ISSN 2221-6413 (Print), ISSN 2223-2559 (Online)
Coden: PJSIB5 60(2) 59-116 (2017)

Pakistan Journal of Scientific and Industrial Research

Series A: Physical Sciences

Vol. 60, No.2, May-June, 2017

(for on-line access please visit web-site http://www.pjsir.org)

Published by
Scientific Information Centre
Pakistan Council of Scientific and Industrial Research
Karachi, Pakistan

Pakistan Journal of Scientific and Industrial Research
Series A: Physical Sciences

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Printed and Published by: PCSIR Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

Editorial Address

Executive Editor
Pakistan Journal of Scientific and Industrial Research, PCSIR Scientific Information Centre
PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan
Tel: 92-21-99261914-99261916, 99261949, 99261917; Fax: 92-21-99261913; Web: http://www.pjsir.org, E-mail: info@pjsir.org

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Pakistan Journal of Scientific and Industrial Research
Series A: Physical Sciences
Vol. 60, No. 2, May-June, 2017

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Short Communication

Pak. j. sci. ind. res. Ser. A: phys. sci. 2017 **60**(2) 59-66

Isolation and Identification of Catechin by a New Method from Food Efficiency Stimulating Plant *Alhagi camelorum*

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(received October 18, 2016; revised February 27, 2017; accepted March 9, 2017)

Abstract: The present article describes a new developed method for the simultaneous determination of (+)-catechin (**1**) and (-)-epicatechin (**2**), separated *via* HPLC. The method has been validated and applied on the real samples and **1** has been detected in both aerial and root parts of *Alhagi camelorum* without any ambiguity of fake positive or negative presence of **1** or **2** by virtue of dual detection system of UV and Mass Spectrometry. The antioxidant capacity was also investigated and a linear correlation has been noticed between the antioxidant capacity and the catechin amount in *A. camelorum* extracts

Keywords: catechin, *Alhagi camelorum*, HPLC, isolation

Introduction

Plants and their extracts have largely been analysed to determine active components, which make them suitable for pharmaceutical industries (Terao *et al*., 1994). (+)-catechin (**1**) and (-)-epicatechin (**2**) are main representatives of one of six flavonoid classes widely analysed by different methods in various samples. They themselves as well as their derivatives are found to be active in curing many diseases. Their antioxidant and free radical scavenger activities are most important (Mendoza-Wilson and Glossman-Mitnik, 2006; Wolfe *et al*., 2003). Different researchers have stressed their role in reducing tumour development and growth (Okabe *et al*., 1999; Gali *et al*., 1994). They have ability to inhibit platelet aggregation and show antibacterial and angio-protective properties (Chang and Hsu, 1989). Normalization of blood pressure, prevention of endothelial dysfunction, insulin resistance in prediabetes stage as well as contribution to beneficial effects on the vascular system has also been attributed to catechins intake (Ihm *et al*., 2009). Their ability to induce selectively Phase I and II metabolic enzymes makes them an important class of drugs (Sohn *et al*., 1994; Vennat *et al*., 1988). Besides these activities, effect of catechins on metabolism has also been reported in different studies (Crespy *et al*., 2003; Donovan *et al*., 2001). Silberberg *et al*. (2005) have studied the effect of catechin on absorption and metabolism in rats with co-administration of quercetin and found significant results. They have also recommended and verified that high nutritional intake of different flavonoids may significantly affect their respective absorption and metabolism. Nevertheless, correlation of food efficiency stimulating property of *Alhagi camelorum* extracts has still been ambiguous. Though it is difficult to quantify the consumption of foods and beverages, but estimation of constituents like catechins in this case may lead to know the actual reason of increased food intake (Auger *et al*., 2004).

Catechin has been found in different plants, likewise it has also been reported that the extracts of *A. camelorum* contain catechin in a quantifiable amount (Teissedre and Landrault, 2000). Extracts of *A. camelorum* have been reported to have different pharmacological activities (Marashdah and AL-Hazimi, 2010) whereas isolation of bio-molecules has also been done. *A. camelorum* has also been reported to have food efficiency stimulating property (Naseri and Mard, 2007) and the presence of flavonoid in *A. camelorum* has already been reported (Shaker *et al*., 2010; Naseri and Mard, 2007). The present study was based on the thought that not only **1** but both compounds (**1**) and (**2**) may be present in *A. camelorum*, which are responsible for the above mentioned activities. It was also aimed to develop and validate new and versatile method for the analysis of

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(**1**) and (**2**) in plant extracts in shorter time with more accuracy and precision.

Materials and Methods

Reference standards of (+)-catechin and (-)-epicatechin were purchased from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile, methanol, formic acid, solid phase cartridge were purchased from Merck (Darmstadt, Germany). Butylhydroxyanisole (BHA) was purchased from BDH Chemicals Ltd (Poole, England). Deionized water was supplied by PCSIR Laboratories. Ethanol purchased from various sugar industries and purified in PCSIR Laboratories was of reagent grade. Raw material of *A. camelorum* was prepared from plant which was collected from vicinity of village Malkani located in district Badin of Sindh province of Pakistan, in February 2010. It was identified in Institute of Plant Sciences, University of Sindh, Jamshoro where a voucher specimen is deposited (*A. camelorum*15460).

Preparation of standards. 25 mg of each (**1**) and (**2**) were weighed and transferred to 25 mL volumetric flask containing 15 mL of methanol. Flasks were shaken and made up to volume by methanol to have 1000 mg/L (stock solution). 0.25 mL from each solution was taken and transferred to a 10 mL volumetric flask. Volume was made up by methanol in order to make 5 mg^2/L concentration of that mixture. From this mixture further dilutions were made in the range of 1.5-25 mg/L.

Extraction procedures. Three different extraction techniques were applied to get extracts from plant material. Root parts were labelled as R and aerial parts as A, while extraction techniques were abbreviated as (Son), (MW) and (Vor) for sonication, microwave and vortex mixing, respectively. Previously dried and powdered plant material (0.2 g) was taken in each technique. In these techniques two extracting solvent systems were used (Iacopini *et al*., 2008) i.e., S1 or S2 comprising of 0.12 M solution of hydrochloric acid in methanol:water or ethanol:water system (7:3 v/v), respectively.

Microwave extraction. Samples of aerial and/or root parts of *A. camelorum* were transferred to pressure controlled Teflon vessels of the Start E Microwave extraction system © 2003 Mile Stone Inc. in triplicate followed by the addition of 10 mL of each solvent system S1 or S2. The programming of microwave extraction system was set in two steps. In the first step, gradient rise of temperature was maintained from ambient to 45 °C within 5 min and in the 2nd step it was kept constant for 15 min using an energy level of 400 W, followed by ventilation for 10 min.

Sonication. Samples were placed in 50 mL conical flasks and solvent S1 or S2 was added. Sonication was performed in ultrasonic bath of Supersonic X-3 Model DSD80A5QS instrument (power 80 Watt) at ambient temperature. Total time of sonication was 15 min for all samples.

Vortex mixing. Samples along with solvent S1 or S2 were placed in 50 mL conical flasks. Flasks were fitted in vortex mixture (manufactured by PCSIR, Karachi-Pakistan) and mixing was allowed for 15 min at ambient temperature.

Liquid chromatographic and mass spectrometric analyses. The LC-MS system used for analysis was consisted of FINNIGUN SURVEYOR units. PDA plus detector for UV detection, Auto-sampler plus injections of samples, LC pump plus for pumping mobile phase and LCQ advantage MAX for mass fragmentation of eluting compounds. X-Caliber 2.0 software programme was used for peak identification and peak integration. For the separation of both isomers, a reverse phase column was used with packing of ODS (Thermo Gold) due to its ability to retain polar compounds. The column was fitted in column oven and temperature of auto sampler as well as oven was kept ambient. Both of the standards (1 & 2) were detected in PDA detector at 280 nm but calculations were made on the basis of total ion chromatogram (TIC) obtained through MS detection. Mobile phase [acetonitrile:methanol 3:7 (A) and 0.1% formic acid (B)] was run in gradient programme by starting at 5% B to reach 25% at 7 min and kept same for 2 min; then 95% of B in two min followed by equilibration time of 4 min.

Method validation. ***Sensitivity.*** Sensitivity of method was determined by limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ were calculated by using following equation:

$$C = K \sigma / Slope$$

Where:

K is 3 and 10 for LOD and LOQ, respectively; standard deviation (s) of response (at the retention time of analytes) of blank sample and slope values were obtained from the equations of straight line constructed by calibration standards.

Specificity. Results of both standards and samples obtained by LC-MS analysis were compared regarding their retention times, UV absorption and mass spectra. The peak purity was assessed by checking UV absorption and mass spectrum of each peak from its start to end. Using X-Caliber software, mass of each peak was extracted by drawing a layout and putting M^{+1} value (291 of 1 and 2) in mass range. Only two peaks for these two isomers were observed in the total ion chromatogram (TIC) chart (Fig. 1).

Accuracy. For accuracy measurement, spiked samples were run along with standard 2 at different concentrations (1.5, 6.25 and 25 mg/L). The addition of the standard 2 into samples was also made prior to all extraction methods (section 2.3) from the plant material for analysis in order to check the interference of 2 with 1.

Precision. Intraday and inter-day precision was carried out by running standard of varying concentration (i.e. 1.5-25 mg/L) seven times in a day and with a gap of seven day, respectively. Retention time and TIC area of peak were taken into account in calculations and the results were taken in % RSD.

DPPH assay. The DPPH assay was performed for the evaluation of antioxidant potential of plant extract as well pure compound (Molyneux 2004). The assay was performed by a reported method (Seeram *et al*., 2006) with slight modifications. Briefly, 1 mL of BHA or plant extract or pure reference standard (1 or 2) was added into 3 mL of DPPH solution in methanol (12×10^{-5} M). To calculate the time effect, absorption of mixtures was measured at 515 nm with 2 min time intervals until reaction reached to a stable level. The % inhibition of DPPH by extract or pure compound was calculated by the following equation:

$$\% \text{ DPPH} = [\text{DPPH}_A - \text{DPPH}_B / \text{DPPH}_A] \times 100$$

Where:

DPPH_A is initial absorption and DPPH_B is the absorption after addition of standard (BH_A) or plant samples or pure compounds 1 and 2.

To calculate the IC50 values calibration curve was prepared for inhibition of DPPH by BHT and a linear equation was generated to calculate the inhibition of DPPH by the sample to its half. Concentration of BHT was taken in µmoles/L while samples were taken as mg/L (known on the basis of LC-MS analysis).

Results and Discussion

Before developing the chromatographic separation method, three different extraction methods have been followed as described in experimental part.

Extraction techniques. Three different methods of extraction (sonication, microwave and vortex mixing,) with two solvent systems S1 and S2 (0.12 M HCl either in methanol:water or ethanol:water system) were adopted for the analysis of catechin in real samples of roots and aerial parts of *A. camelorum.* In all of these techniques, 15 min time was enough to extract the plant material. Longer time does not have any impact on recovery with any of the solvent system. The recovery of (**1**) was different from roots and aerial parts by applying different extraction techniques. The maximum recovery of (**1**) from aerial parts was found by microwave technique; while from the roots, vortex mixing as well as sonication was found to be best technique for maximum recovery (Table 1). These results are in agreement with previous findings by Quan *et al*. (2006).

