. On the other hand, iron concentration showed a zigzag pattern as shown in Fig. 3. In case of manganese, concentration increased till 2006 and then suddenly decreased in 2007. An increasing tendency was observed for EC from the year 2004 to 2007.

Variations between locations. The distribution of different parameters was not uniform in surface water of Dhanmondi Lake. Significant variations in iron, manganese, zinc and phosphate concentrations were observed with regard to sampling stations throughout the study period (Fig. 4).

Therefore, it was very difficult to find out a particular sampling station having the highest or the lowest contamination point in a particular season during the monitoring period. It also indicates that contamination sources are not fixed and these differences can be related to tourist activities. It can be also seen in this figure that no significant variations between the sampling stations were observed in case of sulphate, chloride, fluoride and electrical conductivity.

Conclusion

This study was focused on the evaluation of water quality of Lake and consequently on the determination of the pollution level of this aquatic environment. Seasonal variations did not show any clear pattern and were difficult to explain. A possible explanation might be made based on the fact that the lake is acceptor of both regular and non-regular pollution pulses. An increasing tendency was observed in case of electrical conductivity, phosphate, chloride and sulphate, whereas the level was unchanged in case of fluoride during the last four years of the study. Concentration of zinc, iron and manganese decreased in 2007 as compared to the previous year.

References

Azim, A. 2005. Study on Trace Elements and other Micronutrients in the Water Samples from the Rivers Buriganga and

Turga. *M.Sc Thesis*, 127 pp. University of Dhaka, Bangladesh.

Chowdhury, M.N., Akhter, S., Khan, M.M.K., Quraishi, S.B. 2005. Studies on water quality parameters in flood water. *Journal of Bangladesh Academy of Sciences,* **29:** 195-200.

Conde, J.E., Garcia-Montelongo, F.J. 2004. Heavy metals in the coastal environment of Las Galletas, Tenerife, Canary Islands, Spain. *Bulletin on Environmental Contamination and Toxicology,* **73:** 77-84.

Hadi, D.A., Tarafdar, S.A., Akhter, S. 1996. The level of aluminium in drinking water supplies and river water. *Bangladesh Journal of Scientific and Industrial Research*, **31:** 41-46.

Hadi, D.A., Tarafdar, S.A., Mia, Y. 1991. The study of nitrate, nitrite and phosphate in some river water around Dhaka City. *Nuclear Science and Applications*, **3:** 69-75.

Hossain, Z. 2005. Study on Quality of Lake Water in Dhaka City: Monitoring of Seasonal Variation of Water Quality Parameters for Evaluation of Water Pollution. *M.Sc. Thesis*, pp. 151, Gogonath College, Dhaka, Bangladesh.

Maroof, F.B.A., Hadi, D.A., Khan, A.H. 1985. Cadmium level of surface water of the Dhaka City. *Nuclear Science and Applications*, **16&17**, Series B: 145-146.

MEF, 1997. Drinking water quality criteria. In: *A Compilation of Environmental Laws (Bangladesh Gazette Additional 28)*, M. E. Huq (ed.), pp. 52-54, Department of Environment, The Ministry of Environment and Forests, Bangladesh.

Nojiri, Y., Kawai, T., Otsuki, A., Fuwa, K. 1985. Simultaneous multi element determination of trace metals in lake waters by ICP emission spectrometry with preconcentration and their background levels in Japan. *Water Research*, **19:** 503-509.

Quraishi, S.B., Chowdhury, M.N., Khan, M.M.K, Akhter, S. 2006. Impact of flood on some water quality parameters of lake waters in Dhaka City area. *Nuclear Science and Applications*, **15:** 82-85.

Topalian, M.L, Rovedatti, M. G., Castane, P.M., Salibian, A. 1999. Pollution in a low land river system. A case study: The Reconquista River (Buenos Aires, Argentina). *Water, Air and Soil Pollution,* **114:** 287-302.

Biological Sciences

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Salt Tolerance Evaluation of Rice (*Oryza sativa* L.) Genotypes Based on Physiological Characters Contributing to Salinity Resistance

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Abstract. Seven newly developed rice cultivars i.e., KS-133, DR-83, DR-64, BR-601, Gomal, JP-5 and Gomal-6, were evaluated for salinity tolerance in a glasshouse along with three varieties of known salinity tolerance i.e., KS-282 (tolerant), IR-6 (medium tolerant) and Basmati-385 (susceptible). Based on the survival percentage at 50 mol/m^3 sodium chloride salinity imposed at seedling stage, rice cultivars KS-133, Gomal, and DR-83 showed high survival comparable to that of salinity tolerant cultivars like KS-282, and were thus placed in tolerance range. Survival percentage of JP-5, Gomal-6 and DR-64 remained in medium tolerance range (35 to 38%) as that of IR-6. The rice cultivar BR-601 showed only 13% survival and was found to be as sensitive towards salinity as Basmati-385. The results of rice survival in saline medium showed good uniformity and the check varieties showed results corresponding to those found elsewhere. Sodium (Na^+) and potassium (K^+) concentrations in the third leaf showed variations among different rice cultivars under salinity. There was an inverse correlation between varietal leaf Na^+ *vs* survival percentage (r = -0.808) and Na^+ *vs* leaf chlorophyll (r = -0.857). The correlation between K^+ and final survival percentage was direct (r = 0.744) and also leaf chlorophyll *vs* survival (r = 0.952). The shoot fresh and dry weights were greater in the rice genotypes having higher final survival percentage under saline conditions. Therefore, in addition to final survival percentage, the higher shoot fresh and dry weight under salinity could be also used as criterion for evaluation of salinity tolerance of rice.

Keywords: salinity, rice, chlorophyll, salinity tolerance

Introduction

Salinization of agricultural soils is one of the major abiotic stresses reducing crop productivity worldwide. Over 6 % of the global land area and over 20% of the irrigated land are currently affected by salinity (Munns, 2005). As irrigated system supplies roughly one-third of the world food supply, therefore, addressing the problem of salinity is of great concern especially with an increasing global population. Rice is one of the most important food crops, but the yield of the grain is very susceptible to salinity (Akbar *et al.,* 1985). In Pakistan out of 6.8 million hectares of salt-affected land, over 1.5 million hectares are under rice cultivation (Khan, 1998). Thus, selection of rice cultivar having salt tolerant potential that would grow over a range of soil salinity is a prerequisite for generating income for rice farmers having sizable salt-affected lands.

Salinity resistance in rice is a complex character and many factors contribute to such resistance as occurs in species. Physiological studies suggest that in rice, restriction of sodium entry, higher potassium uptake, plant vigour, tissue tolerance to absorbed ions and water-use efficiency are the main factors contributing towards salinity resistance (Yeo *et al.,* 1990). Reduction in shoot growth under salinity limits the volume of tissue for the uptake of newly arriving salt and once it starts, the situation worsens. Accumulation of Na^+ and Cl^- in the leaves has been found to reduce photosynthetic activity, with ultra-structural and metabolic damage (Flowers *et al.,* 1985). Salinity tolerance in rice can be enhanced by reducing the influx of excessive amounts of sodium chloride in the transpiration stream. The salt concentration in the shoot can be reduced by lowering sodium transport to the shoot and/or increasing plant vigour. Normally the more vigorous plants under non-saline conditions show greater resistance to salts (Yeo and Flowers, 1986). Some traditional cultivars and landraces are more tolerant to various abiotic stresses than elite cultivars. These cultivars are good source of tolerant traits; however, they generally have poor agronomic traits.

Maximizing the salt tolerance of crop species mainly depends on two factors: availability of genetic variation to tolerance and exploitation of the genetic variation by screening and selection of plants with superior performance under the applied stress (Yamaguchi and Blumwald, 2005; Shannon *et al.,* 1994). Sensitivity of rice to salinity varies with the stage of growth. Generally, it is very sensitive to salinity stress at

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young seedling stage and less at reproductive stage (Lutts *et al.*, 1995). Hence, for selection a specific growth stage that is more sensitive to salinity stress should be targeted. Rice is considered to be generally salt sensitive; there is genetic variation for salt tolerance at critical stages in the cultivated gene pool (Moradi *et al.*, 2003; Yeo and Flowers, 1983). Therefore, selection of highly salt tolerant rice cultivars could be expected to provide useful material for breeding and for experimental comparison with unselected lines, in order to examine possible mechanism of salt tolerance. In the present study, we have reported the screening of some newly developed rice cultivars for overall performance (survival) and other physiological characters, such as leaf chlorophyll, Na^+ and K^+ concentrations and biomass accumulation under salt stress.

Materials and Methods

Plant material and growth conditions. The experiment was conducted in a glasshouse with temperature controlled between 25 ºC to 35±3 ºC. Seven newly developed rice cultivars, along with three varieties of known salinity tolerance were used in the study. The three rice varieties of known salinity tolerance used as check were; KS-282 (salt resistant), IR-6 (moderate resistant) and Basmati-385 (salinity sensitive). Sterilized rice seeds were germinated and seedlings were grown in a sand culture. Seven days old seedlings were transplanted into black boxes filled with 5 L rice culture solution (Yoshida *et al.*, 1976). The solution was renewed once a week. Each cultivar was replicated thrice in separate boxes having 30 plants per replicate. At day 11, the solution was salinized with NaCl at a concentration of 50 mol/m^3 (total electrolyte concentration resulting in electrical conductivity of 6 ds/m). The concentration of 50 mol/m^3 NaCl is an established and useful working level for eliciting a wide range of varietal response (Yeo *et al.*, 1990).

Leaf sodium, potassium and chlorophyll concentrations. Six days after salinization, third leaves of 10 plants of each cultivar was analyzed for sodium (Na^+), potassium (K^+) and chlorophyll concentration as described by Din (1997). Chlorophyll contents were extracted in 80% ethanol and calculated according to Arnon (1949).

Plant growth and survival tests. After salinization for 24 days, 30 plants (10 from each replicate) of each cultivar were harvested and the shoot fresh and dry weights were recorded. The number of dead plants was recorded every day after the first plant had died and continued till more than 50% plants of the sensitive check variety, Basmati-385, were dead; a plant was considered dead when it was totally bleached. The final survival was calculated by subtracting the number of dead plants from the total number of plants and expressed as percentage.

Results and Discussion

Survival rate was used as an indicator of genotypic performance to salinity. Based on the survival in saline medium, the cultivars were divided into three tolerance ranks. Survival of KS-133, Gomal and DR-83 was as high as that of KS-282 and therefore, these three varieties were ranked as tolerant. Survival percentage of JP-5, Gomal-6 and DR-64 was 35-36% as that of IR-6, and so these were ranked as medium tolerant, while, BR-601 showed only 13% survival as that of the sensitive, Basmati-385 (Table 1).

The concentrations of Na^+, measured 5 days after salinization, showed differences in various rice cultivars (Fig. 1). There was an inverse correlation between leaf Na^+ and survival

Table 1. Ranking of salinity tolerance of rice cultivars based on the survival under NaCl salinity (50 mM)

Salinity rank	Rice genotypes	Survival (%)
Resistant	KS-133	66
Resistant	Gomal	72
Resistant	DR-83	56
Resistant	KS-282	63
Medium	JP-5	36
Medium	Gomal-6	36
Medium	IR-6	35
Medium	DR-64	29
Sensitive	Bas-385	13
Sensitive	BR-601	13

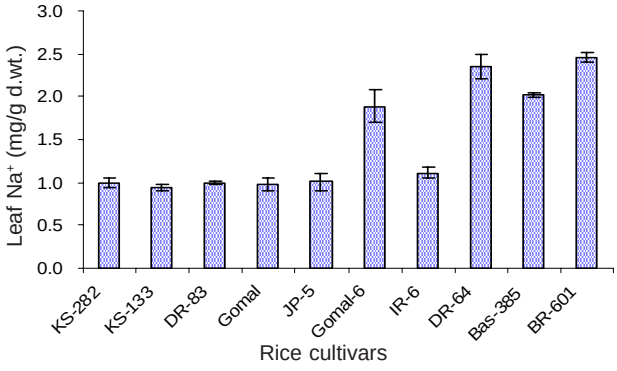

Fig. 1. Mean Na^+ concentration (mg/g dry weight) of third leaf of rice cultivars under NaCl (50 mol/m^3) salinity. Leaves were sampled 5 days after salinization. Each bar represents standard error of the mean.

(r = -0.808). Leaf K^+ showed good correlation with the salinity tolerance of rice cultivars (Fig. 2) where correlation with survival under salinity was (r=0.744, Table 2). Total chlorophyll contents measured in the third leaf after salinization was greater in the tolerant as compared to the sensitive (Fig. 3). The shoot fresh and dry weight showed good correlation with the final survival under NaCl salinity (Table 2). KS-133, Gomal and DR-83 had greater shoot fresh and dry weights and DR-64 had the least (Fig. 4).

In this study survival was used as a quantification of genotypic performance to salinity. This assessment criterion is used in the field for evaluation of salinity damage during mass screening of rice cultivars (IRRI, 1996). Based on the survival in saline medium, the cultivars were divided into three tolerance classes (Table 1). Generally, survival or visual assessment of salt damage is the criterion for overall measurement of plant performance. The characteristics would normally be chosen on their better correlation with overall performance. Yeo *et al.* (1990) concluded that survival under salinity strongly correlates with the salinity resistance of rice. In the present research study, the survival experiment was repeated three times and the results showed good uniformity; the check

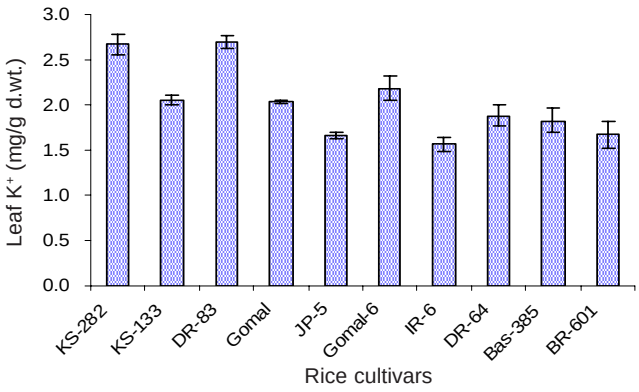

Fig. 2. Mean K^+ concentration (mg/g dry weight) of third leaf of rice cultivars under NaCl (50 mol/m^3) salinity. Leaves were sampled 5 days after salinization. Each bar represents standard error of the mean.

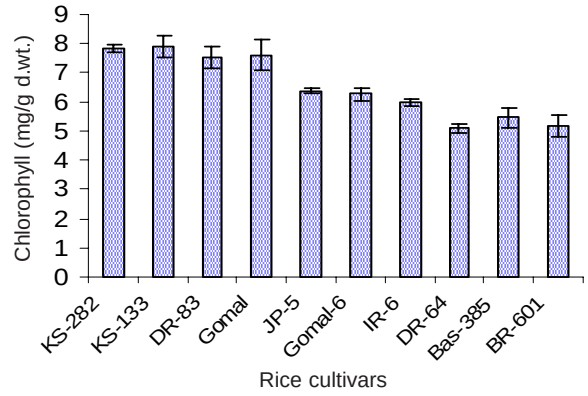

Fig. 3. Chlorophyll concentration (mg/g dry weight) of third leaf of rice cultivars under NaCl salinity (50 mol/m^3). Leaves were sampled 5 days after salinization. Each bar represents standard error of the mean.

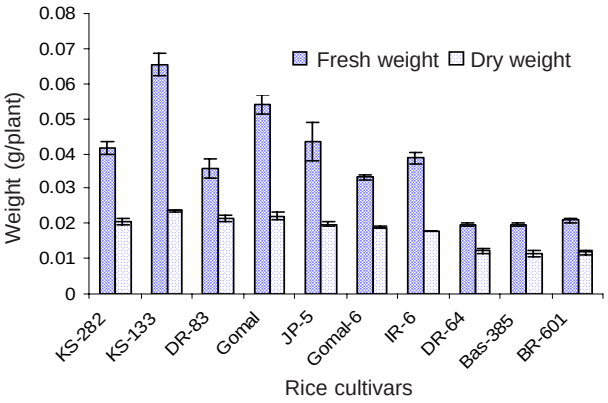

Fig. 4. Mean fresh and dry weights (g/plant) of shoots of rice cultivars, when grown for 24 days in NaCl (50 mol/m^3) salinity. Each bar represents standard error of the mean.

varieties, KS-282 and Basmati-385, showed corresponding salinity tolerance as found earlier (Khan and Abdullah, 2003) under field conditions.

In monocots, generally salinity tolerance is associated with the ability of plant to exclude Na^+ from shoot tissues (Tester

Table 2. Correlation coefficient (r) for relationship between overall performance (survival) and individual traits of the available data

	Shoot Na^+	Chlorophyll	SFW	SDW	K^+
Survival	0.808 (-)* (n = 30)	0.952 (+)* (n = 30)	0.762 (+) (n = 30)	0.886 (+) (n = 30)	0.744 (+) (n = 30)
Chlorophyll	0.857 (-) (n = 30)				

* = signs + and – represent the positive and negative correlation, respectively.

and Davenpor, 2003). In the present study, there were differences in the leaf Na^+ concentration among the rice cultivars; those having higher leaf sodium had poor survival rate (Table 1). Potassium accumulation also showed good correlation with the salinity tolerance of rice cultivars (r = 0.744). In rice, genotypic variations in Na^+ and K^+ uptake have already been reported; low concentration of Na^+ and higher K^+ were correlated with the salinity tolerance under salinity stress (Babu *et al.*, 2007; Kader *et al.*, 2006; Walia *et al.*, 2005). The chlorophyll contents measured in the third leaf after salinization was greater in the tolerant as compared to the sensitive varieties (Fig. 3). There was inverse correlation between leaf chlorophyll and Na^+ concentration (r = -0.857). Yeo and Flowers (1983) established inverse relationship between leaf chlorophyll and Na^+ concentration. The shoot fresh and dry weights were the greatest in the tolerant genotypes followed by the medium tolerant varieties and the least in sensitive rice cultivars (Fig. 4) and showed high correlation with the final survival under saline conditions (Table 2). Sankar *et al*. (2006) recorded the highest total biomass and vigour index in salt tolerant cultivars. The greater plant vigour (shoot fresh and dry weights) may provide dilution of salt concentrations with growth and tolerance within the tissues; these are the traits that may be expected to be helpful in achieving greater salinity tolerance (Yeo and Flowers, 1986). Richards (1983) concluded that vigour is essential for plant survival and productivity under saline environment. Akbar *et al.* (1985) identified more vigorous accessions, which were non-dwarf land races, as the most salt resistant in mass screening trials.

Conclusion

The data demonstrate that rice cultivars with low sodium and high potassium uptake could lead to higher survival of rice under saline conditions. The additional aspects of less chlorophyll damage under such conditions are the potential of resistant cultivars. Chlorophyll content could be used as an index of salt tolerance for selection of rice tolerance against salinity stress. It also demonstrates that amount of biomass of rice seedlings under NaCl salinity could be used as a criterion in ranking for salinity tolerance.

References

Akbar, M., Khush, G.S., Hellerislamber, D. 1985. Genetics of salt tolerance in rice. In*: Rice Genetics, Proceedings of the International Rice Genetics Symposium* IRRI, pp. 399-409, Manila, Phillipines.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts: polyphenol-oxidase in *Beta vulgaris. Plant Physiology,* **24:** 1-15.

Babu, S., Sheeba, A., Yogameenakshi, P., Anbumalarmathi, J., Rangasamy, P. 2007. Effect of salt stress in the selection of salt tolerant hybrids in rice (*Oryza sativa* L.) under *in vitro* and *in vivo* condition. *Asian Journal of Plant Sciences,* **6:** 137-142.

Din, J. 1997. Effect of Hormonal Pretreatments on the Response of Rice (*Oryza sativa* L.) and Wheat (*Triticum aestivum* L.) to Salinity. *D. Phil. Thesis,* pp. 25-26, University of Sussex, UK.

Flowers, T.J., Duque, E., Hajibagheri, M.A., McGonigle, T.P., Yeo, A.R. 1985. The effect of salinity on leaf ultra structure and net photosynthesis of two varieties of rice: Further evidence for cellular component of salt-resistance. *New Phytologist*, **100:** 37-43.

IRRI, 1996. *Standard Evaluation System for Rice*, The International Rice Research Institute, Los Banos, Manila, Philippines.

Kader, M.A., Seidel, T., Golldack, D., Lindberg, S. 2006. Expressions of OsHKT1, OsHKT2, and OsVHA are differentially regulated under NaCl stress in salt-sensitive and salt-tolerant rice (*Oryza sativa* L.) cultivars. *Journal of Experimental Botany,* **57:** 4257-4268.

Khan, G.S. 1998. *Soil Salinity/Sodicity Status in Pakistan.* pp. 1-59, Soil Survey of Pakistan, Lahore, Pakistan.

Khan, M.A., Abdullah, Z. 2003. Salinity-sodicity induced changes in the reproductive physiology of rice (*Oryza sativa* L.) under dense soil conditions. *Environmental and Experimental Botany*, **49:** 145-157.

Lutts, S., Kinet, J.M., Bouharmont, J. 1995. Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *Journal of Experimental Botany,* **46:** 1843-1852.

Moradi, F., Ismail, A.M., Gregorio, G., Egdane, J. 2003. Salinity tolerance of rice during reproductive development and association with tolerance at seedling stage. *Indian Journal of Plant Physiology,* **8:** 105-116.

Munns, R. 2005. Genes and salt tolerance: bringing them together. *New Phytologist*, **167:** 645-663.

Richards, R.A. 1983. Should selection for yield in saline regions be made on saline or non-saline soils? *Euphytica,* **32:** 431-438.

Sankar, P.D., Subbaraman, N., Narayanan, S.L. 2006. Ranking of salt tolerant rice lines based on germination and seedling growth under salt stress conditions. *Research on Crops,* **7:** 798-803.

Shannon, M.C., Grieve, C.M., Francois, L.E. 1994. Whole plant response to salinity. In: *Plant-Environment Interaction,* R.E. Wilkinson (ed.), pp. 199-244, Marcel Dekker Inc., New York, USA.

Tester, M., Davenpor, R. 2003. Na^+ tolerance and Na^+ transport in higher plants. *Annals of Botany,* **91:** 503-527.

Walia, H., Wilson, C., Condamine, P., Liu, X., Ismail, A.M., Zeng, L., Wanamaker, S.I., Mandal, J., Xu, J., Cui, X., Close, T.J. 2005. Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. *Plant Physiology,* **139:** 822-835.

Yamaguchi, T., Blumwald, E. 2005. Developing salt-tolerant crop plants: challenges and opportunities. *Trends in Plant Sciences,* **10:** 615-620.

Yeo, A.R., Yeo, M.E., Flowers, S.A., Flowers, T.J. 1990. Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *Theoretical and Applied Genetics,* **79:** 377-384.

Yeo, A.R., Flowers, T.J. 1986. Salinity resistance in rice (*Oryza sativa* L.) and a pyramidal approach to breeding varieties for saline soils. *Australian Journal of Plant Physiology,* **13:** 161-167.

Yeo, A.R., Flowers, T.J. 1983. Varietal differences in the toxicity of sodium ions in rice leaves. *Physiologia Plantarum,* **59:** 189-195.

Yoshida, S., Forno, D.A., Cock, J.H., Gomez, K.A. 1976. *Laboratory Manual for Physical Studies of Rice,* 3rd edition, International Rice Research Institute, Los Banos, Laguna, Philippines.