Determination of catechins in *A. camelorum.* The activities (e.g., food efficiency stimulation, antioxidant etc.) as well as external effects e.g., infections (Bandoniene and Murkovic, 2002) are associated with variation of concentration of both (**1**) and (**2**). Besides this, the difference in bioavailability of these both isomers also matters (Ghassempour *et al*., 2011). Thus, due to immense importance of (**1**) and (**2**) in pharmaceutical industry, a reliable chromatographic method was necessary to be developed, which may overcome the fatigues of previously reported methods such as complex mobile phase systems with higher flow rates (Donovan *et al*., 2006), longer time of analysis

Table 1. Catechins content in sample of *A. camelorum*

Sample	mg/100g
A-Son-S1	4.34 ± 0.05
A-Son- S2	7.06 ± 0.06
A-MW- S1	7.90 ± 0.06
A-MW- S2	10.46 ± 0.05
A-Vor- S2	8.49 ± 0.04
A-Vor- S1	8.76 ± 0.06
R-Son- S1	9.43 ± 0.05
R-Son- S2	10.02 ± 0.03
R-MW- S2	9.12 ± 0.08
R-MW- S1	9.87 ± 0.08
R-Vor- S2	10.33 ± 0.06
R-Vor- S1	9.33 ± 0.02

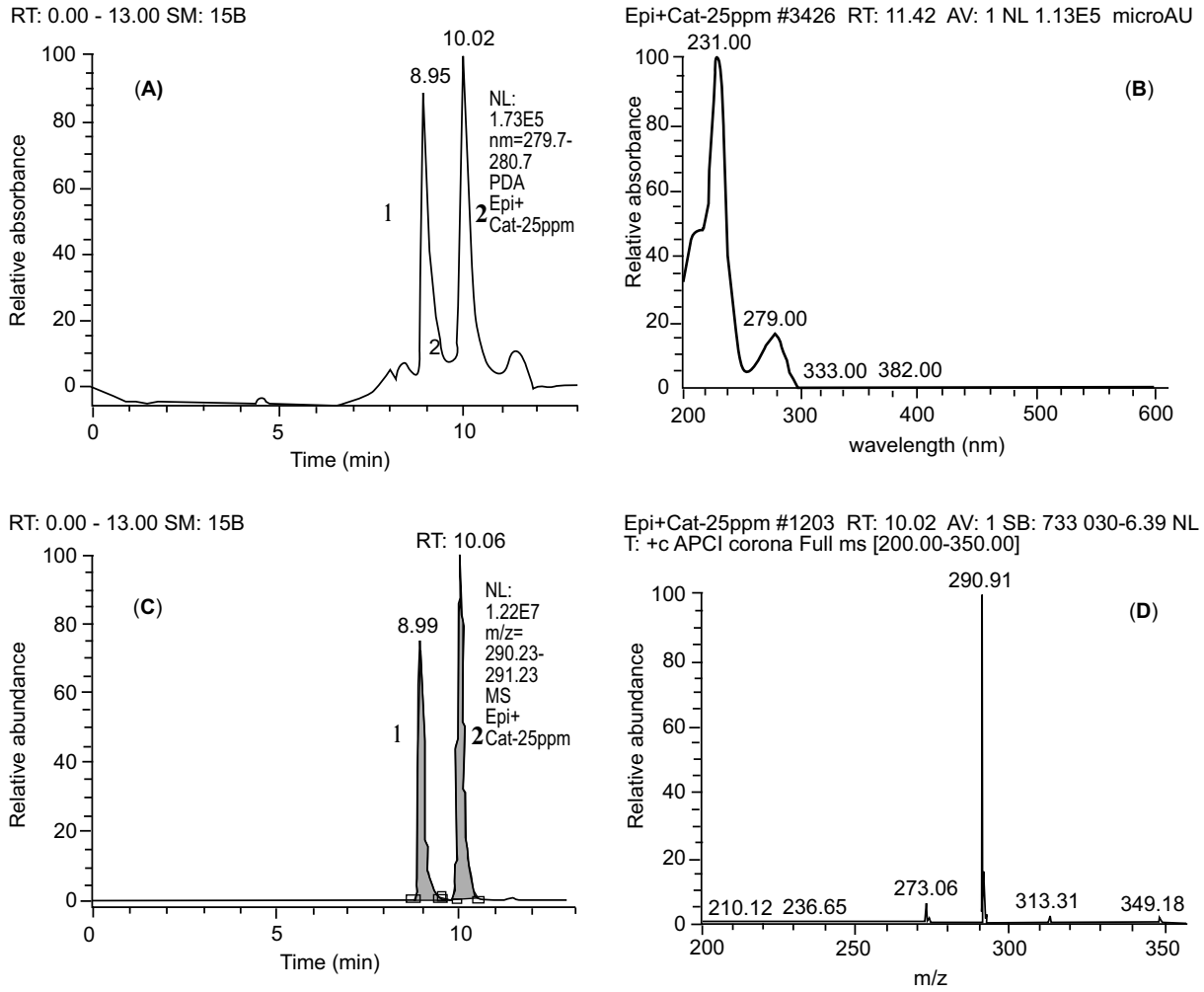

Fig.1. LC-MS profile of pure reference standards **1** and **2**. A= PDA chromatogram; B= UV spectra; C= total ion chromatogram; D= Mass spectrum (for 1 and 2).

(Dias *et al*., 2010) and higher detection limits (Soares *et al*., 2004; Viñas *et al*., 2000).

Separation of both isomers (**1**) and (**2**) (Fig. 1) in standard samples was performed by high-performance liquid chromatography (HPLC), whereas (**1**) was detected, confirmed and quantified (Table 1) in plant samples on the basis of both HPLC and total ion chromatogram (TIC) of mass spectrometry. In initial trials, concentration of solvent B was kept constant and solvent A was altered by changing concentration of acetonitrile. When isomers got separated with reasonable resolution; then mobile phase was selected as mentioned in experimental section. To have best resolution in short time, gradient programming was altered by changing percentage of solvent A from 95 to 25. Going below 25% concentration of solvent A, increased the retention time with some better resolution; while going above 25% concentration of A decreased the resolution. Therefore, programming of gradient was optimized at 25% concentration of A. Because (**1**) and (**2**) have similar response in all detection systems therefore; their separation is necessary even if someone has to determine one of these two isomers. The chance of false positive and false negative results may come out if one of them is taken as reference compound and determined in any sample. To avoid such calamity, proposed method is fair enough to separate both isomers and any one of these two can accurately be determined in any real sample. For verification, real samples were run accordingly by this method and found that *A. camelorum* contains only (**1**) as previously reported by Teissedre and Landrault (2000). The real samples of *A. camelorum* and standard sample of (**1**)

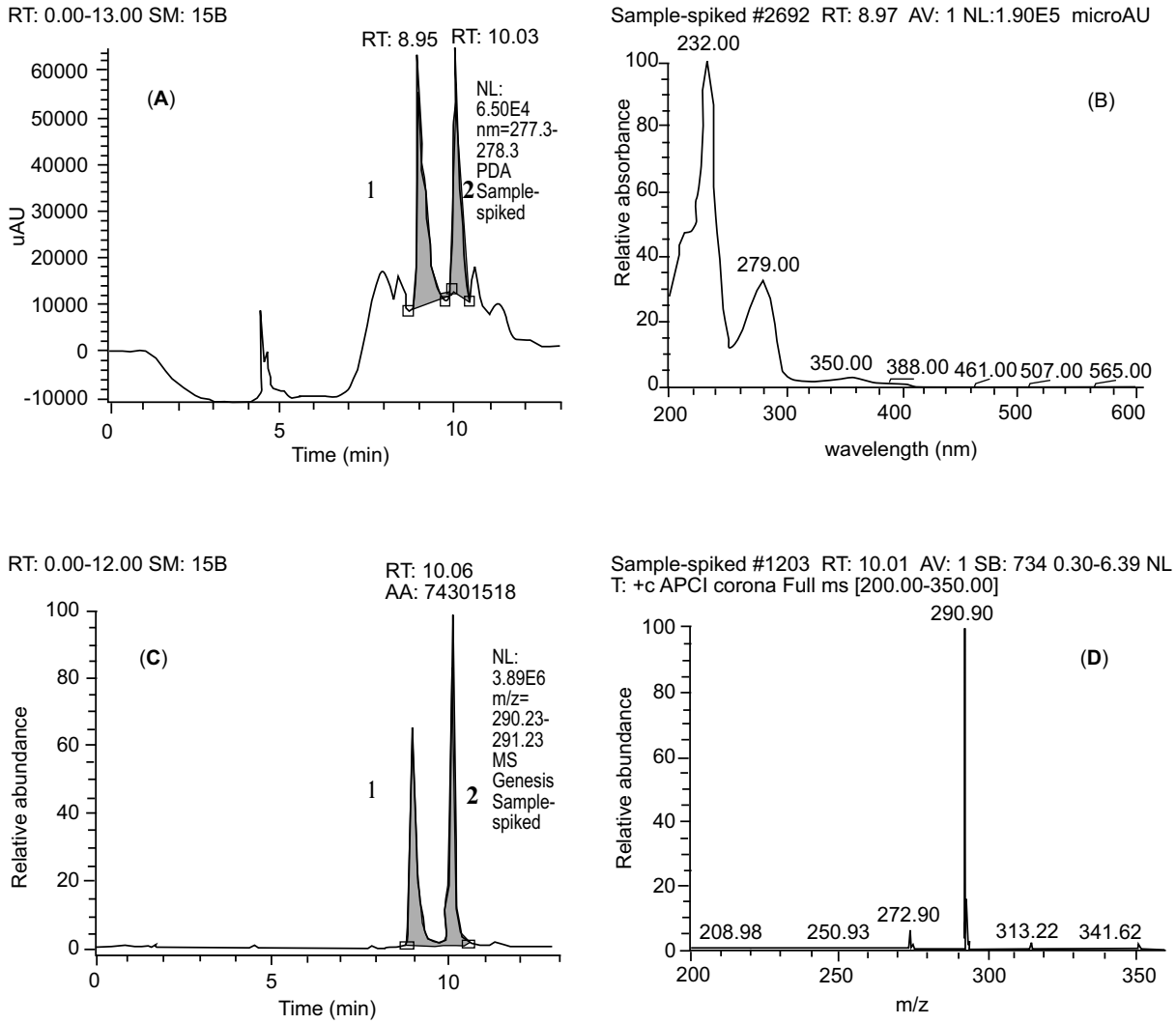

Fig. 2. LC-MS profile of sample. **1** is naturally present and spiked by adding **2;**. A= PDA chromatogram; B= UV spectra; C= Total Ion Chromatogram; D= Mass spectrum (for 1 & 2).

were spiked with the standard sample of (**2**); results showed that neither sample matrix nor 1 had interference with (**2**) (Fig. 2). It verified that proposed method may be a better choice for the analysis of (**1**) and (**2**), where accurate determination of either of these both isomers is required.

Validation of analytical method. For quantification of (**1**) and (**2**) in *A. camelorum* LC-MS method was validated. The concentration of reference standard was in the range of 1.5-25 mg/L having linear calibration curve with r2>0.99. LOD was 0.001 mg/L for both (**1**) and (**2**), while LOQ was 0.003 mg/L for (**1**) and (**2**),

Table 2. Validation data using MS detection

Compound	Molecular weight	M^{+1}	Intercept	Slope	Correlation coefficient	Linearity interval (mg/L)	RSD (%)	Detection limit (mg/L)	Quantification limit (mg/L)
1	290	291	4×106	617959	0.9994	1.5-25	0.2	0.001	0.003
2	290	291	6×106	8×106	0.9987	1.5-25	0.9	0.001	0.003

Table 3. Antioxidant capacity of BHA, reference standards and samples measured by DPPH free radical assay

Compound/Sample	* IC_{50}	
BHA	5.00	±0.2
1	7.25	±0.3
2	7.54	±0.3
A-Son-S1	5.80	±0.3
A-Son- S2	3.71	±0.2
A-MW- S1	2.80	±0.0
A-MW- S2	0.60	±0.0
A-Vor- S2	2.40	±0.1
A-Vor- S1	2.00	±0.1
R-Son- S1	1.60	±0.1
R-Son- S2	1.00	±0.1
R-MW- S2	1.00	±0.0
R-MW- S1	1.20	±0.1
R-Vor- S2	0.70	±0.0
R-Vor- S1	1.90	±0.1

*Concentration of BHA, reference compounds/samples (mg/L).

respectively. Data obtained during method validation studies are summarized in Table 2.

DPPH scavenging capacity. Antioxidant capacities of plant extract, pure antioxidant standards and pure compounds (**1**) and (**2**) were evaluated. All plant extracts were capable of scavenging the free DPPH radical. The IC_{50} i.e., the amount of plant extracts, needed to inhibit the DPPH activity by 50%, was considered as measure of the antiradical capacity of plant extracts. The results of DPPH assay performed to calculate antiradical activities are listed in Table 3. Highest potential of free radical scavenging was shown by the extract from root sample prepared in solvent S2 by vortex mixing; while the extract from aerial parts prepared in S2 by microwave extraction method was found to be a potent radical scavenger. It has also been noticed that there is a close correlation between the amount of (**1**) present in the extracts of *A. camelorum* and the DPPH capacity as shown in Table 1-3. The plant extract samples with higher yield of (**1**) show greater DPPH capacity as compared to those with lower yield. This trend is in agreement with the reported literature (Molyneux, 2004). It may be due to the antioxidant nature of (**1**) present in higher amounts in the samples.

Conclusion

The present study demonstrates a validated analytical method for quantitative determination of catrchin (**1**) and epicatechin (**2**) in plant samples. Initially, optimization of instrumental parameters was achieved by different trials and then the method has been applied on real samples of *A. camelorum*. For extraction optimization, six samples from aerial and root parts of the plant were prepared with different extraction solvent systems and techniques and it has been observed that the high yield of (**1**) could be obtained by microwave extraction in S2 from aerial parts and by vortex mixing in S1 from root parts of *A. camelorum*. Besides this, free radical scavenging capacity of the *A. camelorum* extracts has also been determined by DPPH assay showing a close correlation with the amount of (**1**). From the results, it has been concluded that the developed method for the determination of (**1**) and (**2**) in the real plant extracts is more convenient, less time consuming, reliable and reproducible in terms of RSD, LOD and LOQ. Due to its simplicity and shorter time of analysis with more accuracy, the method could be a better choice for routine analytical measurements in pharmaceutical industries or in quality control laboratories for accurate determination of catechin or epicatechin when one or both are present in samples.

Acknowledgement

Support during this work by the PCSIR Laboratories Complex Karachi,Pakistan as well as National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro,Pakistan and H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi,Pakistan is acknowledged. The authors are also thankful to Prof. Dr. Tahir Rajpoot, Director of Institute of Plant Science, University of Sindh for identification of plant.