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Parasitic Contamination in the Table Vegetables Planted in Shiraz Plain, Iran

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Abstract. Contamination with parasites of the vegetables grown in Shiraz plains and irrigated by urban and industrial sewage-laden Shiraz Rookdhaneh Khoshk River and seasonal Soltanabad River was studied. It was found that 31.5% of the farms irrigated by the river water directly, 30.9% of the farms irrigated by water of nearby located shallow wells and 33.7% of the farms using water from the wells at a distance of one kilometer from the River were contaminated by *Ascaris* ova. 32.20% vegetables of farms irrigated by the wells located near Soltanabad river were contaminated with insects and larvae and 24.5% with *Ascaris* worm. After *Ascaris* ova, the larvae of different insects, *Strongyloides* parasite, *Sterocoralis* and *Trichostrongylus* were the contaminants most present.

Keywords: parasites, irrigation, vegetables, Shiraz rivers, *Ascaris*

Introduction

Table vegetables, particularly those irrigated with raw sewage, play an effective role in the transfer of parasites specially the soil parasites and thus in spreading contagious and parasitic diseases to the consumers (Shariatpanahi, 2001; Fereydoun, 1987). The vegetables irrigated with Firoozabad-Tehran creek in 1989 and 1990 and table vegetables used in Yasooj in 1996, were found to be contaminated with parasitic worm eggs (Sarkari, 1997; Vosooghi, 1990). In south Louisiana in USA, where city and industrial sewage contaminated the agricultural lands, vegetables such as spinach, parsley, onion, asparagus, spearmint, tomato, pea, carrot and cabbage were found to be contaminated with heavy metals, the latter being 1.60% more than the permissible limit of the American National Health Society. Moreover 28.20% of the farms were contaminated with the parasitic eggs found in sewage (Ramelow *et al*., 1992).

In Japan, the researchers of Agriculture College, Tokyo University, in 1962 conducted necessary tests for finding the contamination of leek, parsley, sweet basil, spearmint and green pepper growing in the fields which were irrigated with city and industrial raw sewage and found these vegetables, heavily contaminated with parasites whereas the content of heavy metals was 2.13% more than the standards (Chino *et al*., 1991).

The researches conducted in Iran show that the vegetables may transfer eggs of worms such as *Ascaris*, *Trichocephalus*, *Hymenolepis nana*, *Taenia*, *Fasciolia hepatica*, larvae of worms such as *Trichostrongylus* and hookworms, unicellular creatures such as *Antamoeba histolytica*, *Giardia lamblia*, *Toxoplasma gondii* which cause amoebiasis, giardiasis, (lambliasis) and toxoplasmosis and other diseases (Fereydoun, 1987).

Though irrigation method should be defined in relation to the amount of water used and the type of plants being irrigated, as each plant needs a different rate of water depending on the environment and geographical conditions such as temperature, raining rate, latitude etc., it was observed that these factors are ignored in Shiraz plain irrigation; here the irrigation system is deep water which is traditional and uses about 4,000-12,000 cubic meter water per hectare, yearly. This system causes problems relating to drainage of water, environmental contamination, agricultural damages and soil errosion etc. (Rastegar, 1992).

The present study was undertaken to find the extent of contamination of vegetables irrigated directly by Shiraz Roodkhaneh Khoshk River and Soltanabad River and by nearby lying well water. Taking into consideration the diseases, such as diarrhoea, resulting from the above mentioned practice, it is intended to propose safe means of growing vegetables and prevent regional contamination of vegetables with parasites and their transfer to the consumers.

Materials and Methods

City sewage is discharged into Shiraz Roodkhaneh Khoshk River passing through the city centre. Some districts of the city do not have proper sewage disposal means and the factories beside the river also dump their waste products

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into it. Due to disposal of garbage into the river, some agricultural irrigation places have high BOD (the index indicating sewage contamination) related to city sewage (WHO, 1989).

This study covers table vegetables planted in the farms around Shiraz where the vegetables are consumed either raw or cooked. The areas studied included Gheisar Aboonasr, Mehraghan, Eghbal Abad, Torkan, Nasirabad, Kooshkak, Mahfiroozan, Dasht Khezr, Noortaban, Khaljooy and Sharifabad villages, mountainous region and Kaftarak village, all of them located beside Roodkhaneh Khoshk River.

Parameters taken into consideration were area of the land under cultivation, type and amount of harvest, fertilizer and disinfectants, herbicides, fungicides and means of irrigation including deep and semi-deep wells and river.

The land for cultivation of table vegetables, domestic animals provender, wheat, barley and potato was about 1,372 hectares of which 128 hectares was under cultivation of the provender including alfalfa, 168 hectares that of barley, wheat and maize and 100 hectares were used for planting of potato. The land used for cultivation of vegetables was 276 hectares of which 90 hectares were irrigated with river and 186 hectares irrigated mostly by shallow wells.

Samples (140) of different vegetables cultivated in Kaftarak and Soltanabad were selected on the basis of the factors such as means of irrigation including shallow wells, wells within one km radius of the river and the river itself; one type of vegetable from each farm was sampled. 45 samples were taken from farms irrigated with river and 95 samples, from farms irrigated with deep, semi-deep and shallow wells.

The following vegetables, 250-500 g of each, were sampled:

a) Sweet basil, leek, spearmint, cress, purslane, parsley lettuce, tarragon, common dill and spinach (eaten raw).

b) Radish, tomato, cucumber, eggplant, green pepper, carrot, onion, cabbage and squash (eaten cooked except carrot)

c) Green bean, pea and okra (eaten cooked).

The samples were collected with gloved hands and put into nylex bags and carried to the related laboratories (Gholami and Mohammadi, 1998). Domestic animals provender was sampled in the same way.

Results and Discussion

Samples of 58 shallow, semi deep and deep wells were subjected to bacteriological tests. Results indicated that 98% of the wells were contaminated with coliform, 100% of which were soil coliform. Physical and chemical properties of water did not conform to the standards, except that of 12 deep wells lying at a distance of 1,500 m from the river.

Findings show that the parasitic contamination of table vegetables depends on location of the place/farm to be irrigated, source of irrigation and type of the parasites (Table 1). Most of the farms around Roodkhaneh Khoshk river were contaminated with "*Ascaris*" and larvae of different insects. 31.50% farms irrigated directly by river (Fig. 1), 31% farms irrigated by the wells located around the Roodkhaneh Khoshk river (Fig. 2) and 33.70% by the wells within one kilometer radius of the river were contaminated with ascaris eggs; mostly the fertilizer used was human faeces (Table 1); bacteriological tests of shallow regional well water indicated faecal coliform contamination, 3 to 45 MPN/100 mL, and total coliform, 70 to 800 MPN/100 mL, whereas, total coliforms in deep well water were 5 to 45 MPN/100 mL. Physical and chemical specifications of the water used for irrigation of Shiraz plains and Roodkhaneh Khoshk farms were not within the standard limits necessary for agriculture with BOD more

Table 1: Parasitic contamination in the vegetables according to the irrigating source and the type of parasite

Parasite	Roodkhaneh Khoshk water		Wells near Roodkhaneh Khoshk		Well within radius of 1 km from the river		Wells near Soltanabad river	
	No. of parasites	%	No. of parasites	%	No. of parasites	%	No. of parasites	%
Trichostrongylus	35	15.5	19	16.8	13	16.3	12	19.6
Strongyloides stercoralis	32	14.2	13	11.5	19	23.7	10	16.4
Ascaris lumbricoides	71	31.5	35	31	27	33.7	15	24.6
Trichocephalus	4	1.78	0	0.0	0	0.0	0	0.0
Oxyure	19	8.5	0	0.0	2	2.6	4	6.6
Different insect larvae and eggs	64	28.5	46	40.7	19	23.7	20	32.8
Total	225	100.0	113	100.0	80	100.0	61	100.0

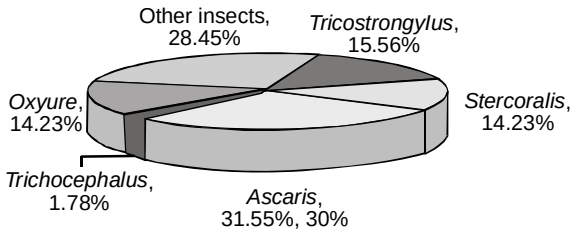

Fig. 1. Pollution (%) of vegetables irrigated *via* water of Khoshk river with different parasites.

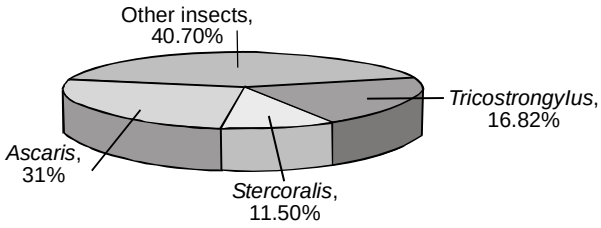

Fig. 2. Pollution (%) of vegetables irrigated *via* wells close to Khoshk river with different parasites.

than 100 mg/L. Also the level of some heavy metals was not within the range applicable for agricultural use. Water EC (electrical conductivity) of some well water was high, but all the wells had coliform contamination.

In transfer of parasites, three factors are involved: source of infection, means of transfer and a sensible host, which means the process depends on the way of distribution of the parasite in a defined place at a defined time. The means by which the parasite reaches from the source to the host are clear (Neva and Brown, 2001). Some parasites reach the host through direct contact whereas others have a more complicated life cycle and need to pass through some growth stages such as free life or need an intermediate host to become infective. The transfer is accomplished through direct and indirect contact, such as by means of food, water, soil and vertebrates and arthropodes. Studies show that rate of contamination with some worms in different parts of Iran is high; in Iran, around 32 human parasites were found, some of which were very common and frequent such as *Ascaris* (Mahvi, 1996). The vegetables, contaminated with parasite eggs, transfer them to the consumers (Monzavi, 1985). Raw sewage, human faeces and contaminated water, used as fertilizer and for irrigating farms, are dangerous sources of contamination for the consumers of the final products because traditional and deep water systems are still used here as the sources of potable water.

Tests showed that all the table vegetables cultivated in Shiraz plains and irrigated with Roodkhaneh Khoshk river, and the wells close to the river were contaminated with one or several faecal parasites. The range of pollution in vegetables irrigated directly by the river was (with the exception of 2% in cabbage) from 6% (in gourd) to 35% (in green beans) (Fig. 3) and in those irrigated by close-by lying wells was 4% (in gourd cabbage and onion) to 10% (in green beans) (Fig. 4). Thus it is clear that most of the contamination relates to the means of irrigation and use of man faeces (US EPA, 1981).

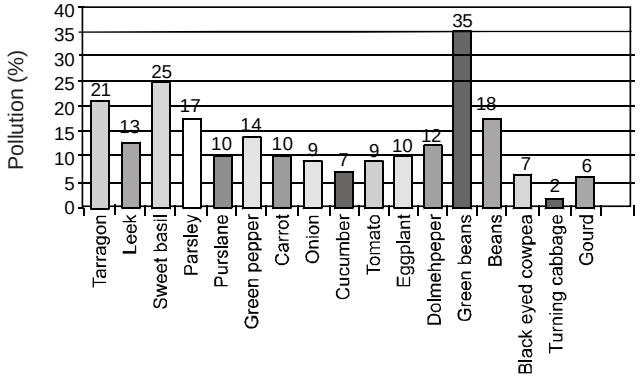

Fig. 3. Parasitic pollution (%) of different vegetables irrigated *via* water of Khoshk river.

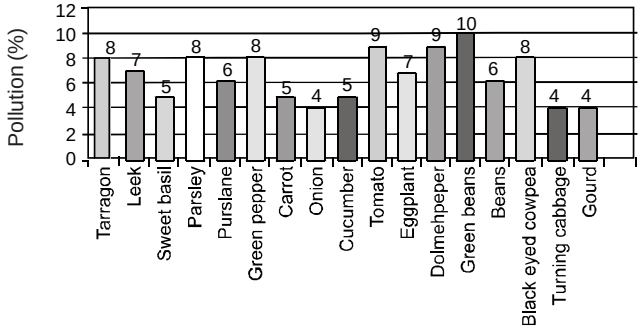

Fig. 4. Parasitic pollution (%) of different vegetables irrigated *via* wells close to Khoshk river.

The land measuring 276 hectares is used for cultivation of table vegetables and is irrigated with Roodkhaneh Khoshk river; these raw vegetables are distributed in Shiraz and other adjoining cities. Also the wells located within a radius of one km of the river, when tested bacteriologically, whenever less deep and farther from the river, were more contaminated; it shows that the wells were influenced by the river. The distant well water had parasitic eggs found in human faeces (used in the farm) and the differences were as follows:

a) The parasitic contamination was more in the vegetables of farms irrigated with the river water directly.

b) Contamination of the vegetables with parasite eggs was less in the farms irrigated by hand and shallow wells farther from the river than the farms irrigated directly by the river water .

c) Contamination in the farms, irrigated by deep wells more than one km distant from the river, was less than the above-mentioned two cases.

So, it is clear that the river water contaminated by sewage played main role in contaminating the consumable raw vegetables with parasite eggs.

It was found that green beans, sweet basil and tarragon were more contaminated with the eggs and larvae of *Ascaris* and *Trichostrongylus* than with the other insects. Also the farms irrigated by the shallow wells and the wells, within a radius of 50 m of the river, had vegetables contaminated with parasite eggs similar to the farms irrigated by the river water.

It was also found that the farms irrigated by the wells around the seasonal river, Soltanabad, had eggs of strongylosis, sterocoralis, ascaris and trichostrongylosis, more than other parasite eggs.

The contamination of vegetables with parasite eggs of farms irrigated by Roodkhaneh Khoshk river was found to be more frequent as compared to those irrigated by the wells beside the seasonal river, Soltanabad.

Conclusion

The results show that the vegetables cultivated in Shiraz plain, irrigated/fertilized by sewage-containing water, play an important role in transferring important parasites such as ascaris, trichostrongylus, trichocephalus, *S. stercoralis* and oxyure. Thus sewage and human fertilizers have been the cause of contamination of farm vegetables irrigated by such means. Irrigation of agricultural farms with such sources of contamination is harmful from hygienic point of view of the consumers of crops, grown in such farms.

It is therefore, proposed that the standards and regulations relating to the irrigation water used specially for raw vegetable crops should be observed. It is recommended that unrefined as well as refined sewage may not be used for irrigating vegetable crops and for fertilization; instead animal fertilizer, whose conditions of maintenance have been observed, be used.

References

Chino, M., Moriyama, K., Saito, H., Mori, T. 1991. The amount of heavy metals derived from domestic sources in Japan. *Water, Air and Soil Pollution*, **57:** 829-837.

Fereydoun, A. 1987. *Medical Helminthology*, vol. **1** & **2**, 3rd edition, Danesh Pajouh Publications, Tehran, Iran.

Gholami, M., Mohammadi, H. 1998. *Water and Wastewater Microbiology*, Hayan Publications, Tehran, Iran.

Mahvi, A.H. 1996. *Sewage Filtration in Torrid Zone*, Jahad Daneshgahi Publications Organization, Tehran, Iran.

Monzavi, M.T.I. 1985. *Water Supply*, 4th edition, Tehran University Publications, Tehran, Iran.

Neva, F.A., Brown, H.W. 2001. *Basic Clinical Parasitology* (Translated by A. Athari), Pezhman Publications, Tehran, Iran.

Ramelow, G.J., Biven, S.L., Zhang, Y., Beck, J.N., Young, J.C., Callahan, J.D., Marcon, M.F. 1992. The identification of point sources of heavy metals in an industrially impacted water way by periphyton and surface sediment monitoring. *Water, Air and Soil Pollution*, **65:** 175-190.

Rastegar, M. 1992. *Dry Farming*, 1st edition, Behramand Publications, Tehran, Iran.

Sarkari, B. 1997. Examining parasitic contamination of the vegetables used in Yasuj city. *Journal of Yasuj University of Medical Sciences*, **3:** 15-18.

Shariatpanahi, M. 2001. *Principles of Quality and Treatment of Water and Wastewater*, 6th edition, Tehran University Publications, Tehran, Iran.

US EPA, 1981. *Process Design Manual for Land Treatment of Municipal Wastewater*, US Environmental Protection Agency, Rept. 625 11-77-008, US. Govt. Printing Office, Washington DC., USA.

Vosooghi, M. A. 1990. Examining the Worm Parasitic Eggs Contamination of the Vegetables Irrigated by Firoozabad River, *MS. Thesis*, Tehran Medical Sciences University, Tehran, Iran.

WHO, 1989. *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture*, WHO Technical Report Series 778, World Health Organization, Geneva, Switzerland.

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Microbiological Quality of Drinking Water and Beverages in Karachi, Pakistan

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Abstract. Microbiological assay of 780 water samples and 1220 beverage samples (412 branded and 808 unbranded), collected from 490 different schools, both government (98 schools) and private (392 schools), situated in different areas of the city of Karachi, was conducted for bacterial heterotrophic plate count, total coliforms, faecal coliforms, *E. coli*, faecal streptococci, *Pseudomonas* and *Salmonella* species. The counts ranged from 0 to 2.5×10^5 cfu/mL and from 0 to 10^6 cfu/mL in water and beverage samples, respectively. About 36% of water samples and 48% of unbranded beverage samples were contaminated with the indicator and the pathogenic bacteria; all the branded beverage samples were found fit for human consumption from microbiological viewpoint.

Keywords: drinking water, beverages, microbiological quality

Introduction

Raw water itself does not contain large number of micro-organisms. Drinking water contains assimilable organic compounds that allow a certain degree of bacterial growth (Exner *et al*., 2005). Improperly installed hand pumps permit infiltration of contaminated surface water, whereas unclean storage devices and other factors contribute to disease cycle, malnutrition and high mortality. Infectious diseases caused by pathogenic bacteria are the most common and wide spread health risk associated with drinking water. Some of the pathogens, which are transmitted through contaminated drinking water, lead to severe and sometimes life threatening diseases particularly in children. The potential of drinking water to transport microbial pathogens to large number of population, causing subsequent illness, is well documented in different countries.

The most common and widespread risk associated with drinking water is its contamination by human or animal excreta. The potential consequences of microbial contamination necessitates that its control be of paramount importance. Pathogens in drinking water, presenting serious risk of diseases, include *Salmonella* sp., pathogenic *Escherichia coli*, *Pseudomonas*, *Vibrio cholerae* etc. Faecal specific bacteria such as coliforms, faecal coliforms and *E. coli* are the parameters of importance in monitoring faecal pollution.

The survival and growth of microorganisms in processing environments of foods, such as beverages, sherbets, ice creams, ice-lollies, etc. may lead to contamination of the finished products, resulting in reduction of microbiological safety and quality (Kohnen *et al*., 2005). However, several studies have demonstrated that the total counts and number of pathogens may get reduced due to the acidity and the effect of CO_2 during storage. (Mugochi *et al*., 1999; Simango and Rukure, 1992; Sheth *et al*., 1988; Zschaler, 1979; King and Nagel, 1975; 1967).

In Pakistan people lack access to adequate supply of safe water for daily use. The basic sanitary facilities are very poor and are not available for half of the population. Sources of microbial contamination of water and beverages include raw materials, processing equipment or utensils, human activities, sanitation practices, workers or handlers, waste materials, animal and insect pests and microbial growth niches. Chemical composition of foods and beverages and the environmental factors, such as water activity, pH, temperature, etc., determine the types of organisms that can grow there.

The present study was undertaken keeping in view the hazards of polluted or contaminated drinking water and the effect of its use in beverages and ice lollies etc. The survey provides base line data for authorities to set the guidelines for microbiological quality of water and beverages within the country.

Materials and Methods

Sample collection. A total of 780 water and 1220 beverage samples were collected from 490 different schools/educational institutes of Karachi, located in various areas including those inhabited by low income, middle class and upper middle class population, as well as rich and wealthy people. Both the government and the private institutions were included in the

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study, out of these 98 were government schools whereas 392 were private institutions. Insulated ice chest with ice packs was used for collection and transportation of samples. The collected samples were labelled with date and laboratory code. Other necessary information about the samples, like area or location of school, collection point etc. was recorded on prescribed forms. Samples were collected in sterilized screw-capped glass bottles.

Beverage samples were categorized as branded and unbranded. The unbranded beverages consisted of gola-gandas (local name for an item made by crushed ice and additives), sherbets (drinks) and carbonated and other drinks sold on vending carts (thailas). All the water and beverage samples were tested microbiologically for their heterotrophic plate counts, total coliforms, fecal coliforms, *E. coli* 0157:H7, faecal streptococci, *Pseudomonas* and *Salmonella* species.

Heterotrophic plate count (HPC), coliforms and faecal coliforms were tested according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1998; ISO 9308-1: 2000; 9308-2: 1990; 6222: 1988a; 8199: 1988b). *E. coli* 0157:H7 was tested by serological kit method (Pro-Lab Diagnostics) according to Thompson *et al*. (1990) and faecal streptococci were determined using ISO method 7899-1 (1984). Other parameters were tested according to on-line Bacteriological Analytical Manual of US FDA (2006; 2002; 2001). *Salmonella* was confirmed using antisera (Pro-Lab Diagnostics).

Results and Discussion

Figures 1 and 2 present total heterotrophic plate count (HPC) in water and beverage samples, respectively. The heterotrophic plate count in water and beverage samples ranged from 0 to 2.5×10^5 cfu/ml and from 0 to $<10^6$ cfu/ml, respectively. HPC may be used to assess the general bacterial content as well as the efficiency of water treatment. The HPC standards for drinking water vary a lot from country to country. According

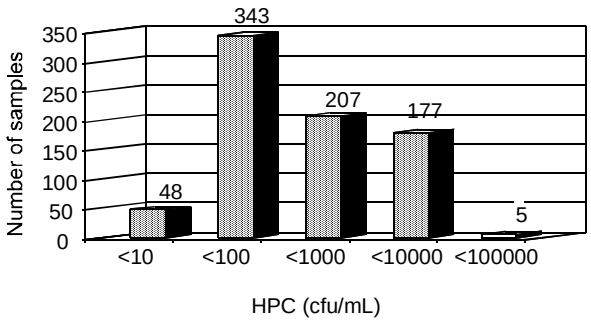

Fig. 1. Heterotrophic plate counts of water samples.

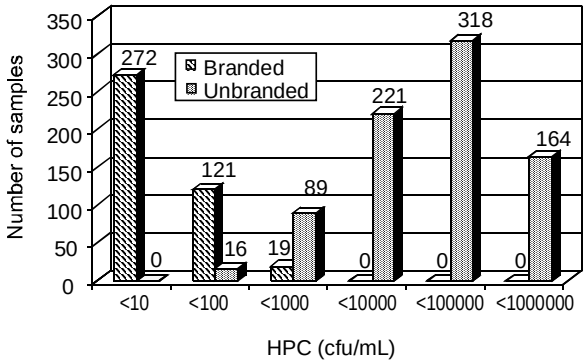

Fig. 2. Heterotrophic plate counts of beverage samples.

to WHO (1999) guidelines for drinking water quality, the limit for HPC is 100 cfu/mL which has also been adopted by Pakistan Standards and Quality Control Authority (PSQCA; 2004).