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Pak. j. sci. ind. res. Ser. A: phys. sci. 2017 **60**(2) 67-61

Chemical Characterisation of Himalayan Rock Salt

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(received November 3, 2016; revised January 11, 2017; accepted January 20, 2017)

Abstract. Present study involves the chemical evaluation of rock salt samples collected from the plugging sites of Himalayan salt (Khewra salt mines and Kalabagh salt mines) for their moisture content, water insoluble matter, calcium, magnesium, sulphate content and trace minerals such as Fe,Cu,Cd,Pb,As,Ag and Zn determined by atomic absorption spectroscopy. Moisture content of Khewra and Kalabagh salt samples ranged from 0.03 wt. % to 0.09 wt. % and 0.06 % to 0.08 %, respectively. Water insoluble matter ranged from 0.08 wt. % to 1.4 wt. % and 1.5 wt. % to 2.8wt. % for Khewra and Kalabagh salt samples, respectively. Sulphate content for Khewra salt sample was from 0.39 % to 0.91 % and for Kalabagh salt mines from 0.75 wt. % to 0.95wt. %. For Khewra salt mines calcium ranged 0.15wt. % to 0.32wt. % and for Kalabagh salt samples from 0.1 wt. % to 0.27wt. %. Magnesium ranged from 0.11 wt. % to 0.35wt. % for Khewra salt mines, while for Kalabagh salt samples its range was 0.18wt. % to 0.89wt. %. Trace metals had the concentration ranges between 0.2 to 1.85 mg/kg for copper; between 0.21 to 0.42 mg/kg for manganese; between 0.04 to 0.06 mg/kg for zinc; between 0.12 to 0.18 mg/kg for arsenic and between 0.03 and 0.05 mg/kg for lead while cadmium content was either below the method's detection limits or in very trace amounts. The results show that the concentrations of all the parameters studied are below the limits set by World Health Organization (WHO) and Food and Agriculture Organization (FAO). Therefore, it can be concluded from the paper that the Himalayan salt from the plugging sites of Khewra and Kalabagh salt mines are safe to use.

Keywords: atomic absorption spectrophotometer, minerals, water insoluble matter, sulphate

Introduction

Mining of salt for consumption and food preservation has been practiced for millions of years (Eftakhari *et al*., 2014). Underground mining of salt in Austria and Romania is from new Stone Age (Zarei *et al.,* 2011). Salt is also obtained from the salt lakes. It is found that salt from lakes and seas is with more minerals, less purity and higher water insoluble matter, calcium, magnesium and sulphate content (Pourghesari *et al.*, 2014). Table salt is sold in many forms in market such as refined, unrefined and fortified salts. Unrefined rock salt after mining is ready to use after minor mineralogical operations which involve mainly the removal of dust and mud etc. (Heshmati *et al*., 2014). Usage of unrefined salts is in its oldest uses. It is also referred to as the industrial salt. A few mining operations used in mining are packing and its transport (Celik and Oehlenchlar, 2007).Unrefined salts are 96 % pure and have some essential trace minerals such as Mg, Ca, S, N and I etc. Unrefined salt is still the preferable choice of consumers in developing countries despite of the fact that several health agencies have discouraged its usage (Al-Rajhi, 2014). In order to give a whitish look and to increase its shelf life and purity, table salt has undergone through various mineralogical operations. Refined salts are usually 99% pure (Eftekhari *et al.*, 2014). Beside the Na^+ and Cl^-ions in the common salt, some other inorganic trace minerals such as Ca, Mg, Fe and S are also present. Proportion of these minerals is higher in unrefined salts (Tandon and Singh, 2000).

Himalayan rock salt deposits are among the largest and oldest salt deposits of the world located in Pakistan .Its discovery goes back in 320BC when horses from the troops of Alexander licked the salt. But it became functional for exploration in Mughal reign (Sedivy, 2009). Eighty two billion tonnes (Elsagh and Rabbani, 2010) to 600 billion tonnes (Elsagh, 2012) of rock salt is estimated from these salt deposits. Many researchers have studied mineral characterisation and chemical evaluation of these salt deposits (Hassan and Mohyuddin 2016; Cheraghali *et al*., 2010) but very little is known about their impact on the public health. Parameters used for these evaluations were moisture; water insoluble particles heavy metals, Mg^{2+}, SO_4^{2-}, Cl^-, Na^+, and K (Hassan and Mohyuddin, 2016; Pourgheyshari *et al*., 2014).

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Environmental samples like food, salt, water and soil are getting contaminated day by day so a complete chemical evaluation of these environmental samples is very essential (Khaniki *et al*., 2007). Present work involves characterisation of % purity, sulphate, moisture, calcium and magnesium content and minerals (Fe,Cu,Cd, Pb,As, Ag and Zn) in the unrefined table salt samples collected from major Himalayan rock salt plugging sites of Khewra and Kalabagh salt mines in Pakistan to insight their chemical nature. Present work also involved the comparison of unrefined salt samples with the standards regarding human health.

Materials and Methods

Collection of Samples. Salt samples from the plugging sites of Himalayan rock salt (Khewra and Kalabagh) were collected from 25cm below the mining sites. Sample excavation was done with the help of traditional axe used for the mining. Eight samples each of 4kg of weight and rectangular form were collected from the mining site of Khewra salt mines. The same procedure was employed for seven samples collected from the Kalabagh salt mines. After collection they were packed into the Polyethylene bags. In laboratory salt samples were then crushed and sieved to 80 meshes then transferred to air tight glass containers.

Sample preparation. Unrefined salt samples were crushed, sieved to 80 meshes and then stored in the air tight glass containers. One gram of each of sample was taken to dissolve in 100 mL of double distilled water. Residue was separated by filtration. Volume of filtrate was made up to 250 mL to be analysed (ASTM, 2002).

Moisture content. Sample (0.05g) was placed in an oven to calculate its moisture content using a dried and previously tarred moisture dish. This tarred moisture dish was placed in oven at the temperature of 110°C for 2 h. After heating it was cooled in a dessicator. After cooling, this dish was weighed. Percentage of moisture in sample was calculated by the following formula.

$$\text{Weight \% of moisture} = \frac{A}{B} \times 100.$$

Where:

A = weight in grams loss after drying.
B= weight in grams of salt sample.

Atomic absorption analysis. Atomic absorption spectrophotometer (AAS) having Model Hitachi Z8000 with specifications for studied metals enlisted in Table 1 was used for analysis. In 10 mL of HNO_3 and 5 g of sample was dissolved to make slurry. This slurry was then covered with watch glass for 30 min. Volume of the sample was made 1litre by dissolving doubly distilled water in 1000 mL flask. This sample solution was then heated at 110 °C for 15 min and then refluxed for 30 min without boiling. Sample solution was then cooled to add 5 mL of concentrated HNO_3. Sample solution was then again refluxed for 30 min. During refluxing most of the samples gave no brown fumes confirming that HNO_3 had completely reacted. While samples that gave brown fumes were added to 5 mL of concentrated HNO_3 and further stirred for 30 min until no brown fumes.

Table 1. Specifications of metals by atomic absorption spectrophotometer

Metals	$\lambda_{(max)}$	Flame gases	Sensitivity	Maximum lamp current
Cd	228.8	Air-acetylene	1.5	8
Cr	357.9	Nitrous oxide	4	12
Cu	324.8	Air-acetylene	4	10
Fe	248	Air-acetylene	5	30
Mn	279.5	Air-acetylene	2.5	20
Ni	232	Air-acetylene	7	30
Pb	283.3	Air-acetylene	20	15
Zn	213.9	Air-acetylene	1	10

Results and Discussion

Moisture content for Khewra salt mines range from 0.03 to 0.09% (Table 2) and salt samples from Kalabagh salt mines also had the moisture content in the range of 0.06 to 0.08 % (Table 3). Moisture content of both types of salt samples had lower values than the previously reported result by Chen *et al*. (2011) having average content 0.549% and by Usman and Filli (2011) having 0.649% in food grade salt. Sharif *et al*. (2007) reported the content of moisture in unrefined rock salt from Khewra salt mines in the range of 0.9 to 1.2% (Fig. 1). About 1% of moisture is considered as a permissible limit in food grade salt so present results were in this limit and found to be safer to use. Lower moisture content in all rock salt samples suggests them to be free flowing, minimum lump formation and being crystalline. Water insoluble matter was from 0.02 to 1.4% in all salt samples from Khewra salt mines (Table 2). Salt samples from the Kalabagh salt mines had relatively larger values of water insoluble matter ranging from

Table 2. Analytical parameters of Khewra salt

Sodium chloride %	Moisture	water insoluble	(Ca^{++}) (%)	(Mg^{++})	(SO_4^{-2})
96.4	0.08	1.4	0.15	0.23	0.9
97	0.06	1.2	0.2	0.22	0.9
98	0.09	1.2	0.2	0.15	0.9
97.4	0.07	0.02	0.15	0.23	0.91
96.3	0.07	1.06	0.24	0.21	0.77
98.9	0.06	0.09	0.19	0.11	0.36
97.8	0.06	0.87	0.32	0.35	0.54
98.6	0.03	0.06	0.24	0.21	0.39

1.5 to 2.8% (Table 3). When comparing the water insoluble matter of both the salt mines, it is evident that Kalabagh salt mines had a larger value. When the present results were compared with the previously reported results of water insoluble matter in table salt then it was found that the values were obtained by our research were slightly higher than the results reported by Sharif *et al*. (2007); Usman and Filli (2011) and Chen (2011).

Table 3. Analytical parameters of Kalabagh salt

Sodium chloride %	Moisture	Water insoluble	(Ca^{++})%	(Mg^{++}) %	(SO_4^{-2})
			%		
96	0.06	1.5	1.2	0.81	0.77
93.3	0.07	2	1.27	0.89	0.82
92	0.75	2.1	0.26	0.23	0.78
93	0.06	2.4	0.2	0.25	0.75
92	0.07	2.1	0.24	0.2	0.95
95	0.08	2.8	0.26	0.18	0.92
96	0.06	1.6	0.15	0.2	0.94

Table 4. Concentrations of trace minerals in Khewra salt

Sample	Fe	Cr	Zn	Mn	Cu	Cd	Pb	Se	Ag	As
				mg/kg						
1	1.2	ND	0.02	ND	0.03	ND	ND	0.03	0.02	ND
2	1	0.42	0.11	0.04	ND	ND	0.1	0.05	ND	0.03
3	1.85	0.22	0.13	ND	0.03	ND	ND	0.04	ND	ND
4	1.2	0.22	0.02	0.06	0.01	ND	ND	0.03	0.01	ND
5	1.8	0.24	0.12	ND	0.03	ND	0.04	0.02	ND	0.04
6	0.2	0.23	0.17	0.05	0.02	ND	ND	0.03	0.02	ND
7	0.4	0.22	0.14	ND	0.03	ND	0.05	0.02	ND	0.05
8	0.2	0.21	0.04	ND	0.05	ND	ND	0.02	ND	ND

ND=not detected

However, all the values of all the salt samples were in the range set by Codex Alimentarius Commission. Sulphate content of Khewra salt sample ranged from 0.36 to 0.91% while for Kalabagh salt mines its range was 0.75 to 0.95%. Present results of research showed that Kalabagh salt mines had relatively higher values of sulphate content. Sharif *et al*. (2007) found the %age of sulphate content in Khewra salt mines in the range from 0.28 to 0.58%.So present results were in accordance with the previously reported literature. According to Codex Food Standard, food grade sodium chloride should be 97% pure. Looking at the %age purity of all the salt samples, it makes clear that out of eight salt samples from Khewra, seven salt samples were above this limit. Only one salt sample had a variation of just 0.6 % (Fig.1). In Kalabagh salt samples however, no salt sample was as pure as 97% (Fig.2). Maximum purity of these salt samples were 96%. These results

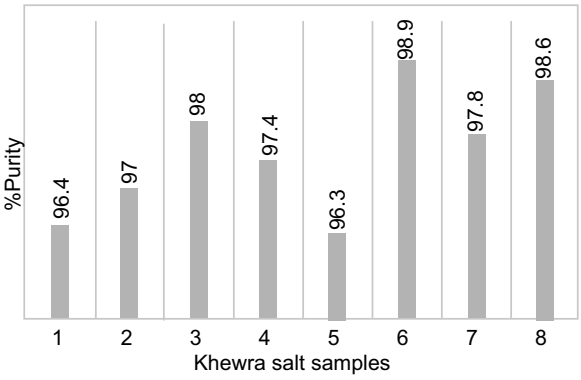

Fig. 1. Weight % purity of salt samples collected from Khewra salt mines.

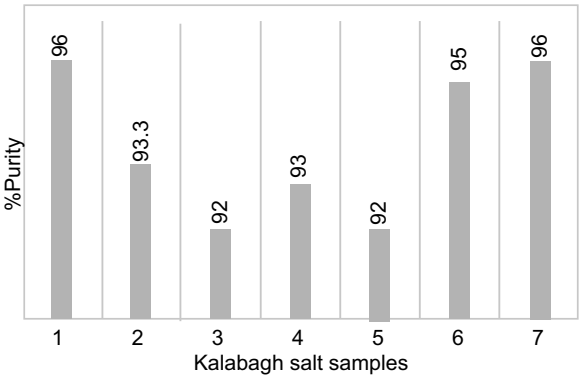

Fig. 2. Weight % purity of salt samples collected from Kalabagh salt mines.