However, WHO guidelines for drinking water quality are intended to be used as a basis for the development of national standards in the context of local or national environmental, socio-economical and cultural conditions (WHO, 1999). The HPC standard for drinking water in Sri Lanka is 10,000 cfu/mL (SLS, 2001; 1995), which is a pretty relaxed standard, compared to the stringent standard of WHO. According to Canadian standard, HPC is not considered as a parameter of drinking water quality. A review study conducted by Allen *et al*. (2004) reveals that there is no evidence to support health-based regulations of HPC concentrations.

The national standards should be influenced by national priorities and economic factors. Stringent standards could limit the availability of water supplies, which is a significant consideration in regions of water shortage. However, public health must never be endangered. For this very reason, it is suggested that the WHO standard for HPC must not be adopted as the criterion for rejection of line water samples for drinking purpose and the acceptance limit must be adopted according to Sri Lankan standard or the Canadian guidelines. The presence of indicator organisms and/or pathogens only must be considered as the criterion for declaring a sample unfit for human consumption. However, we do support adoption of WHO guidelines for bottled water according to which the samples must not contain any count at all as the manufacturers/bottlers are claiming it to be pure and charging extra money for that.

Figure 2 presents total bacterial counts in beverage samples. According to the PSQCA (2002) standard PS: 1654-2002 R, the freshly prepared carbonated drinks may contain <100 bacteria per mL of the sample whereas the count must

decrease to <30 within three days of storage. The counts in the branded samples were mostly within the limit of PS standard with only 19 of the 412 tested samples having counts exceeding 100 mL.

Coliform organisms are recognized as suitable microbial indicators of drinking water quality. Coliforms include heterogeneous lactose fermenting bacteria found in faeces and environment. Detection of coliforms suggests inadequate treatment, post-treatment contamination or excessive nutrients. Although coliforms may not always be related to the presence of faecal contamination or pathogens, they are useful for monitoring the microbial quality of public water supplies. Presence of coliforms in the absence of faecal coliforms suggests the use of secondary indicators like faecal streptococci for confirmation of faecal contamination.

Figures 3 and 4 present the occurrence of organisms of public health significance in water and beverage samples, respectively. The microbiological analyses revealed that a total of 36% water samples and 48% of unbranded beverages were unfit for human consumption due to the presence of organisms of public health significance i.e. indicator organisms and/or pathogenic bacteria. The branded samples, on the other hand were all free from organisms of public health significance.

In the present study, a total of 269 out of 780 water samples (34.5%; Fig. 3) and 549 out of 808 unbranded beverages samples (45%, Fig. 4) were found contaminated with coliform bacteria (Noble *et al*., 2004, 2003; Reasoner and Galdreich, 1985).

Thermotolerant (faecal) coliform group comprises of *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter*, which are able to ferment lactose at 44-45 °C. Out of these organisms, only *E. coli* is specifically of faecal origin; other thermotolerant coliforms may originate from organically enriched waters such as industrial effluents or from decaying plant materials and soils.

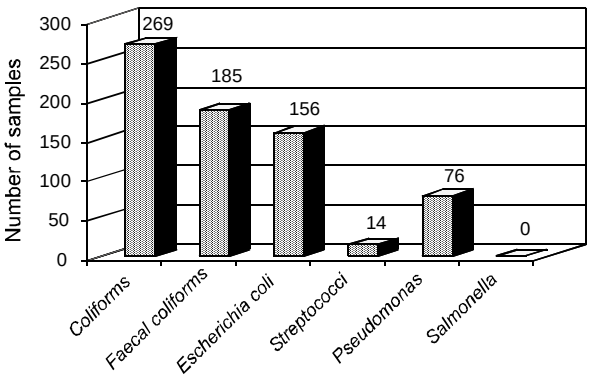

Fig. 3. Incidence of organisms of public health significance in water samples.

Out of 780 water samples, 185 (23.5%) and 156 (20%) (Fig. 3) and out of 808 unbranded beverages, 549 (45%) and 463 (38%) (Fig. 4) samples were found contaminated with faecal coliforms and *E. coli*, respectively.

The presence of *Pseudomonas aeruginosa* in potable water also indicates serious deterioration in bacteriological quality and is often associated with complaints about taste, odour and turbidity linked to low rates of flow in distribution system and a rise in water temperature.

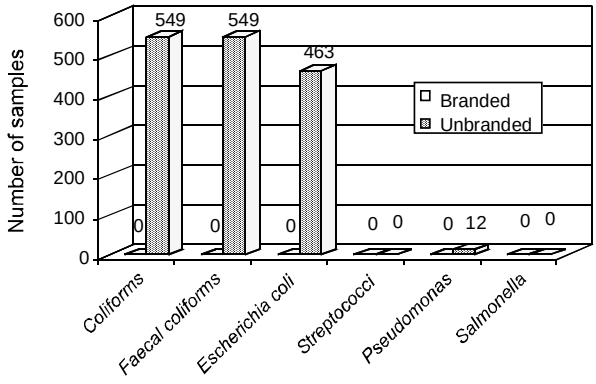

Fig. 4. Incidence of organisms of public health significance in beverage samples.

Conclusion

The present study has shown that the microbiological quality of water and locally made beverages vended in schools is not satisfactory and approximately 36% water samples and 48% unbranded beverage samples were found to be unfit for human consumption due to the presence of organisms of public health significance i.e. indicator organisms and/or pathogenic bacteria.

Water sources must be protected from contamination by the human and animal excreta and other wastes to protect the community from the risks of outbreaks of intestinal and other infectious diseases. Moreover, effective treatment and regular monitoring must be carried out in order to protect the water reservoirs. Furthermore, strict sanitary and hygienic regulations must be imposed on the local manufacturers of beverages; the sanitary conditions of the vending carts and the beverages being sold through them must also be effectively monitored.

References

Allen, M.J., Edberg, S.C., Reasoner, D.J. 2004. Heterotrophic plate count bacteria - what is their significance in drinking water. *Intternational Journal of Food and Microbiology*, **92:** 265-274.

APHA, 1998. Microbiological examination. In: *Standard Methods for the Examination of Water and Wastewater*, 20th edition, American Public Health Association, Washington DC., USA.

Exner, M., Vacata, V., Gebel, J. 2005. Public health aspects of the role of HPC - An introduction. In: *Heterotrophic Plate Counts and Drinking Water Safety*, J. Bartram Cotruvo, M. Exner and A. Glasmacher (eds.), AITBS Publishers, New Delhi, India.

ISO 9308-1:2000(E). Detection and enumeration of *Escherichia coli* and coliform bacteria. Part 1. Membrane Filtration Method.

ISO 9308-2:1990(E). Water quality - detection and enumeration of coliform organisms, thermotolerant coliform organisms, and presumptive *Escherichia coli*. Part 2: Multiple Tube (Most Probable Number) Method.

ISO 6222:1988a. Enumeration of viable microorganisms. Colony count by inoculation in or on nutrient culture medium.

ISO 8199:1988b. General guide to enumeration of microorganisms by inoculation in or on culture medium.

ISO 7899-1:1984. Detection and enumeration of faecal streptococci-Part 1: Method by enrichment in a liquid medium.

King, Jr., A.D., Nagel, Ch. W. 1975. lnfluence of carbon dioxide upon the metabolism of *Pseudomonas aeruginosa*. *Journal of Food Science*, **40:** 362-366.

King, Jr., A.D., Nagel, Ch. W. 1967. Growth inhibition of a *Pseudomonas* by carbon dioxide. *Journal of Food Science*, **32:** 575-579.

Kohnen, W., Teske-Keiser, S., Meyer, H.G., Loos, A.H., Pietsch, M., Jansen, B. 2005. Microbiological quality of carbonated drinking water produced with in-home carbonation systems. *International Journal of Hygiene and Environmental Health*, **208:** 415-423.

Mugochi, T., Parawira, W., Mpofu, A., Simango, C., Zvauya, R. 1999. Survival of some species of *Salmonella* and *Shigella* in mukumbi, a traditional Zimbabwean wine. *International Journal of Food Science and Nutrition*, **50:** 451-455.

Noble, R.T., Leecaster, M.K., McGee, C.D., Weisberg, S.B., Ritter, K. 2004. Comparison of bacterial indicator analysis methods in stormwater-affected coastal waters. *Water Research*, **38:** 1183-1188.

Noble, R.T., Moore, D.F., Leecaster, M.K., McGee, C.D., Weisberg, S.B. 2003. Comparison of total coliform, fecal coliform and enterococcus bacterial indicator response for ocean recreational water quality testing. *Water Research*, **37:** 1637-1643.

PSQCA, 2004. Pakistan Standard Specification for Drinking Water. PS: 4639-2004 (R), Pakistan Standards & Quality Control Authority, Standards Development Centre.

PSQCA, 2002. Pakistan Standard Specification for Carbonated Beverages. PS: 1654-2002 (R), Pakistan Standards and Quality Control Authority, Standards Development Centre.

Reasoner, D.J., Geldreich, E.E. 1985. A new medium for the enumeration and subculture of bacteria from potable water. *Applied Environmental Microbiology*, **49:** 1-7.

SLS, 2001. Sri Lankan Standards 894, specification for packaged bottled drinking water.

SLS, 1995. Sri Lankan Standards 1038. Specification for bottled natural mineral water.

Sheth, N.K., Wisniewski, T.R., Franson, T.R. 1988. Survival of enteric pathogens in common beverages: an *in vitro* study. *American Journal of Gastroenterology*, **83:** 658-660.

Simango, C., Rukure, G. 1992. Survival of bacterial enteric pathogens in traditional fermented foods. *Journal of Applied Bacteriology*, **73:** 37-40.

Thompson, J.S., Hodge, D.S., Borczyk, A.A. 1990. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O157. *Journal of Clinical Microbiology*, **28:** 2165-2168.

US FDA, 2006. *Bacteriological Analytical Manual*, An Online publication of Center for Food Safety & Applied Nutrition of USFDA. Chapter 3 (2001), Chapter 4 (2002), Chapter 5 (2006).

WHO, 1999. *Guidelines for Drinking-Water Quality*. *Health Criteria and Other Supporting Information*, vol. **2**, 2nd edition, AITBS Publishers, World Health Organization, Geneva, Switzerland.

Zschaler, R. 1979. Influence of carbon dioxide on the microbiology of beverages. *Antonie van Leeuwenhoek* **45:** 158-160.

Short Communication

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Feeding Inter-Relationship of *Caranx hippos* (Linneaus), *Chrysichthys nigrodigitatus* (Lacepede), *Ethmalosa fimbriata* (Bowdich) and *Mugil cephalus* (Linneaus) in Lagos Lagoon, Nigeria

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Abstract. Study of the feeding inter-relationship of *Caranx hippos, Chrysichthys nigrodigitatus, Ethmalosa fimbriata* and *Mugil cephalus* in the Lagos Lagoon, Nigeria, revealed that algae and diatoms formed the main food items of the four fish species; other food items were crustaceans, molluscs and detritus. Utilization of nearly identical food items suggested inter-specific competition for food.

Keywords: feeding habits, *Caranx hippos, Chrysichthys nigrodigitatus, Ethmalosa fimbriata, Mugil cephalus*, Nigeria

Study of food and feeding habits of fish requires continuous research, since successful fishery management, aquaculture and capture fishery programmes are based on it (Oso *et al*., 2006).

Caranx hippos, Chrysichthys nigrodigitatus, Ethmalosa fimbriata and *Mugil cephalus* are some of the fish species readily available in the Lagos Lagoon, Nigeria in West Africa, and make up an important part of artisanal fisheries. Several studies of the food and feeding habits of these four fish species have been made, some of which include the work of Oronsaye and Nakpodia, (2005) and Blay (1995). However, information on the feeding inter-relationship of these species is lacking. In this paper, a report on the feeding inter-relationship among the four fish species is presented.

Forty specimens of the above-mentioned four fish species were caught in the Lagos lagoon each month during February to May 2001. Body weights and lengths of fish were measured and the stomach contents were studied. The organisms found were identified to the species level and analyzed by numerical and frequency of occurrence method.

Analysis of the food of *C. hippos* revealed diatoms and algae to form the major food items. Other food items were molluscs (*Aloidis trigona*, bivalve shell and *Tympanotonus fuscatus*), crustaceans (*Calanus finmarchicus*, shrimp and shrimp parts), fish (eggs, bones, scales and flesh) and detritus and other unidentifiable matter (Fig. 1).

Diatoms formed the major food items of *C. nigrodigitatus*. Other food items were molluscs (*Aloidis trigona*, bivalve shell and *Tympanotonus fuscatus*), crustaceans (shrimp parts, *Calanus finmarchicus*, cladocera and crab appendages), fish (fins, scales, eggs and bones), algae, plant material and unidentifiable matter (Fig. 2).

Diatoms were the major food items of *E. fimbriata* as well. Other food items were crustaceans (*Calanus finmarchicus*, shrimp parts, isopods and cladocera), fish (bones, scales and eggs), algae, plant materials and unidentifiable matter (Fig. 3).

Major food items in the gut of *M. cephalus* were diatoms. Other food items were crustaceans (*Calanus finmarchicus* and shrimp parts), fish (scales and bones), algae, plant materials and unidentifiable matter (Fig. 4).

Analysis of the food items in the gut of four fish species revealed that *C. hippos* did not feed on fish in February and April, molluscs in April and did not feed on plant material at all. *C. nigrodigitatus* fed on plant material in Februaury and fish in May. *E. fimbriata* did not feed on fish in February and April, on plant material in April and did not feed on molluscs at all throughout the months studied. *M. cephalus* did not feed on fish in April and May and did not feed on molluscs at all.

The study reveals the food and feeding habits of the four fish species. *M. cephalus* is a plankton feeder, feeding mainly on algae and diatoms (Ramirez-Luna *et al*., 2008). In this study the important food item of *M. cephalus* comprised of diatoms, while other food items were algae, crustaceans, plant material and detritus. The food items of *C. nigrodigitatus* included plant materials, molluscs, crustaceans, fish and detritus. Dada and Araoye (2008) also discovered similar food items in the stomach of *C. nigrodigitatus*. Ajah *et al*. (2006) reported

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Fig. 1. Food items of Caranx hippos.

Fig. 2. Food items of Chrysichthys nigrodigitatus.

Fig. 3. Food items of Ethmalosa fimbriata.

Fig. 4. Food items of Mugil cephalus.

the food items of C. nigrodigitatus to include gastropods, nematodes, diatoms and crustaceans. However, in this study, nematodes were not recorded.

From this study, it appears that there is likely to be inter-specific competition for food among the four fish species, due to the fact that they all seem to have the same important food item, i.e. diatoms, in common and thus competition for diatoms was high. C. hippos was an exception which had algae as another important food. It can, therefore, be concluded that there was an inter-relationship in the food and feeding habits of the four fish species. This inter-relationship would lead to a high competition for food in the Lagos lagoon.

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References

Ajah, P.O., Georgewill, M.N., Ajah, M.O. 2006. The food and feeding habits of five freshwater and brackish-water fish species in Nigeria. African Journal of Aquatic Science, **31:** 313-318.

Blay, J. (Jr.) 1995. Food and feeding habits of four species of juvenile mullet (Mugilidae) in a tidal lagoon in Ghana. Journal of Fish Biology, **46:** 134-141.

Dada, J.O., Araoye, P.A. 2008. Some aspects of the biology of Chrysichthys nigrodigitatus (Pisces: Siluroidae) in Asa Lake, Ilorin, Nigeria. Nigerian Journal of Fisheries, **5:** 73-84.

Oronsaye, C.C., Nakpodia, F.A. 2005. A comparative study of the food and feeding habits of Chrysichthys nigrodigitatus and Brycinus nurse in a tropical River. Pakistan Journal of Scientific and Industrial Research, **48:** 118-121.

Oso, J.A., Ayodele I.A., Fagbuaro, O. 2006. Food and feeding habits of Oreochromis niloticus (L) and Sarotherodon galilaeus (L) in a tropical reservoir. World Journal of Zoology, **1:** 118-121.

Ramirez-Luna, V., Navia, A.F., Rubio, E.A. 2008. Food habits and feeding ecology of an estuarine fish assemblage of Northern Pacific Coast of Ecuador. Pan-American Journal of Aquatic Sciences, **3:** 361-372.

Technology

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Production and Characterization of Chitosan from Shrimp (*Penaeus semisulcatus*) Shell Waste of UAE

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Abstract. Chitosan was prepared from shrimp (*Penaeus semisulcatus)* shell waste by a chemical process involving demineralization, deproteinization and deacetylation; conversion of chitin to chitosan (deacetylation) was achieved by treatment with concentrated sodium hydroxide solution (55%) at room temperature (25 ºC). The present study was undertaken to evaluate the influence of deacetylation process during chitosan production on the physicochemical and functional properties of shrimp shell chitosan. Four experimental chitosan samples were prepared with deacetylation for 40 h, for 50 h, with and without stirring as well as for 60 h and were subjected to physicochemical and functional characteristic analysis. Change in duration of deacetylation process yielded some differences in each characteristic; deacetylation for 40 h led to lower viscosity, solubility, water/fat binding capacity and degree of deacetylation and for 60 h resulted in increase in solubility but decrease in viscosity. Stirring during deacetylation process led to lower viscosity, higher degree of deacetylation and higher fat binding capacity of the product. In contrast non-stirred sample produced product with lower degree of deacetylation and higher viscosity. It was concluded that duration of deacetylaylation process should be monitored constantly for optimal chitosan production depending on its intended usages in food, pharmaceutical and biomedical industries.

Keywords: shrimp shell waste, deacetylation, chitosan, chitin

Introduction

Chitosan is a fiber-like substance derived from chitin, a homopolymer of ß-(1→4)-linked N-acetyl-D-glucosamine. Chitin is widely distributed in marine invertebrates, insects, fungi, and yeast (Subasingle, 1995; Austin *et al.*, 1981); however, it is not present in higher plants and higher animals. Generally, the shells of selected crustaceans consist of 30-40% protein, 30-50% calcium carbonate and calciumc phosphate and 20-30% chitin (Acosta *et al.*, 1993; Knorr, 1984). Chitin is widely available from a variety of sources among which, the principal source is shellfish waste such as that of shrimps, crabs and crawfish (Rinaudo, 2006; Allan and Hadwiger, 1979). It also exists naturally in a few species of fungi (Franco *et al.*, 2004; Andrade *et al.*, 2000; Chung *et al.*, 1994). Chitin and chitosan have similar chemical structures (Fig. 1). Chitin is made up of a linear chain of acetylglucosamine groups while chitosan is obtained by removing enough acetyl groups (CH_3-CO) from the molecule so that it becomes soluble in most diluted acids. This process is called deacetylation. The actual difference between chitin and chitosan is the acetyl content of the polymer. Chitosan having a free amino group is the most useful derivative of chitin (No and Meyers, 1992).

Chitosan is a non toxic, biodegradable polymer of high molecular weight (Zhang and Neau, 2001; Tomihata and Ikada,

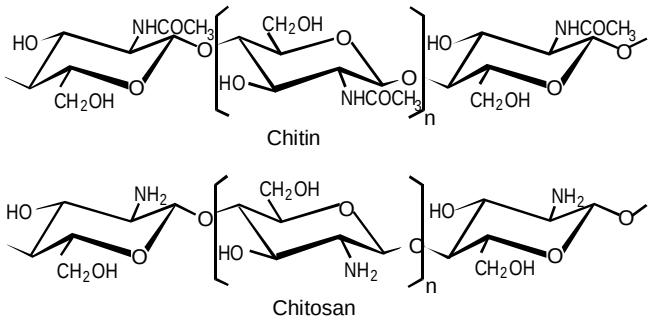

Fig. 1. Structure of chitin and chitosan.

1997). Over the last several years, chitinous polymers, especially chitosan, have received increased attention as one of the promising renewable polymeric materials for their extensive applications in the pharmaceutical and biomedical industries for enzyme immobilization and purification, in chemical plants for wastewater treatment and in food industries for use in food formulations as binding, gelling, thickening and stabilizing agent (Prashanth and Tharanathan, 2007; Franco *et al.*, 2004; Knorr, 1984).

Traditional isolation of chitosan from crustacean shell waste consists of four basic steps: demineralization, deproteinization, decolourization and deacetylation (Galed *et al.*, 2008; No and Meyers, 1995). Several procedures have been developed and proposed by many researchers over the years for preparation of chitosan from different crustacean shell wastes (Galed

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et al., 2008; No and Meyers, 1995; No *et al.*, 1989). Some of these formed the basis of chemical processes for industrial production of chitosan. But most of the reported processes were carried out with 45% concentrated sodium hydroxide solution at 100 °C or higher temperature with autoclaving (Galed *et al.*, 2008; Prashanth and Tharanathan, 2007; Domard and Rinaudo, 1983; Horton and Lineback, 1965). Therefore, the specific objectives of this work were to develop an optimum shrimp shell chitosan production process at room temperature (25 °C) with increased alkali strength without decolourization step and to study the influence of deacetylation process on the physicochemical and functional properties of shrimp shell chitosan.

Materials and Methods

Shrimp shell chitosan production. *Penaeus semisulcatus*, *Metapenaeus mastersii* and *Penaeus latisulcatus* are the shrimp species found in UAE waters of which *Penaeus semisulcatus* is the most common and commercially important species. Undersized shrimp shell waste of *Penaeus semisulcatus* was obtained from a commercial shrimp shell processor of Dubai, UAE. Upon receipt, shells (head, body and tail) were washed under running warm tap water to remove soluble organics, adherent proteins and other impurities. The shells were then dried in the oven (Mammert, Germany) at 70 °C for a period of 24 h or longer until completely dried shells were obtained. The moisture content of dried shell was 0.48%. To obtain a uniform size product, the dried shell was ground through a centrifugal grinding mill and sifted with 20-mesh (0.841 mm) and 40-mesh (0.425 mm) sieves. Dried ground shell powder was placed in opaque plastic bottles and stored at room temperature until used. The production of chitosan from shrimp shell waste was carried out with a modified method of No *et al.* (1989). The dried shrimp shell powder (5 kg) was demineralized with 8-10% hydrochloric acid at ambient temperature with a solid to solvent ratio of 1:15 (w/v), in an acid resistant vessel with stirrer for 20-22 h until demineral-i-zation was completed. The demineralized shells were deproteinized with 8-10% sodium hydroxide solution for 20-22 h at 65 °C with constant stirring or without stirring at a solid to solvent ratio of 1:10 (w/v). Samples were then washed with tap water and dried under vacuum for 2-3 h until the powder was crispy. Removal of acetyl groups from chitin was achieved by using concentrated sodium hydroxide solution (55%) with a solid to solvent ratio of 1:10 (w/v). Samples of four experimental shrimp shell chitosans were prepared. The chemical reactions were carried out at room temperature (25 °C). Duration of deacetylation process was 40 h for sample C_{40}, 50 h for C_{50S} (with magnetic stirring), 50 h for C_{50WS} (without stirring) and 60 h for C_{60}. The resulting chitosans were washed to neutrality in running tap water, rinsed with distilled water, filtered and dried at 60 °C for 24 h in the oven. The obtained shrimp shell chitosan was white to off white in colour and it was not necessary to decolourize or bleach it.