Table 5. Concentrations of trace minerals in Kalabagh salt

sample	Fe	Cr	Zn	Mn	Cu	Cd	Pb	Se	Ag	As
					mg /kg					
1	1.8	0.34	0.1	ND	0.03	ND	0.1	ND	ND	ND
2	1.6	0.34	0.12	0.04	0.04	ND	0.04	ND	0.02	0.01
3	1.9	0.37	ND	0.03	0.04	ND	0.06	ND	ND	ND
4	1.8	0.31	ND	ND	0.02	ND	0.03	ND	ND	0.01
5	1.8	0.34	ND	ND	0.03	ND	ND	0.03	ND	0.02
6	1.75	0.38	ND	ND	0.03	ND	ND	0.04	0.01	0.01
7	1.72	0.22	ND	0.05	0.02	ND	0.1	0.04	ND	ND

ND=not detected

imply that there should be some mineralogical operations in order to improve the purity of Kalabagh salt samples. Unrefined rock salt samples should be undergone through some chemical or physical means to improve its purity level. WHO allows a maximum of 5g/kg of calcium in food grade salts. Results confirmed that the Kalabagh salt samples had a relatively higher concentration of calcium. Blaurockbusch (1996) reported the content of calcium to be 0.349µg/g while Sharif *et al*. (2007) reported the 0. 25mg/kg of sulphate content for Khewra salt mines. So obtained results were in agreement with the values previously reported in literature and also within the legal limits of maximum consumption by WHO. Content of magnesium was in the range of 0.11 to 0.35% for Khewra salt mines while for Kalabagh salt mines its range was in the range of 0.18 to 0.89%. Previously magnesium content was found 0.4% by Bergner (1997) and 0.12 % by Sharif *et al*. (2007). All the values were lower than the maximum human consumption limit of 3g/kg set by WHO. Iron range from 0.2 to 1.85mg/kg for Khewra and from 1.6 to 1.9 mg/kg for Kalabagh samples. Previously iron in Khewra salt mines samples were reported from 0.24 to 0.62 mg/kg by Sharif *et al*. (2007). So obtained results of Khewra and Kalabagh salt mines were within the limit of maximum human consumption and previously reported data. Copper between 0.01 to 0.05mg/kg for Khewra salt mines while for Kalabagh salt mines its range was from 0.02 to 0.04mg/kg in two salt samples. Out of 7 salt samples from the Kalabagh salt samples 5 were without any concentration of copper. Copper in table salt samples has been reported by Soylak *et al*. (2008) in the range of 0.17 to 0.47µg/g and by Usman and Filli (2011) in the range of 0.1 to 2.0 µg/g. Codex Alimentarius Commission recommended that the concentration of copper in food grade salt should be more than 2 mg/kg and recommended that daily allowance for copper is150-600 µg/day (Soylak *et al*., 2008). Thus from the obtained results and permitted level of copper regarding the maximum consumption by human body, it can be concluded that copper in the Khewra and Kalabagh salt samples is below this level. Out of eight salt samples of Khewra salt samples, 3 samples had the manganese concentration ranging from 0.04 to 0.06 mg/kg while in Kalabagh salt samples, out of 7 salt samples, 4 were without manganese and three salt samples had the concentrations 0.03 to 0.05 mg/kg. A maximum of 0.5mg/kg of manganese is reported by Lentech (2004). Present research results have confirmed that manganese is in lower concentrations as recommended daily allowance for the manganese is 20-90µg/day (Sharif *et al*., 2007). Range of chromium was from 0.21 to 0.42 mg/kg in Khewra salt while. Kalabagh salt samples had a slightly higher value of 0.22 to 0.38 mg/kg. All the results were in the limits of maximum human consumption set by Codex Alimentarius Commission. Lead in Khewra salt mines were 0.04 to 0.1mg/kg while it was 0.03 to 0.1 mg/kg for Kalabagh salt mines. Previously reported lead in Khewra salt mines were reported in the range of 0.02 to 0.10 mg/kg so present results were in accordance with the earlier literature. Previously zinc in Khewra salt mines were reported in the range of 0.12 to 0.18 mg/kg.During the present research its concentration was 0.02 to 0.17 mg/kg in Khewra salt mines and 0.1 to 0.12 for Kalabagh salt mines. Out of 7 salt samples of Kalabagh salt mines 5 were without zinc content. Concentration of arsenic in present research was 0.03 to 0.05 mg/kg in Khewra salt mines and 0.01 to 0.02 mg/kg in 4 out of 7 Kalabagh salt samples. Codex Alimentarious Legislation has 0.5 µg/g of arsenic as permissible limit.

Conclusion

Present research was conducted in order to evaluate the unrefined rock salt chemically from two major mining sites of Himalayan Rock salt deposits of Pakistan. During the whole research emphasis has been given on the comparison of results with the international standards regarding their maximum limits of human consumption set by Codex Alimentarius Commission, World Health Organization (WHO) and Food and Agriculture Organization (FAO). As the literature reported contamination of heavy metals with the table salts from Iran, Turkey, Greece, Brazil, Croatia, India, Portugal and Kingdom of Saudi Arabia present research results were also compared with the reported literature.

Analytical parameters had revealed that although the %age purity of all the salt samples for Khewra salt are more than 97% pure set by Codex but there should be some improved mineralogical and refining processes for it to be implied as food grade salt. Kalabagh salt samples are less pure so they must also be undergone through rigorous refining and mineralogy to raise its purity level up to 97%. All essential and non essential minerals were below the maximum limit of human consumption set by Codex Alimentarius Commission. Unrefined rock salts are concluded to be more nutritionally important than the refined ones.

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Pak. j. sci. ind. res. Ser. A: phys. sci. 2017 **60**(2) 72-78

Radiological Parameters Due to Radon-222 in Soil Samples at Baghdad Governorate (Karakh), Iraq

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(received February 8, 2017; revised May 25, 2017; accepted May 26, 2017)

Abstract: Measurements of radon concentration, effective radium content, potential alpha energy concentration (PAEC) and annual effective dose (AED) were estimated for soil samples in fifteen locations of the Baghdad governorate (Karakh) in the central part of Iraq. In this survey we used the can technique, containing nuclear track detector (CR-39). The obtained values of radon concentration measurements were generally low, ranging from 38.12±13.46 to 94.51±16.5 Bq/m^3, with an average 66.07 Bq/m^3, while the effective radium content varied from 5.80±0.21 Bq/kg to 14.39±0.33 Bq/kg with an average 10.09 Bq/kg. The average of the PAEC and AED were assessed to be 7.14 mWL 1.66 mSv/y, respectively. The results of the present study shows that the radium content are lower than the allowed limit reported by Organization for Economic Cooperation and Development (OECD) that is equal to 370 Bq/kg. In general, it is seen that the AED limit was within the recommended reference level (3 mSv/y to 10 mSv/y) of the World Health Organization. Also, it is found that there is a strong correlation (R^2= 1) between radon concentrations and effective radium content. The results obtained from this study indicate that the locations of Karakh has background radioactivity(radon concentrations) levels within the natural limits.

Keywords: radon levels, soil samples, Baghdad governorate, CR-39 detector

Introduction

Radon-222 is a gaseous radioactive isotope with a half-life of 3.82 days. It is resulted from the decay of ^{226}Ra radionuclide, which is in turn, the decay product of ^{238}U series. The soil's rock can be considered as the major source of radon in the atmosphere (i.e., at least 80%) (Michael, 2003). The rocks contain portion of Uranium, where the disintegration of ^{238}U through ^{226}Ra gives^{222}Rn. In this context, some types of rocks namely, granites, dark shale, light coloured volcanic rocks, sedimentary rocks and metamorphic rocks derived from rocks have a high uranium content (Virk and Singh, 1993). Because the radon is a gas, it is being characterised by having high mobility incomparably with the uranium and radium. When the radon is inhaled into the lungs, this may cause certain ionization damages when it strikes the lung tissue. Throughout time, this damage might cause lung cancer (Guo *et al*., 1992). Radon is found in all soils and rocks to some extent, but the amount may vary in different parts of the country at different times of the year. It consists in the ground by the radioactive decay of small amounts of radium which itself is a decay product of the uranium. Gas rises to the surface and in the open air it is quickly diluted to low and harmless concentrations in the atmosphere. However, once it seeps into the enclosed space, such as a building, it can accumulate to dangerous levels, depending on the concentration of radon in the underlying soil and the details of the construction of the building. Radon can also be introduced indoors by way of ground water supplied from a well or from building materials that contain traces of radium (Virk and Singh, 1993). The radon gas is well recognized as a radiation hazard source of excess lung cancer across underground miners (IAEA,2003). Radon poses grave health hazards to people living in normal houses and buildings, where about 60,00,000 homes in the United States have radon levels above 4 pCi/L, which is the remediation level recommended by EPA (2003). Also, it helps in mineral exploration (Uranium/Thorium), earthquake prediction, study of volcanic activities, and search for geothermal energy sources. It has proved to be a good friend and a powerful enemy at the same time (Khan, 1991). CR-39 detector is one of the most many-sided, Sensitive and widely used (SSNTDs) at the present time. It has been recognized by Cartwright and his colleagues as the track detector (Cartwright *et al*., 1978). CR-39 can detect protons of energy up to 10 MeV and has a wide energy range for α-particles detection (0.1-20) MeV. CR-39 is an appropriate detector

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when it is compared with other detectors used for radon measurement. CR-39 detector was used in this work so that a reasonable result can be obtained (Nabil and Hamed, 1995). Some authors have recently used solid state nuclear track detector and other techniques to measure radon concentrations in soil at different countries (Tawfiq and Jaleel 2015; Wedad and Adel, 2013; Ali, 2011; Ramola *et al*., 2008).

The aim of this work was to measure the radon concentration in soil gas of some locations of Baghdad governorate (Karakh) using solid state nuclear track detector (CR-39). In addition the PAEC and AED values were estimated.

Materials and Methods

Area of study. The area of Baghdad city is around 5159 km^2 and about 7 million people are living in Baghdad which represents 24% of Iraqi population (Al-Adili, 1998). The urban area (i.e., study region) lies between 33°346'-33°285' N latitudes and 44°269'- 44°375' E longitudes covering an area 1350 km^2 and it has mean elevation of 40 m ((Iraqi Meteorological Agency, 2010; Hamza and Yacoub, 1982). Fig. 1 shows the map of Baghdad governorate.

Fig. 1. Map of Baghdad governorate.

The soil of Baghdad town is characterised that it is not homogeneous, variable laterally and vertically because of the impact of human action, notably agriculture, wherever many ways employed in irrigation and drainage to cause a fast variation of the soil from one space to another in Baghdad (Hatab, 1985). Additionally it has been found that the soil of the study space containing the deposits of chlorides and sulphates, and this can be as a result of dry climate, low rainfall and poor irrigation of agricultural land. Therefore, the soil of Baghdad characterise the character of mixed and irregular alluvial sand (Adli, 1998).

Collection and preparation of samples. To measure radon concentrations in soil surface 15 soil samples were collected from different sites, one sample average from each point, was taken by digging a hole at a depth of 10-15 cm from the ground surface. The soil texture for all samples was very similar. . The studied locations in Baghdad city are shown in Fig. 2 and Table 1. The samples were crushed and dried. All of these samples

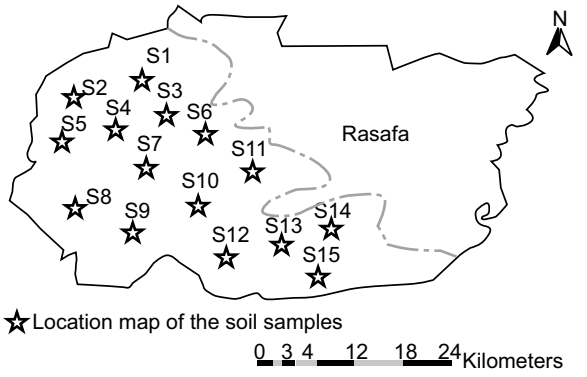

Fig. 2. Map of the studied area showing the locations of soil samples in Baghdad area.

Table 1. The studied locations in Baghdad city

Sample code	Region	Coordinate	
		Longitude (°)	Latitude (°)
S1	GAZALIYA	44.269 to 44.283 E	33.346 to 33.339 N
S2	HURRYA	44.312 to 44.326 E	33.346 to 33.339 N
S3	ADEL	44.276 to 44.290 E	33.339 to 33.332 N
S4	JAMIAH	44.295 to 44.315 E	33.325 to 33.318 N
S5	AMEL	44.314 to 44.341 E	33.267 to 33.260 N
S6	KHADRA	44.276 to 44.290 E	33.339 to 33.332 N
S7	SHORTA	44.305 to 44.312 E	33.246 to 33.238 N
S8	YARMOUK	44.334 to 44.348 E	33.396 to 33.289 N
S9	MANSOUR	44.341 to 44.355 E	33.318 to 33.310 N
S10	HARTHYA	44.370 to 44.377 E	33.310 to 33.305 N
S11	MAMOON	44.365 to 44.385 E	33.332 to 33.325 N
S12	JIHAD	44.290 to 44.304 E	33.274 to 33.267 N
S13	AALAM	44.312 to 44.326 E	33.224 to 33.217 N
S14	SHUALA	44.334 to 44.341 E	33.382 to 33.375 N
S15	QADISIYAH	44.345 to 44.375 E	33.295 to 33.285 N

dried in an oven at 150 °C for one hour to ensure that any significant moisture was removed. After that a sieve with diameter holes 250 μm was used to obtain a homogeneous powder.