Physicochemical and functional properties. ***Measurement of nitrogen.*** Nitrogen of the crawfish chitosan was determined using a microprocessor-based, software-controlled instrument Model-TruSpec CN (Model # FP-428 Leco Corporation, USA). There were three phases during an analysis cycle, i.e., purging, burning and analysis. The encapsulated sample was purged of any atmospheric gases that had entered during sample loading. During the burning phase, the sample was dropped into a hot furnace (850 °C) and flushed with pure oxygen for a very rapid combustion. Finally, in the analysis phase, the remaining combustion product (nitrogen) was measured by the thermal conductivity cell. The final result was displayed as percent nitrogen.

Ash. Ash of the crawfish chitosan was calculated according to the standard method # 923.03 (AOAC, 1990). 2.0 g of chitosan (triplicate) were placed into previously ignited, cooled, and tarred crucible. The samples were heated in a muffle furnace preheated to 600 °C for 6 h. The crucibles were allowed to cool in the furnace to less than 200 ºC and then placed in desiccator with a vented top. Crucibles were cooled, weighed and ash content was recorded.

Degree of deacetylation. Chitosan samples prepared in the form of KBr discs were studied for the degree of deacetylation (DD) (Kassai, 2008; Khan *et al.*, 2002). The prepared chitosan KBr discs were kept in desiccators for 12 h and then placed in sealed plates before scanning. The DD of chitosan was established using a FTIR (Fourier Transform Infrared Spectroscopy) instrument (Model # M2000, Midac Corp. USA) with frequency of 4000-4/cm. The degree of deacetylation (DD) of the chitosan was calculated using the baseline reported by Khan *et al.* (2002). The computation equation for the baseline is given below:

$$DD = 100 - [(A_{1655} / A_{3450}) \times 100 / 1.33]$$

where A_{1655} and A_{3450} are the absorbance at 1655 cm^{-1} of the amide-I band as a measure of the N-acetyl group content and at 3450 cm^{-1} of the hydroxyl band as an internal standard to correct for disc thickness. The factor '1.33' denotes the value of the ratio of A_{1655}/A_{3450} for fully N-acetylated chitosan.

Viscosity. Viscosity of chitosan was determined with a Brookfield viscometer (Model DV-II + Brookfield Engineering

Laboratories Inc., Stonghton, MA.). Chitosan solution was prepared in 1% acetic acid at 1% concentration on dry basis. Measurement was made in duplicate using a No. 27 spindle at 50 rpm on solutions at 25 °C with values reported in centipoise (cP) unit.

Solubility. Crawfish chitosan sample (0.1 g in triplicate) was placed in a centrifuge tube (known weight) then dissolved with 10 ml of 1% acetic acid for 30 min. The solution was then centrifuged at 10,000 rpm for 10 min. The supernatant was decanted. The undissolved particles were washed in distilled water (25 ml) then centrifuged at 10,000 rpm. The supernatant was removed and undissolved pellets were dried at 60 °C for 24 h. Finally, the particles were weighed and the percentage solubility was determined.

Water binding capacity (WBC). WBC of chitosan was measured using a modified method of Knorr (1982). Initially a centrifuge tube containing 0.5 g of sample was weighed, 10 ml of water was added and mixing was carried out on a vortex mixer for one min to disperse the sample. The contents were left at ambient temperature for 30 min with intermittent shaking for 5 s every 10 min and then centrifuged (Model # Z383K, HERMLE-National Labnet Company, USA) at 3,500 rpm (6,000 × *g*) for 25 min. After the supernatant was decanted, the tube was weighed again. WBC was calculated as follows:

WBC (%) = [water bound (g)/ initial sample weight (g)]× 100. All experiments were carried out in triplicate.

Fat binding capacity (FBC). FBC of chitosan was measured using a modified method of Knorr (1982). Initially a centrifuge tube containing 0.5 g of sample was weighed, 10 ml of oil (three types of oil were used namely soybean, corn and sunflower oils) were added and mixing was carried out on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with shaking for 5 s every10 min and then centrifuged at 3,500 rpm (6,000 × *g*) for 25 min. After the supernatant was decanted, the tube was weighed again. FBC was calculated as follows:

FBC (%) = [fat bound (g)/ initial sample weight (g)] × 100. Experiments were performed in triplicate.

Statistical analysis. All experiments were carried out in triplicate, Average values (means) and standard deviations were reported. Mean separations were analyzed using the ANOVA and Tukey's student range tests at á = 0.05.

Results and Discussion

Yield. Yield was calculated as the dry weight of chitin obtained from 5 kg of dried shrimp shell powder. The yield of chitin was 20% and that of chitosan ranged from 16-19%. The highest yields were obtained from sample C_{40} (19%), followed by C_{50WS} (18%), C_{60} (17%), and C_{50S} (16%). Results are shown in Table 1. Brzeski (1982) reported about 14% yield of chitosan from krill and 18.6% from prawn waste (Alimuniar and Zainuddin, 1992). The yield of chitosan obtained (15-18%) is lower than that (approximately 23%) of chitin reported in the literature (No and Meyers, 1989). This may be due to loss of sample mass/weight during deacetylation process as we used here 55% concentrated sodium hydroxide solution, whereas in other methods 45% sodium hydroxide solution was used. The moisture content of the shrimp shell chitosan, determined by the gravimetric method (Black, 1965), was in the range of 0.3% to 0.4% (Table 1).

Table 1. Proximate analysis of shrimp shell and commercial chitosans (dry weight basis)

Sample	Yield (%)	Moisture (%)	Nitrogen (%)	Ash (%)
C_{40}	19	0.4 (0.25)[a]*	8.33 (0.02)[a]*	0.29 (0.07)[a]*
C_{50S}	16	0.3 (0.20)[a]	8.19 (0.01)[a]	0.3 (0.99)[a]
C_{50WS}	18	0.4 (0.25)[a]	8.11 (0.05)[a]	0.3 (0.23)[a]
C_{60}	17	0.4 (0.22)[a]	7.91 (0.05)[a]	0.3 (0.98)[a]
Sigma 91**		2.5 (0.11)[b]	8.23 (0.09)[a]	1.5 (0.25)[a]

* = numbers in parentheses are standard deviations; means with different letters in each column are significantly different ($P < 0.05$); ** Sigma 91 is a commercial crab chitosan.

Nitrogen content. Nitrogen content of the shrimp shell chitosan samples varied between 7.91% and 8.33% on a dry basis, showing no significant differences ($P > 0.05$) in nitrogen content, but the values were slightly higher than that (7.06% to 7.97%) reported by No and Meyers (1995), for chitosan from crab and shrimp shell on a dry basis. This is probably due to the presence of protein residues as mentioned by Rutherford and Austin (1978). Protein is bound by covalent bonds forming stable complex with chitin and chitosan. Thus, it is very difficult to achieve 100% deproteinization. Even with complete deprotinization, nitrogen was still present since chitosan has the amino ($-NH_2$) group.

Ash. Table 1 shows the ash content of shrimp shell chitosan in the range of 0.29-0.3%. Ash measurement is an indicator of the effectiveness of the demineralization step for removal of calcium carbonate. Elimination of demineralization step results in products having 31-36% ash (Bough *et al.*, 1978). Some residual ash of chitosans may affect their solubility, consequently contributing to lower viscosity, or can affect other more important characteristics of the final product. A high

quality grade of chitosan should have less than 1% of ash content (No and Meyers, 1995). An ash content of less than 1% from crab chitosans has been reported by No and Meyers (1995). The results presented in Table 1 indicate that the resultant chitosan sample was completely demineralized and contained less than 1% ash.

Degree of deacetylation. The degree of deacetylation (DD) of the studied shrimp shell chitosan samples ranged from 55% to 76% (Table 2). According to No and Meyers (1995), DD of chitosan ranges from 56% to 99% with an average of 80%. Sample C_{50S} (76%) had the highest DD, followed by C_{50WS}, C_{60} and C_{40} (75%, 74%, and 55%, respectively).

As in the Table 2, C_{40} had a very low solubility and viscosity which may be due to the lower DD value. Therefore, comparison among samples C_{50S}, C_{50WS} and C_{60}, sample C_{50S} gave lower viscosity (136.6 cP) and higher DD (76%) value which are very important characteristics of chitosan. The medical and pharmaceutical applications of chitosan as antitumor, hemostatic, hypocholesterolemic, antimicrobial and antioxidant depends mostly upon DD and solubility (Jian *et al.*, 2008; Muzzarelli and Muzzarelli, 2005). However, we expected that samples C_{50S}, C_{50WS} and C_{60} would have higher DD with higher solubility but the values obtained were lower than the expected ones. According to Kassai (2008) and Khan *et al*. (2002) , the IR spectroscopic method is commonly used for the estimation of chitosan DD values for its advantages: it is relatively fast and does not require dissolution of the chitosan sample in an aqueous solvent. DD values are not only highly dependent on the source and method of purification (No *et al.*, 1989) but also on the type of analytical methods employed, sample preparation and type of instrument used; other conditions may also influence the analysis of DD (Kassai 2008; Khan *et al*., 2002).

Viscosity. The viscosity of chitosan solutions, reported in the literature, generally ranges from 60 to 780 cP (Alimuniar and Zainuddin, 1992). This range of viscosity was also observed by Cho *et al.* (1998) for five commercially available chitosans. The results of viscosity, solubility and degree of deacetylation of our shrimp shell chitosans are shown in Table 2.

Bough *et al*. (1978) stated that viscosity of chitosans varied considerably from 60 to 5,110 cP depending on the species. Our shrimp shell samples had viscosity ranging from 90.7 to 170.2 cP. C_{40} had the lowest viscosity (90.7 cP) comparable to that of other samples as of lower solubility may be due to incomplete deacetylation of the sample. Whereas C_{50WS} had a very high viscosity (170.2 cP) (Table 2). Some factors affect viscosity during the production of chitosan such as the degree of deacetylation, molecular weight, concentration, ionic strength, pH and temperature, etc. Moorjani *et al*. (1975) reported that viscosity of chitosan decreased with increasing time of demineralization. The viscosity of chitosan in acetic acid tends to increase with decreasing pH but decrease with decreasing pH in HCl. Intrinsic viscosity of chitosan is a function of the degree of ionization as well as ion strength (Bough *et al.*, 1978). Deproteinization with 3% NaOH and elimination of the demineralization step in chitin preparation, decreased the viscosities of the final chitosan samples (Bough *et al*., 1978). Moorjani *et al.* (1975) stated that it is not desirable to bleach the material at any stage since bleaching considerably reduces the viscosity of the final chitosan product. Our product, prepared without bleaching step, gave lower viscosity which was desirable for preservation of foods against microbial deterioration, formation of biodegradable films and medical applications (Liu *et al.*, 2008; Zeng *et al.*, 2008).

Solubility. Three shrimp shell chitosan samples demonstrated excellent solubility ranging from 98.01 to 99% with no significant difference (Table 2), except sample C_{40}, which showed comparatively lower solubility (60.3%); it may be due to lower degree of deacetylation. Brine and Austin (1981) noted that lower solubility values suggested incomplete removal of protein and acetyl group. Since solubility of chitosan depends on the removal of acetyl group from chitin therefore lower DD value and the presence of protein contaminants remaining in the sample during the analysis process could adversely interfere with the results.

Water binding capacity (WBC). Water binding capacity of shrimp shell and commercial chitosans are shown in Table 3. WBC differed among crawfish chitosan samples, ranging from 299.6 % to 745.4%. There were no significant differences in

Table 2. Viscosity, solubility and degree of deacetylation of shrimp shell and commercial chitosans

Sample	Viscosity (cP)	Solubility (%)	Degree of deacetylation (%)
C_{40}	90.7 (5.07)*[a]	60.3 (0.61)[a]	55
C_{50S}	136.6 (2.09)[b]	98.2 (0.66)[b]	76
C_{50WS}	170.2 (3.66)[c]	98.01 (0.45)[b]	75
C_{60}	154.29 (2.69)[d]	99.00 (0.56)[b]	74
Sigma 91**	380.15 (3.44)[e]	89.88 (0.42)[c]	74

* = numbers in parentheses indicate standard deviation; means with different letters in each column are significantly different ($P < 0.05$);
** Sigma 91 is a commercial crab chitosan.