Irradiation of the detectors. The weight of 10 g of soil were taken from each location and placed in plastic can (container). Then, the container were sealed with a tape and kept for one month before investigation to allow secular equilibrium to be earned between ^{222}Rn and its parent ^{226}Ra in uranium-series. These containers of 7 cm mouth diameter, 4 cm bottom diameter and 8.5 cm height were made of polypropylene. CR-39 detector with dimensions of (1×1) cm^2 and 1 mm thick (manufactured by Pershore Moulding Ltd. U.K.) was placed at the middle of the underside of the cover and affixed with an adhesive tape. The edges of the cover were taped and sealed to prevent radon from leaking. The CR-39 detector recorded the presence and effects of alpha particles which resulted from the disintegration of radon gas. The distance between the surfaces of the sample and CR-39 was 7.5cm and the radius was 2.5 cm as shown in Fig. 3. The dimensions can minimize the effect of thoron gas. The long-term method of 21 day exposure was applied before removing the CR-39 exposing them to the chemical etching procedure. The background was measured by using three empty containers and counting under the same time for the sample measurements.

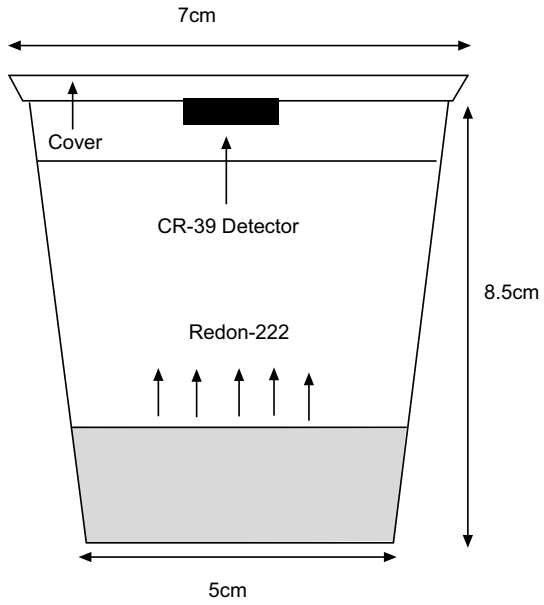

Fig. 3. The Can technique with CR-39 used for radon measurements in soil samples (Mustafa, 2006).

Chemical etching and microscopic scanning. The detectors after the exposure time were etched in a 6.25 N aqueous solution of NaOH maintained at 60 °C in a water bath for 6 h, which was the normally employed etching time. The detectors were then taken out from the etching, rinsed with distilled water and dried in air. The number of tracks (Track/mm^2) was counted using an optical microscope with (400x).

Calculation of radon concentrations and effective radium content. The density of the tracks (ρ) in the detectors was calculated using the following relation (Heiyam *et al*., 2016; Morelli *et al*., 2015):

$$\rho = \frac{N}{A} \qquad (1)$$

where:

ρ is the track density(track /mm^2), N is average number of total tracks and A is area of field view. Radon concentration in soil samples under the study was obtained by creating a comparison between track densities registered (in unit track/mm^2) on the detectors of the sample which is the standard geological sample, and radon concentration can be calculated as the following relation:

$$\frac{C_x}{\rho_x} = \frac{C_s}{\rho_s} \qquad (2)$$

where:

C_S and C_X are specific activity (Bq/m^3) of radon concentration for standard and sample, respectively. ρ_s and ρ_X: track density (Track/mm^2) for standard and sample, respectively. From eq.(2) can be found:

$$C_x = C_s \frac{\rho_x}{\rho_s} \text{ and slope} = \frac{\rho_s}{C_s} \qquad (3)$$

slope for radon is=33.3 track/mm^2 per Bq/m^3, as shown in Fig. 4.

The effective radium content in unit (Bq/kg) can be calculated by following equation (Sonkawade *et al*., 2008):

$$C_{Ra} = \left(\frac{\rho}{kT_e}\right)\left(\frac{hA}{M}\right) \qquad (4)$$

where:

ρ is track density in (track/mm^2) , K is calibration factor in (track/mm^2. h) per (Bq/m^3), T_e is irradiation time in hour, h is the distance between the detector and top of

the solid sample in m, M is the mass of the soil sample in kg and A is the area of cross-section of the can in m^2.

Calculation of PAEC and AED. The PAEC values (in mWL) were calculated using the following equation (Shakir and Azam, 2012; ICRP,1993):

$$\text{PAEC (mWL)} = \frac{C_{Rn} \times F_{Rn}}{3.7} \qquad (5)$$

where:

F_{Rn} is the equilibrium factor for radon and has a value of 0.4 as suggested by UNCSEAR (1999).

The value of AED due to the exposure to radon and progeny in the soil of studied area were calculated using the formula (UNSCEAR, 2000):

$$\text{AED}\left(\frac{\text{msv}}{\text{y}}\right) = \text{C}\left(\frac{\text{Rq}}{\text{m}^s}\right) \times \text{F} \times \text{t(h/y)} \times \text{D}\frac{\text{nSv}}{\text{h}} \quad \text{per}\left(\frac{\text{Bq}}{\text{m}^s}\right) \qquad (6)$$

where:

C is the measured radon concentration (in Bq/m^3), *F* is the average of equilibrium factor for radon and progeny which is value 0.4 (ICRP, 1981), t is the indoor occupancy (7008 h/y), and *D* is the dose conversion factor, which convert activity concentration to effective dose rate, *D*= 9 nSv/h per Bq/m^3.

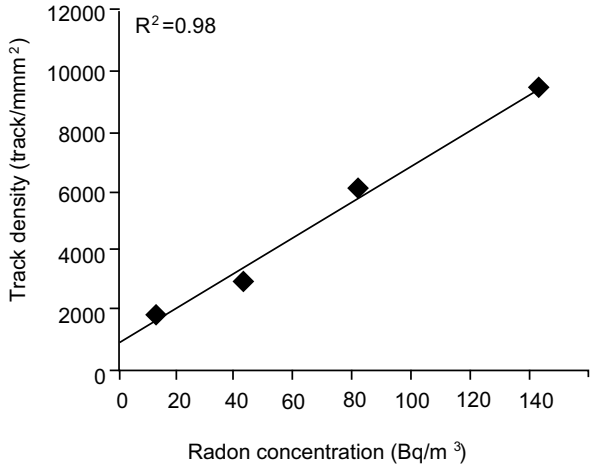

Fig. 4. The relation between track density and the radon concentrations for standard geological soil samples (Mustafa, 2006) .

Results and Discussion

In the present work, fifteen locations for soil samples collection at Baghdad (Karakh) have been selected to achieve this study. Table 2 illustrated the radon concentrations and effective radium content in these locations in unit of Bq/m^3 and Bq/kg, respectively. Potential alpha energy concentration and annual effective dose calculated values are shown in Table 3. The values of radon concentration in studied area varies from (38.12±13.46) Bq/m^3 to (94.51±16.5) Bq/m^3 with an average of (66.07) Bq/m^3, while the values of effective radium content varied from (5.80±0.21) Bq/kg to (14.39±0.33) Bq/kg with an average of (10.09) Bq/kg. The values of radon concentration found to be maximum at SHUALA (S14) and minimum at MAMOON (S11). These results are lesser than the affordable limit, usually ranges from 0.4 to 40 kBq/ m^3 (Ali, 2012; Gabriele, 2007) and shown as a red line in Fig. 5. Also, the maximum value of effective radium content is lower than the allowed limit reported by Organization for Economic Cooperation and Development (OECD), which is around 370 Bq/kg (OECD,1979). In addition, it is found that the emanation rate for radon concentration varied from one location to other. This is due to the nature of the soil or the geological layer of the area. Table 3 and Fig. 6-7 show the calculated average values of the PAEC and AED for soil samples under the study. From Table 3, it can be observed that the range average PAEC for ^{222}Rn were found (4.12-10.21) mWL, while

Table 2. Radon concentration values in soil samples under the study.

Sample code	Radon concentration (Bq/m^3)	Effective radium content (Bq/kg)
S1	60.26±13.42	9.17±0.26
S2	45.22±9.18	6.88±0.23
S3	76.52±15.6	11.65±0.30
S4	62.80±11.25	9.56±0.27
S5	90.01±7.65	13.70±0.32
S6	53.90±14.00	8.20±0.25
S7	82.20±14.81	12.52±0.31
S8	77.31±6.04	11.77±0.3
S9	59.65±9.33	9.08±0.26
S10	84.11±11.19	12.81±0.31
S11	38.12±13.46	5.80±0.21
S12	69.00±10.11	10.51±0.28
S13	41.23±6.21	6.27±0.22
S14	94.51±16.5	14.39±0.33
S15	56.34±12.6	8.58±0.25
Average	66.07	10.09

Table 3. The PAEC and AED values in soil samples under study

Sample code	PAEC (mWL)	AED (mSv/y)
S1	6.51	1.52
S2	4.88	1.14
S3	8.27	1.93
S4	6.78	1.58
S5	9.73	2.27
S6	5.82	1.35
S7	8.88	2.07
S8	8.35	1.95
S9	6.44	1.50
S10	9.09	2.12
S11	4.12	0.96
S12	7.45	1.74
S13	4.45	1.04
S14	10.21	2.38
S15	6.09	1.42
Average	7.14	1.66

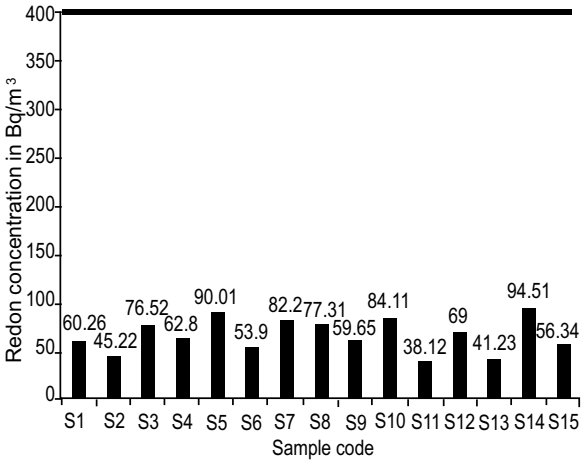

Fig. 5. Contribution of the average of radon concentration in samples under study area.

the range average of AED were (0.96 - 2.38) mSv/y. The observed values of annual effective dose in the present study were below the world range levels (3-10) mSv/y (ICRP, 1993). Figure 8 shows the relationship between the radon concentrations in soil samples under study and effective radium content in the same samples. From Fig. 8 it can be seen the amount of radon concentrations increase and increase the effective radium content, where correlation factor (R^2=1). The results have revealed that the radon concentration in studied area and the associated potential alpha energy concentration dose do not pose risk to human health.

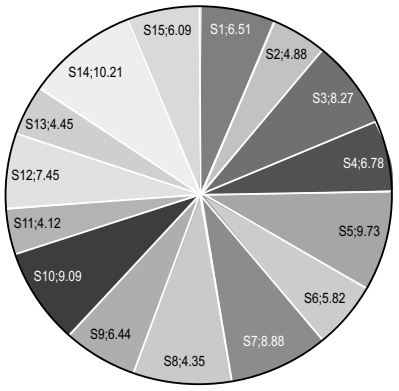

Fig. 6. Average values of PAEC in unit (mWL) for samples in study area.

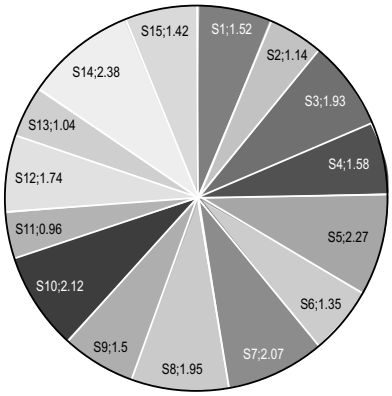

Fig. 7. Average values of AED in unit (mSv/y) for samples in study area.

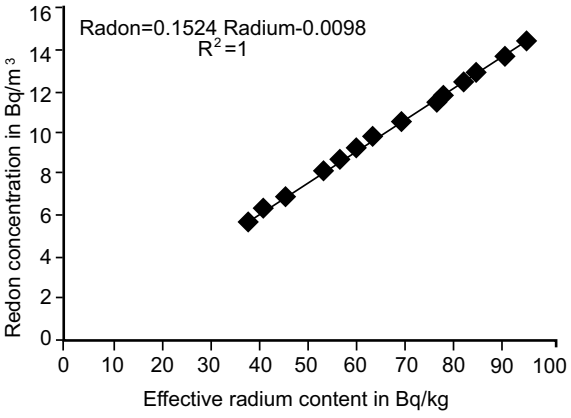

Fig. 8. Correlation between radon concentrations and effective radium content for samples in study area.

The average of radon concentration obtained in this study was compared with other similar studies in literature and this is presented in Table 4.

Table 4. Comparison of the present work study results with previous studies

Country	Average of Radon Concentrations (Bq/m^3)	Reference
India	566	(Sharma *et al*.,2012)
Iraq (Nasiriya)	138.623	(Ibthaj, 2014)
Iraq (Salahaddin)	77.07	(Ridha and Kadhim, 2014)
Turkey	3.4 -138	(Muslim *et al*., 2011)
Pakistan	376	(Faheem and Matiullh, 2008),
Iraq(Baghdad)	66.07	This work

Conclusion

Radon concentrations, PAEC and AED values were estimated for the soil samples of some locations of Baghdad governorate, Iraq. The measured radon concentrations and the calculated annual effective dose values are found to be within acceptable ranges, according to the ICRP (1993). Hence, it can be concluded that the studied area is safe in term of radon health hazards since the levels of radon concentration do not pose any radiation risk especially when the potential alpha energy concentration and the annual effective dose are being considered.

Acknowledgement

Authors would like to acknowledge all those who have contributed in this study. Special thanks to the staff of the Department of Physics at Kufa University.

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Pak. j. sci. ind. res. Ser. A: phys. sci. 2017 **60**(2) 79-84

Dynamic Compression and Thermo-Physical Properties of Some Wood Particles in South Western Nigeria

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(received June 6, 2016; revised January 10, 2017; accepted January 20, 2017)

Abstract. This study examines the dynamic compression and thermo-physical properties of some wood particles obtained from Akure, south local government area, Ondo State, South Western Nigeria. These wood particles are of the species of *Celtis zenkeri* and *Celtis philippensis* of the Ulmaceae family. The samples were possessed into different particle sizes (300, 600 and 850 μm) and subjected to varied compacting pressures (2.6-3.0 MPa). The density and specific heat capacity of the wood samples were determined using weighing displacement methods and temperature dependent model while the thermal diffusivity was estimated from other thermal properties. The results revealed significant variation in the values of the specific heat capacity as a result of change in pressure for all the wood samples considered. The density of wood samples lies between 4.51×10^2 -7.32×10^2 kg/m^3 and the specific heat capacity values obtained for the samples fall within the range of 1.28×10^3-1.33×10^3 J/kg/K. It was also noted that the thermal diffusivity obtained falls within the range of 1.37×10^{-7}-2.10×10^{-7} m^2/s for the wood materials considered. However, the values of the densities, specific heat capacities and thermal diffusivities of the samples were found to change as the compacting pressure increased due to decreased in porosity. The implication of the study is that the materials have potential for use in polymer applications and the fabrication of film based photographic devices.

Keywords: density, specific heat capacity, thermal diffusivity, wood particle

Introduction

Thermal insulation material generally reduces the heat flow by minimizing heat in a system. Thermal properties of a material include; thermal conductivity, thermal diffusivity and specific heat. It is of common knowledge that as the moisture content of a material increases, the thermal conductivity, thermal diffusivity and specific heat also increase (Ajit *et al*., 2013). One of the important parameters for determining the insulation property of a material is the specific heat capacity. It is the amount of heat required to raise the temperature of one kilogram of a material by one degree Kelvin. The specific heat capacity is fairly constant for different wood samples; it increases with temperature and moisture content (Simpson and Tenwolde, 1999). A high specific heat capacity value means high ability of heat retention for an insulating material (Ayugi *et al*., 2011). The thermal diffusivity (λ) of a material is usually estimated from the values of the thermal conductivity (k), density (ρ) and specific heat capacity (c) (Moore, 2011; Glass and Zelinka, 2010; Silva *et al*., 1998; Suleiman *et al*., 1997). Thermal diffusivity is a measure of how quick a material can absorb heat from its surroundings and diffused through crystal lattice of the material; it is the ratio of thermal conductivity to the product of density and heat capacity. Wood is a hard fibrous structural tissue found in the stems, roots of trees and other woody plants. It has been in use for many years as fuel (fire wood) and construction material. It is an organic material, a natural composite of cellulose fibres, embedded in a matrix of lignin which resists compression (Hickey and King, 2001). Sawdust is generally considered as a waste product. It is a by-product of wood, which is produced from sawing of wood (Badejo and Giwa, 1985). Saw dusts are very prevalent around sawmills and wood based industries (Akande, 2001). According to Ogunsawo (2001), the non-utilization of the sawdust create disposal problems, which are burdensome. Owonubi and Badejo (2000) have therefore observed that in order to dispose of the large environment and also cause environmental pollution; many saw miller resort to burning. It however produces smoke and offensive gases like carbon dioxide and carbon monoxide, which are hazardous to human health. Knowledge on the thermal properties of wood is a continuous process as the understanding of the model heat transfer processes in wood and wood based materials

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cannot be overemphasized. For example, the energy design and evaluation of energy performance of wood-frame buildings partially rely on the thermal properties of wood and wood products (Zi-Tao *et al*., 2011; Tenwolde *et al*., 1988). Various properties had been adduced to affect the thermal properties materials which will in turn have attendant influence on their device utilization and applications (Oluyamo *et al*., 2016; Oluyamo and Adekoya, 2015; Oluyamo and Bello, 2014). This research intends to study the effect of dynamic compression on the thermo-physical properties of selected wood particles which may lead to a more beneficial usefulness to the society rather than menace.

Materials and Methods

Sample preparation. The wood materials used in the study are two different wood species growing in the rainforest region, South Western Nigeria. These species are; *Celtis zenkeri* and *Celtis philippensis* of the family Ulmaceae. The samples were collected from different sawmills in Akure, south local government area of ondo State, Nigeria. These wood samples were converted into sawdusts using circular saw machine and further separated into different particle sizes (300, 600 and 850 μm) with the aid of a mechanical test sieve shaker (Fig. 1). The samples were subjected to different compacting pressure (2.6-3.0 *MPa*) and later shaped into circular disc shape using a modified California Bearing Ratio (CBR). The preparation of the samples into disc shapes and particle sizes were carried out at the Department of Applied Geology and Material and Metallurgical Engineering Department of The Federal University of Technology, Akure. Possessed samples were oven dried in the laboratory before further analyses were carried out.

Determination of density and specific heat capacity. The density was measured for each of the sample using the weighing displacement methods (Akpabio *et al*., 2001; Ekpe *et al*., 1996) while Temperature Dependent Model (TDM) was used to determine the specific heat capacity of the wood samples. According to Simpson and Tenwolde (1999), the approximate specific heat capacity of oven-dry wood as a function of temperature is given as

$$c=0.1031+0.003867T \qquad (1)$$

where:

T is the equilibrium temperature.

Determination of Thermal Diffusivity. The thermal diffusivity is given as

$$\lambda=\frac{k}{\rho c} \qquad (2)$$

The thermal conductivity of the two samples at different compacting pressure and particle sizes has earlier been reported by Oluyamo and Adekoya (2015).

Results and Discussion

Density. Variation in densities of the samples has been depicted in Fig. 2-3. The values of the density were

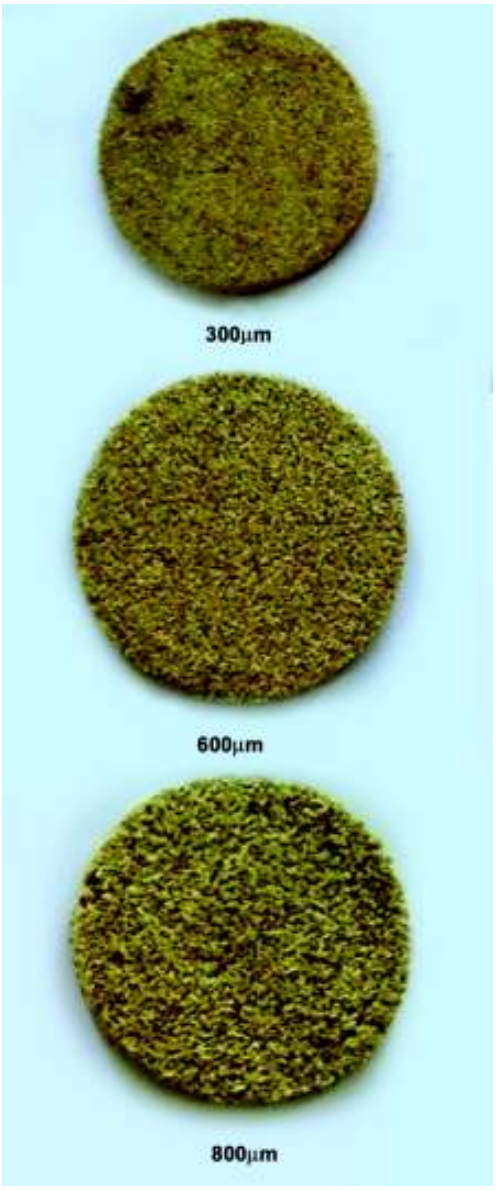

Fig. 1. Final disc shape of the samples for different particle sizes.

found to change as the compacting pressure increased. There also seems to be no definite variation in densities of the material with compacting pressure. However, for *C. zenkeri*, the densities of the wood samples increases as the particle sizes decreases except at 3.0 MPa.

These values of densities of wood samples for different compacting pressures at particle sizes, 300, 600 and 850 µm, respectively are shown in Tables 1-2. The values of the density ranged from 4.51×10^2-6.81×10^2 kg/m^3 for *C. philippensis* (Ita funfun) and 4.7×10^2-7.32×10^2 kg/m^3 for *C. zenkeri* (Ita pupa).The values of the densities were found to be relatively high for 300µm particle size at 2.7 MPa compacting pressure for *C. zenkeri*.

Specific heat capacity. The variations of the various specific heat capacities of the selected wood samples as a function of compacting pressure are displayed in Fig. 4-5. It was observed that the specific heat capacity has no defined trend with the compacting pressure for all the wood samples. The values of the specific heat capacities only fluctuate between minimum and maximum values within the range of compacting pressure.

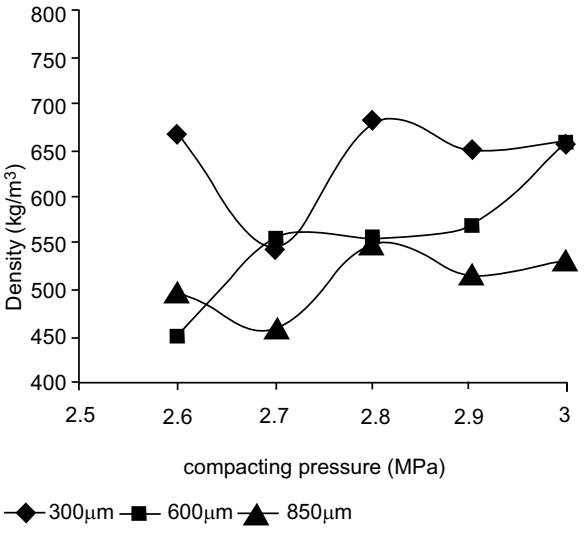

Fig. 2. Graph of density as a function of compacting pressure for *Celtis philippensis* (Ita funfun).

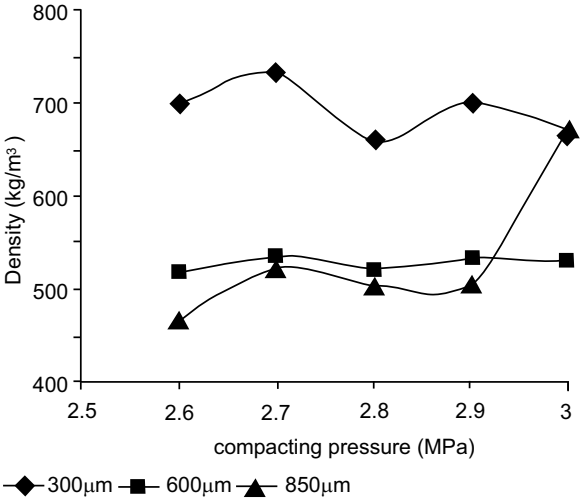

Fig. 3. Graph of density as a function of compacting pressure for *Celtis zenkeri* (Ita pupa).