Table 3. Water binding capacity and fat binding capacity of shrimp shell and commercial chitosans

Sample	WBC (%)	Fat binding capacity (%)		
		Soybean oil	Corn oil	Sunflower oil
C_{40}	299.6 (9.97)*[a]	258.7(8.9)[a]	245.5 (4.8)[a]	255.7 (5.3)[a]
C_{50S}	738.8 (5.6)[b]	587.3 (5.3)[b]	599.2 (8.5)[b]	586.8 (9.9)[b]
C_{50WS}	745.4 (4.9)[b]	571.5 (7.9)[b]	583.6 (7.3)[b]	579.4 (5.6)[b]
C_{60}	732.2 (4.04)[b]	575.8 (6.5)[b]	577.5 (6.7)[b]	566.9 (7.6)[b]
Sigma 91**	538.5 (4.99)[c]	379.7 (5.9)[c]	444.3 (5.3)[c]	398.6 (6.6)[c]

* = numbers in parentheses indicate standard deviation; means with different letters in each column are significantly different ($P < 0.05$); ** = Sigma 91 is a commercial crab chitosan.

WBC between C_{50S}, C_{50WS} and C_{60}. These values were in agreement, except C_{40}, with those reported by Cho *et al*. (1998) where WBC for chitosans ranged from 458% to 805% for five commercial chitosans from shrimp and crab shell. Sample C_{40} had a lower WBC (299.6 %) than that of other samples; it may be due to lower DA value.

Fat binding capacity (FBC). Fat binding capacity (FBC) of four shrimp shell chitosans was measured using three types of oils including soybean, corn, and sunflower oil. The results are shown in Table 3. FBC differed among chitosan products, ranging from 245.5% to 599.2%. Among our crawfish chitosan samples, C_{50S} showed the highest FBC values: 587.3% with soybean oil, 599.2% with corn oil and 586.8% with sunflower oil, although C_{50S} had low viscosity (136.6 cP); C_{50S}, C_{50WS} and C_{60} showed no significant difference in FBC.

The sample C_{40} showed the lowest FBC (245.5%-258.7%) as it was not properly deacetylated; it seems higher deacetylation facilitates oil binding capacity of chitosan. Several workers suggested that the DD of chitosan is an important factor which influences fat binding capacity of chitosan (Shahidi *et al.*, 2002). They suggested that increased DD causes increased electrostatic force between chitosan and fatty and bile acid and increased FBC. Moorjani *et al.* (1975) advocated that changing the sequence of steps, when demineralization is conducted prior to deproteinization and finally deacetylation, results in an increase in FBC than when deproteinization is conducted prior to demineralization and finally deacetylation. Amongst the three types of oil used, soyabean oil generally demonstrated more FBC with shrimp shell chitosan samples, whereas sunflower oil showed the least FBC. Regardless of the type of vegetable oils, the four prepared shrimp shell chitosan samples showed desirable FBC ranging from 566.9% (with sunflower) to 599.2% (with corn) which is in agreement with those (314 to 535% with an average of 417%) reported by No *et al.* (2000). Sample C_{40} showed lower value than the reported value. It seemed degree of deacetylation influenced the fat binding capacity of chitosan.

Conclusion

Throughout the literature on chitosan, the main emphasis is on its quality and physicochemical properties which vary widely with the crustacean species and the preparation methods. Most of the reported preparation methods used high temperature with 45% concentrated alkali and sometimes used autoclave. Based on the reported practice this research study attempted to present a process for the production of shrimp shell chitosan at room temperature (25°C) with increased alkali strength (55%); it could help to develop small industry without wasting energy. This study also demonstrated that the duration of deacetylation process affects the quality of the products. In view of the foregoing, it is our recommendation that for the purpose of achieving uniformity and proper product quality control for particular usage of chitosan, the relationship between the process protocols/conditions and the resulting specific characteristics of chitosan products must be monitored constantly and properly.

References

Acosta, N., Jimenez, C., Borau, V., Heras, A. 1993. Extraction and characterization of chitin from crustaceans. *Biomass and Bioenergy,* **5:** 145-153.

Alimuniar, A., Zainuddin, R. 1992. An economical technique for producing chitosan. In: *Advances in Chitin and Chitosan*, C.J. Brine, P.A. Sanford and J.P. Zikakis (eds.), pp.627-632. Elsevier Applied Science, Essex, UK.

Allan, R., Hadwiger, L.A. 1979. The fungicidal effect of chitosan on fungi of varying cell wall composition. *Experimental Mycology,* **3:** 285-287.

Andrade, V.S., Neto, B.B., Souza, W., Campos-Takaki, G.M. 2000. A factorial design analysis of chitin production by *Cunninghamella elegans*. *Canadian Journal of Microbiology,* **46:** 1042-1045.

AOAC. 1990. *Official Methods of Analysis of Association of Official Analytical Chemists*, 15th edition, AOAC Washington, DC., USA.

Austin, P.R., Brine, C.J., Castle, J.E., Zikakis, J.P. 1981. Chitin: New facets of research. *Science,* **212:** 749-753.

Black, C.A. 1965. *Methods of Soil Analysis*: *Part I. Physical and Mineralogical Properties,* 2nd edition, American Society of Agronomy, Madison, Wisconsin, USA.

Bough, W.A., Salter, W.L., Wu, A.C.M., Perkins, B.E. 1978. Influence of manufacturing variables on the characteristics and effectiveness of chitosan products. 1. Chemical composition, viscosity, and molecular weight distribution of chitosan products. *Biotechnology and Bioengineering*, **20:** 1931-1942.

Brine, C.J., Austin, P.R. 1981. Chitin variability with species and method of preparation. *Comparative Biochemistry and Physiology,* **69B:** 283-286.

Brzeski, M.M. 1982. Concept of chitin/chitosan isolation from Antarctic krill (*Euphausia superba*) shells on a technique scale. In: *Proceedings of the Second International Conference on Chitin and Chitosan,* S. Hirano and S. Tokura (eds.), The Japan Society of Chitin and Chitosan, Sapporo, Japan.

Cho, Y.I., No, H.K., Meyers, S.P. 1998. Physicochemical Characteristics and Functional Properties of Various Commercial Chitin and Chitosan Products. *Journal of Agriculture and Food Chemistry,* **46:** 3839-3843.

Chung, L.Y., Schmidt, R.J., Hamlyn, P.F., Sagar, B.F., Andrews, A.M., Turner, T.D. 1994. Biocompatibility of potential wound management products: fungal mycelia as a source of chitin/chitosan and their effect on the proliferation of human F1000 fibroblasts in culture. *Journal of Biomedical Materials Research,* **28:** 463-469.

Domard, A., Rinaudo, M. 1983. Preparation and characterization of fully deacetylated chitosan. *International Journal of Biological Macromolecules,* **5:** 49-52.

Franco, L.O., Maia, R.C.C., Porto, A.L.F., Messias, A.S., Fukushima, K., Campos-Takaki, G.M. 2004. Heavy metal biosorption by chitin and chitosan isolated from *Cunninghamella elegans* (IFM 46109). *Brazilian Journal of Microbiology,* **35:** 243-247.

Galed, G., Diaz, E., Heras, A. 2008. Conditions of N-deacetylation on chitosan production from alpha chitin. *Natural Product Communications,* **3:** 543-550.

Horton, D., Lineback, D.R. 1965. N-deacetylation, chitosan from chitin. In: *Methods in Carbohydrate Chemistry*, R.L.Whistler and M.L. Wolfson (eds.), pp.403, New York, USA.

Jian, Y., Feng, T., Zheng, W., Qing, W., Yan-Jun, Z., Shi-Qian, C. 2008. Effect of chitosan molecular weight and deacetylation degree on hemostasis. *Journal of Biomedical Materials Research,* **84B:** 131-137.

Kassai, M.A. 2008. Review of several reported procedures to determine the degree of N-acetylation for chitin and chitosan using infrared spectroscopy. *Carbohydrate Polymers,* **71:** 497-508.

Khan, T.A., Peh, K.K., Ch'ng, H.S. 2002. Reporting degree of deacetylation values of chitosan: The influence of analytical methods. *Journal of Pharmacy and Pharmaceutical Sciences,* **5:** 205-212.

Knorr, D. 1984. Use of chitinous polymers in food–A challenge for food research and development. *Food Technology,* **38:** 85-97.

Knorr, D. 1982. Functional properties of chitin and chitosan. *Journal of Food Science,* **47:** 593-595.

Liu, J., Zhang, J., Xia, W. 2008. Hypocholesterolaemic effects of different chitosan samples *in vitro* and *in vivo*. *Food Chemistry,* **107:** 419-25.

Moorjani, M.N., Achutha, V., Khasim, D.I. 1975. Parameters affecting the viscosity of chitosan from prawn waste. *Journal of Food Science and Technology,* **12:** 187-189.

Muzzarelli, R.A.A., Muzzarelli, C. 2005. Chitosan Chemistry: Relevance to the biomedical sciences. *Advances in Polymer Science,* **186:** 151-209.

No, H.K., Lee, K.S., Meyers, S.P. 2000. Correlation between physicochemical characteristics and binding capacities of chitosan products. *Journal of Food Science,* **65:** 1134-1137.

No, H.K., Meyers, S.P. 1995. Preparation and characterization of chitin and chitosan - A Review. *Journal of Aquatic Food Product Technology,* **4:** 27-52.

No, H.K., Meyers, S.P. 1992. Utilization of crawfish processing wastes as carotenoids, chitin and chitosan sources. *Journal of Korean Society of Food and Nutrition,* **21:** 319-326.

No, H.K., Meyers, S.P. Lee, K.S. 1989. Isolation and characterization of chitin from crawfish shell waste. *Journal of Agriculture and Food Chemistry,* **37:** 575-579.

No, H.K., Meyers, S.P. 1989. Crawfish chitosan as a coagulant in recovery of organic compounds from seafood processing streams. *Journal of Agriculture and Food Chemistry,* **37:** 580-583.

Prashanth, R., Tharanathan, R. 2007. Chitin/chitosan: Modifications and their unlimited application potential - an overview. *Trends in Food Science and Technology,* **18:** 117-131.

Rinaudo, M. 2006. Chitin and chitosan: Properties and applications. *Progress in Polymer Science,* **31:** 603-632.

Rutherford, F.A., Austin, P.R. 1978. Marine chitin properties and solvents. In: *Proceedings of the First International Conference on Chitin/Chitosan*, R.A.A. Muzzarrelli and P.R. Austin (eds.), pp.182-192, MIT Sea Grant Programme, Cambridge, MA, USA.

Shahidi, F., Kamil J., Jeon, Y.J., Kim, S.K. 2002. Antioxidant role of chitosan in a cooked cod (*Cadus morhua*) model system. *Journal of Food Lipids*, **9:** 57-64.

Subasingle, S. 1995. The development of crustacean and mollusc industries for chitin and chitosan resources. In: *Chitin and Chitosan*, M.B. Zakaria, W.M. Wan Muda and M.P. Abdullah (eds.), pp. 27-34, Penerbit Universiti Kebangsaan, Malaysia.

Tomihata, K., Ikada, Y. 1997. *In vitro* and *in vivo* degradation of films of chitins and its deacetylated derivatives. *Biomaterials*, **18:** 567-575.

Zeng, L., Qin, C., Wang, W., Chi, W., Li, W. 2008. Absorption and distribution of chitosan in mice after oral administration. *Carbohydrate Polymers*, **71:** 435-440.

Zhang, H., Neau, S.H. 2001. *In vitro* degradation of chitosan by a commercial enzyme preparation: effect of molecular weight and degree of deacetylation. *Biomaterials*, **22:** 1653-1658.