The specific heat capacities of the wood samples depend on the moisture content of the wood and seen to be independent of density and species which is in agreement with previous research (Simpson and Tenwolde, 1999). In addition, materials with high specific heat capacity absorb more energy before they change in temperature than substances with low specific heat capacity (Oladunjoye and Sanuade, 2012; Oyekan and Kamiyo, 2011). The specific heat capacity values obtained ranged between 1.29×10^3 -1.33×10^3 J/kg/K for *C. phillipensis* and 1.28×10^3 – 1.31×10^3 J/kg/K for C. *zenkeri*. The samples have their highest specific heat capacity at 300 µm particle size of 3.0 MPa with *C. philippensis* having

Table 1. Thermal Properties of *Celtis philippensis*

Compacting pressure (MPa)	Density (10^2 × kg/m^3)			Specific heat capacity (10^3 × J/kg/K)			Thermal diffusivity (10^{-7} × m^2/s)		
	300 µm	600 µm	850µm	300 µm	600 µm	850µm	300 µm	600 µm	850µm
2.6	6.70	4.51	4.99	1.29	1.31	1.30	1.54	2.10	1.86
2.7	5.45	5.55	4.60	1.31	1.30	1.30	2.00	1.85	2.05
2.8	6.81	5.58	5.50	1.29	1.30	1.30	1.72	1.93	1.77
2.9	6.51	5.70	5.18	1.31	1.30	1.30	1.77	1.91	1.93
3.0	6.58	6.58	5.31	1.33	1.30	1.30	1.75	1.68	1.96

Table 2. Thermal properties of *Celtis zenkeri*

Compacting pressure (MPa)	Density (10^2×kg/m^3)			Specific Heat Capacity (10^3×J/kg/K)			Thermal Diffusivity (10^{-7}×m^2/s)		
	300 µm	600 µm	850µm	300 µm	600 µm	850µm	300 µm	600 µm	850µm
2.6	7.00	5.20	4.70	1.30	1.29	1.30	1.37	1.68	1.66
2.7	7.32	5.36	5.23	1.31	1.30	1.29	1.38	1.70	1.67
2.8	6.60	5.23	5.05	1.30	1.30	1.31	1.58	1.82	1.74
2.9	7.00	5.33	5.08	1.29	1.28	1.30	1.56	1.93	1.80
3.0	7.00	5.30	6.75	1.30	1.31	1.30	1.70	1.97	1.42

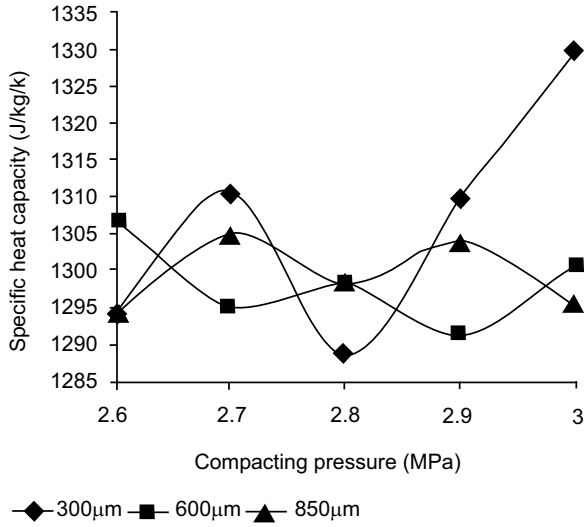

Fig. 4. Graph of specific heat capacity as a function of compacting pressure for *Celtis philippensis* (Ita funfun).

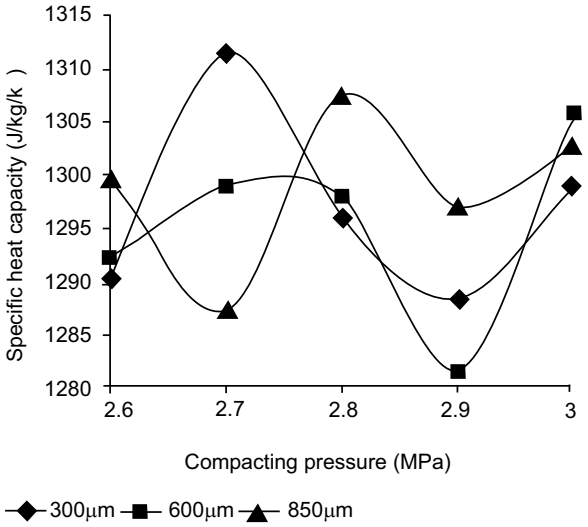

Fig. 5. Graph of specific heat capacity as a function of compacting pressure for *Celtis zenkeri* (Ita pupa).

the highest specific heat capacity value of 1.33×10^3 while *C. zenkeri* has the least for 600 µm particle sizes at 2.9 MPa. These values are within the range obtained by Simpson and Tenwolde (1999) and the values conform to Kärkkäinen (2007) which is 1.34×10^3 J/kg/K at temperature range of 273-373 K. The results of the study reveals that the specific heat capacity values conform to common material used in polystyrene (1.30×10^3-1.50×10^3), polycarbonates (1.20×10^3-1.30×10^3) and cellulose acetate (1.30×10^3 -1.80×10^3) (www.engineering tool box.com, May 2016). Therefore, the particulate materials could be useful in the fabrication of film based photography, eye protection, producing plastics cutlery and dinnerware and smoke detector housing. However, the particle sizes seem to be more suitable in these categories

Thermal Diffusivity. Variations of thermal diffusivities of the selected wood samples are depicted in Fig. 6-7. The values of the thermal diffusivity change gradually as the compacting pressure increases except at 2.9 MPa compacting pressure which increases as the particle sizes increase.

Evidence of slight increase in thermal diffusivities of the wood samples was noticed as particle size increases from 300 µm to 600 µm but decreases monotonically at 850 µm for *c. zenkeri*.

Conclusion

The effects of dynamic compression on thermal properties of wood species were investigated. It was established in the research that the specific heat capacity

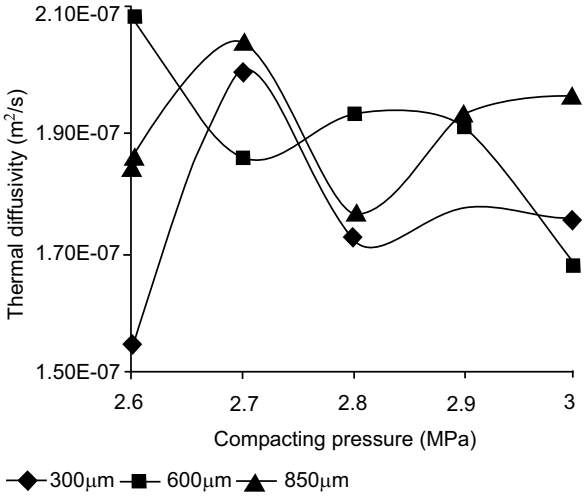

Fig. 6. Graph of thermal diffusivity as a function of compacting pressure for *Celtis philippensis* (Ita funfun).

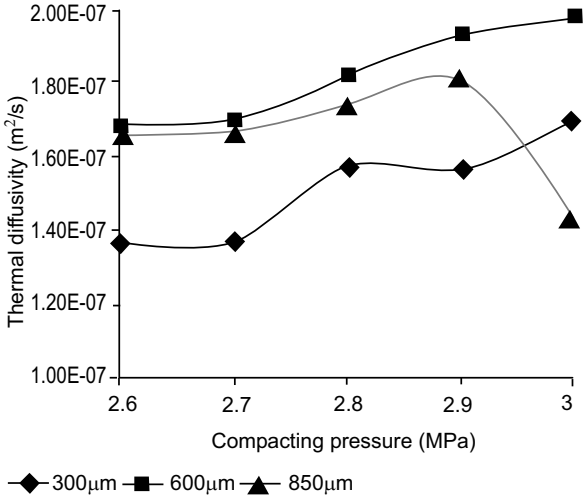

Fig. 7. Graph of thermal diffusivity as a function of Compacting Pressure for *Celtis zenkeri* (Ita pupa).

of wood materials used in the study falls between 1.28×10^3– 1.33×10^3. This range lies within the specific heat capacity values of common materials used in polystyrene, polycarbonates and cellulose acetate. The selected wood materials could find useful applications in insulating and heat resistant devices. The results in the study revealed that compacting pressure and density has an effect on the thermal diffusivities but has no significant effect on specific heat capacity of the wood samples studied.

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A Three-Layer Microstrip Resonator for Complex Permittivity Measurement of Medium Loss Liquids Using 3D-FDTD Simulation

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(received October 15, 2016; revised April 8, 2017; accepted April 27, 2017)

Abstract. A three-layer microstrip resonator is introduced to measure complex permittivities of medium loss liquids. The device is configured such that the first layer containing the liquid under test; a sheet of polytetrafluoroethylene (PTFE) is embedded in the middle layer, and the third layer functions as the base on which the patch is printed. The base layer is inverted on PTFE layer, and reflection coefficient is measured from 2.5 GHz to 5 GHz. The complex permittivities are extracted from the resonant frequency and the 10-dB bandwidth of S-parameter for different combinations of ethanol and methanol. Indeed, a three-layer microstrip resonator allows us to possess an affordable, and yet, high-accuracy electrical device to measure complex permittivities of medium loss liquids. FDTD method is used for analysing the structure and the results obtained by using FDTD method and the experimental data indicate a high degree of similarity.

Keywords: microstrip resonator, microwave chemistry, complex permittivity measurement, 3D-FDTD

Introduction

Complex permittivity measurement is of great significance in bio-electromagnetics, microwave chemistry and microwave engineering. Complex permittivity of some biological dilutions, which is important for millimeter-wave dosimetry studies, has been extracted by means of an open-ended coaxial probe (Zhadobov *et al*., 2012). A microwave resonator has been constructed to characterize the complex permittivity of fruitful solutions in chemistry and biology (Chretiennot *et al*., 2013) and several methods have been recently investigated to measure the dielectric of a ferroelectric composition, which is used in the electronics industry (Queffelec *et al*., 2014). The permittivity of many substances changes with frequency and quantifying of the permittivity of these materials with high resolution along with frequency, specifically microwave frequencies, is of prime concern in different industries (Sarri *et al*., 2012; Osman *et al*., 2008, Komarov *et al*., 2005). Planar circuits have always been one of the best instruments to determine the complex permittivity of materials (Chakyar *et al*., 2017, Yang *et al*., 2016; Ansari *et al*., 2015). They have great advantages including small size, light weight, low power consumption, and easy implementation. On the other hand, the number of methods applicable to measure their permittivities is reduced by the lack of particular shape in liquids. Microstrip resonators, as one type of planar circuits, have shown a good performance in the permittivity measurement of liquids. Given the importance of identification of liquids in applied sciences, resonant methods make a considerable contribution; through exploring the complex permittivity of liquids with an eligible degree of accuracy and sensitivity (Yu *et al*., 2000).

A three-layer microstrip resonator is introduced, by which the complex permittivity of liquids is determined. The resonator is composed of three substrates; the first substrate is considered to measure the sample liquid, the second substrate is fixed by a Teflon (PTFE) sheet which is used to increase the degree of freedom in choosing resonant frequency, and the last is the base layer on which the patch is inversely fabricated. The resonator measures the complex permittivity of medium loss liquids for the frequency near and below 4 GHz.

Materials and Methods

Numerical analysis. Finite-difference time-domain (FDTD) was initially proposed in 1966 by Yee (1966). This modeling technique, which solves Maxwell's

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equations in time domain, has been broadly used to simulate various electromagnetics problems (Sullivan, 2013; Taflone and Susan, 2000). FDTD method is very appropriate to model dispersive material in a wide range of frequencies (Bia *et al*., 2015; Luebbers *et al*., 1990, Yee, 1966). The S_{11} parameter of binary mixtures of ethanol-methanol, which belong to dispersive materials characterised by Debye Law (Bao *et al*., 1996) was simulated by using this time domain method.

A three dimensional FDTD code is written for the resonator. The code is implemented by MATLAB. This code has been used to validate and compare visually the results of the measurements with standard values.

Extraction of complex permittivity components. Notwithstanding, there are some equations that represent resonant frequency and Q-factor are dependent on the real ($\in'$) and imaginary ($\in''$) parts of the complex permittivity of the substrates (Gupta and Srivastava, 2012). To extract the complex permittivity components, an approximate approach was considered which is simpler and more practical than the mentioned complex equations (Gupta and Srivastava, 2012). There is almost an inverse relationship between the resonant frequency f_0 and the magnitude of $\in'$. In the same way, an inverse relationship can be considered between Q-factor and the quantity of $\in''$. Equations (1) and (2) illustrate these issues. The results of simulations and experiments presented confirm these assumptions. In the FDTD code, the values of $\in'$ and $\in''$ are dependent on frequency and are extracted from Debye function (Bao *et al*., 1996).

$$\frac{\in'_1}{\in'_2} \approx \frac{f_2}{f_1} \quad \text{..... (1)}$$

$$\frac{\in''_1}{\in''_2} \approx \frac{Q_2}{Q_1} \quad \text{..... (2)}$$

As reported by Bao *et al*. (1996), the changes of the real and imaginary part of the complex permittivity of ethanol-methanol binary mixtures along the intended frequency range are almost linear in connection with changes in volume fractions of these two liquids. The results are classified in the format of table and any unknown binary mixture of ethanol and methanol can be interpolated linearly from this table. Thus, the values of the real ($\in'_x$) and imaginary ($\in''_x$) part of the complex permittivity can be extracted as:

$$\frac{\in'_x - \in'_1}{\in'_2 - \in'_1} = \frac{f_1 - f_x}{f_1 - f_2} \quad \text{..... (3)}$$

$$\frac{\in''_x - \in''_1}{\in''_2 - \in''_1} = \frac{BW_x - BW_1}{BW_2 - BW_1} \quad \text{..... (4)}$$

Where:

constants of $\in'_1$, $\in''_1$, $\in'_2$ and $\in''_2$ are real, reference data by Bao *et al*. (1966). f_1, f_2, BW_1 and BW_2 are achieved by the FDTD code and f_x and BW_x are obtained from the experimental results.

Minimization of errors of permittivity components. Like many electrical structures, there are some errors and discrepancies between measurement values delivered by device under test and standard ones. One of the most common and not to mention easiest way to minimize these errors is equally shifting primary results to the standard values for each component of the permittivity. But this kind of gradation cannot minimize all the errors since the real part and imaginary part of the complex permittivity in ethanol-methanol binary mixtures change nonlinearly with frequency. Nevertheless, a certain frequency zone may be designated in which both parts of the permittivity approximately vary linearly in the frequency domain. The frequency range from 3.5 GHz to 4 GHz would be an overall proper choice. Therefore, discrepancies between measured and standard values decreased conspicuously. Actually, this was attained because the three-layer microstrip resonator allowed to choose an appropriate frequency domain.

Three-layer microstrip resonator configuration. Among many design challenges, the expenditure in manufacturing a device has constantly been of prime concern. Apart from cost issue, availability of the material, having appropriate features; is a key factor. In many cases, the material, which is appropriate to realize a high-accuracy measuring device, is out of reach and providing the material would not be affordable. Achieving both of these is a difficult task. The three-layer microstrip resonator, however, seems to be able to fulfill this target. The presence of two substrates beside liquid under test enhances the number of materials that can be selected as substrates in association with our demands.

Schematic diagram of the proposed microstrip resonator configuration is shown in Fig. 1. The thickness of sample layer is h_{mut}=1 mm. PTFE is set in middle layer with thickness of h_{tfln}=2 mm. The base layer is a polyester (RO4003) with relative permittivity $\in_r$=3.4 and thickness of h_{base}=0.5 mm. The cupric microstrip patch was designed in 28×20 mm^2 dimensions. The 2×10 mm^2

quarter-wavelength matched the 4×10 mm^2 microstrip transmission line to the patch. Also, SMA connector with flange jack was employed to supply the resonator. All layers were covered with a Teflon-metal enclosure; the metal portion under the structure had the duty of ground plane. Teflon layer above the resonator prevents the device from being aesthetically unpleasant. Both were jointly considered as an enclosure to protect substrates. Total dimensions of the system, as shown in Fig. 1, are 80×70×20 mm^3.

Results and Discussion

The permittivity of different binary mixtures of ethanol and methanol was constructed by the resonator. The sample layer of the structure was filled by the binary mixtures, and then reflection coefficient was measured by a vector network analyzer in the frequency range of 2.5 GHz to 5 GHz (Fig. 2). This test was performed for eleven samples in which the volume fractions of ethanol and methanol varied in a scale of 10% - from 0% to 100%. The results of the parameters were saved and then replotted in addition to FDTD results using MATLAB in order to display visually the similarity between two approaches. Figure 3 shows two specimens of these plots. Due to good assumptions in FDTD code, the simulation speed of this numerical technique would be considerably less than simulator softwares, which can be investigated later. However, the main purpose of providing this numerical method has been offering an available approach to analyze our experimental results and data in Tables 1-2 demonstrate the success of this attempt.

As mentioned before, the complete results including amounts of measured and simulated resonant frequency and 10-dB bandwidth of all samples and errors of $\in'$ and $\in''$ have been provided in Tables 1-2. The errors have been extracted according to equations (3) and (4) and also have been accomplished by minimization method, explained in second section (Minimization of

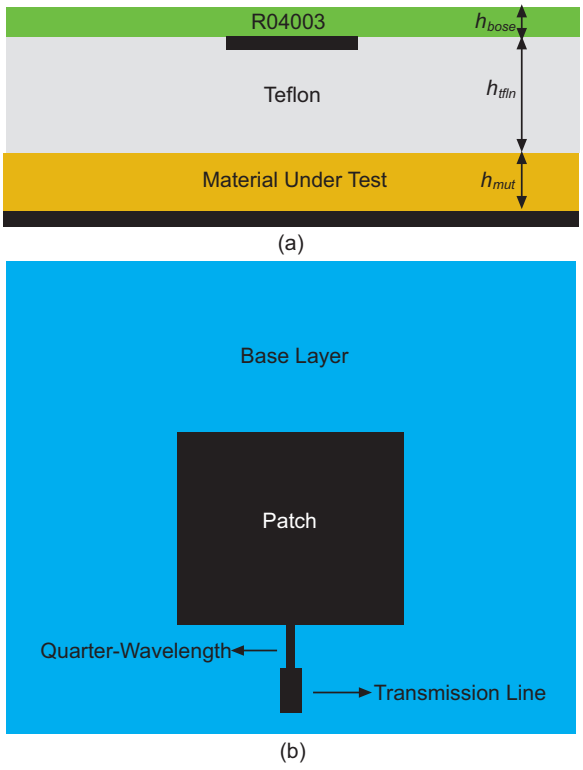

Fig. 1. Configuration of the three-layer microstrip resonator from (a) lateral view and (b) top view.

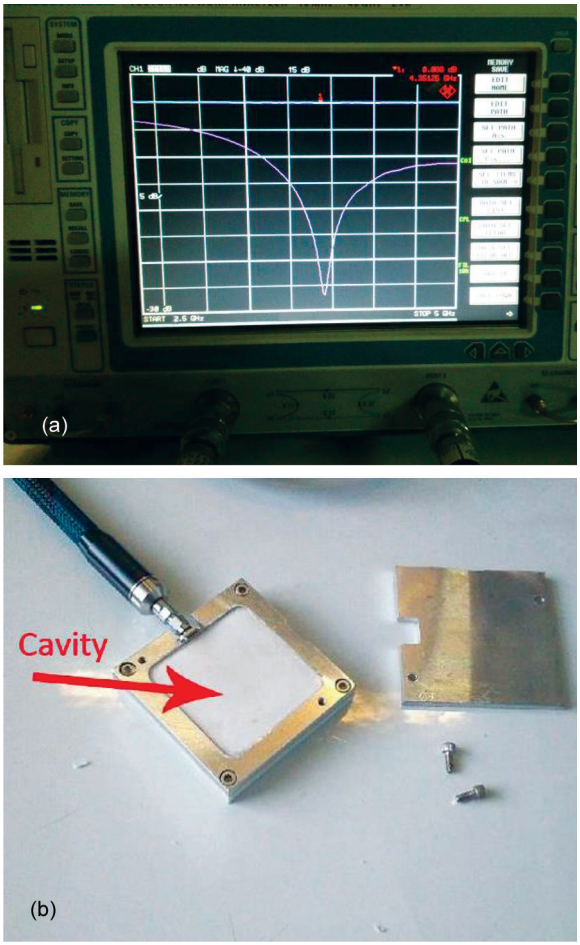

Fig. 2. Photo of (a) the measurement system and (b) the microstrip resonator.

errors of permittivity components). The considerable achievement obtained by the new resonator was regarding the errors of the imaginary ($\in''$) part of the complex permittivity in the binary mixtures which keep limited. The resemblance between the 10-dB bandwidths of the two curves in Fig. 3 demonstrates this claim. Capability in choosing a proper resonant frequency, which varied between 3.5 GHz and 4 GHz, in a three-layer microstrip resonator led to acquire such an improvement. In this frequency area, both components of the complex permittivity showed almost linear changes with respect to changes in volume fractions of ethanol-methanol. In future works, the effect of feed system and matching can also be considered on the accuracy of measurements in the three-layer microstrip resonator by applying different types of feeding techniques.

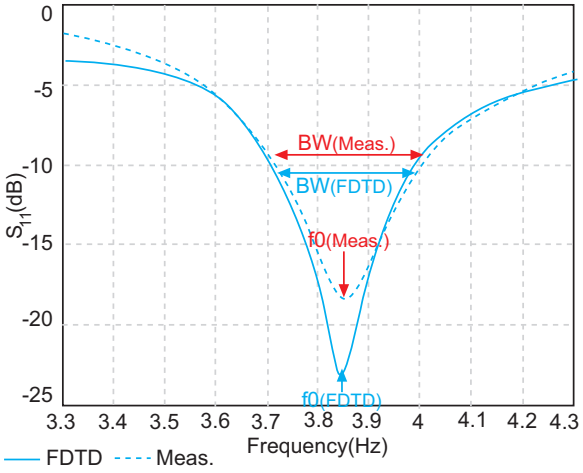

Fig. 3. Measured and simulated curves of reflection coefficient for binary mixture of ethanol 80% - methanol 20%.

In Tables 1-2 the errors of the real and imaginary part of the complex permittivity have been given as:

$$\text{Error}_{\in^x}\ (\%) = \frac{\in^x_{data} - \in^x_{meas}}{\in^x_{data}} \times 100 \ \ldots\ldots\ldots\ (5)$$

Where:

$\in^x_{data}$ is the permittivity component retrieved from (Bao *et al.*, 1996), and $\in^x_{meas}$ is obtained from measured results and equations (3) and (4).

In order to indicate improvement in measuring the complex permittivity of the binary mixtures of ethanol and methanol, the measured results by the three-layer microstrip resonator has been compared to the result of

Table 1. Results of measured and simulated resonant frequency of samples and measurement errors of ($\in'$)

Material under test	f_0(GHz)-FDTD	f_0(GHz)-Measurement	Error ($\in'$)
Ethanol 1.0 - Methanol 0.0	3.886	3.907	0.7%
Ethanol 0.9 - Methanol 0.1	3.868	3.889	1.2%
Ethanol 0.8 - Methanol 0.2	3.851	3.873	0.8%
Ethanol 0.7 - Methanol 0.3	3.838	3.859	2.9%
Ethanol 0.6 - Methanol 0.4	3.830	3.854	-1.2%
Ethanol 0.5 - Methanol 0.5	3.819	3.843	-1.5%
Ethanol 0.4 - Methanol 0.6	3.811	3.835	-1.2%
Ethanol 0.3 - Methanol 0.7	3.804	3.828	-2.8%
Ethanol 0.2 - Methanol 0.8	3.800	3.824	-2%
Ethanol 0.1 - Methanol 0.9	3.795	3.819	-1.8%
Ethanol 0.0 - Methanol 1.0	3.789	3.813	-1.6%

Table 2. Results of measured and simulated 10-dB bandwidth of samples and measurement errors of ($\in''$)

Material under test	BW(MHz)-FDTD	BW(MHz)-Measurement	Error ($\in''$)
Ethanol1.0 -Methanol0.0	426	427.3	2.8%
Ethanol0.9-Methanol 0.1	419	420.1	1.6%
Ethanol0.8-Methanol 0.2	405.1	406.9	0.7%
Ethanol0.7-Methanol 0.3	392.9	395.1	0.3%
Ethanol0.6-Methanol 0.4	380.4	382.5	0.8%
Ethanol0.5-Methanol 0.5	364.1	367.6	-1.1%
Ethanol0.4-Methanol 0.6	353	358.5	-3.2%
Ethanol0.3-Methanol 0.7	346	351	-1.8%
Ethanol0.2-Methanol 0.8	336	342	-4.1%
Ethanol0.1-Methanol 0.9	331	334.5	-0.7%
Ethanol0.0-Methanol 1.0	322.9	326.8	-0.9%

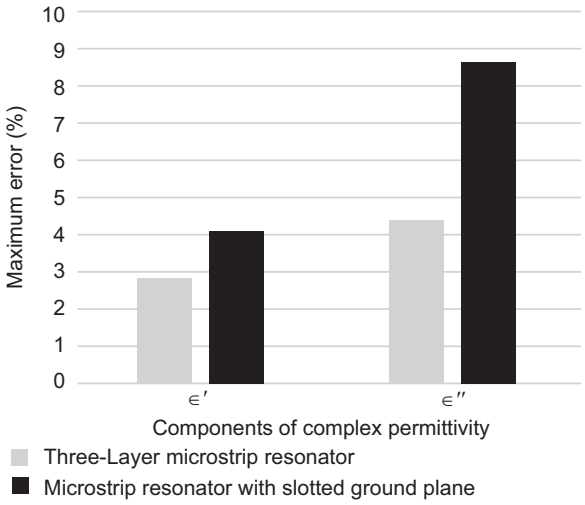

Fig. 4. Maximum measurement errors for the real and imaginary part of the complex permittivity in the three-layer microstrip resonator and a microstrip resonator with slotted ground plane.

a microstrip resonator with slotted ground plane (Liu and Pu, 2008), and the maximum errors of the real and imaginary part of the complex permittivity measured by both instruments have been plotted in Fig. 4.

Conclusion

A three-layer microstrip resonator was demonstrated in order to measure the complex permittivity of binary mixtures of ethanol-methanol. FDTD simulations and measurements were applied to illustrate the accuracy of the structure. The maximum measurement errors for the real and imaginary part of the complex permittivity were produced 2.9% and 4.1%, respectively, indicating the high quality of this resonator. In fact, the presence of this configuration allowed us to choose its operating frequency and materials used in the resonator. The former increased the precision of measurements; the later decreased the expenditure of the structure.

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Pak. j. sci. ind. res. Ser. A: phys. sci. 2017 **60**(2) 90-95

Biosorption of Chromium (VI) and Calculations of Langmuir's and Freundlichs Isotherms

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(received June 16, 2016; revised January 25, 2017; accepted January 30, 2017)

Abstract. In the present study yeast biomass has been successfully used as biosorbent for removal of Cr from aqueous solution. Yeasts of *Saccharomyces cerevisiae* are effective biosorbents for heavy metal ions and it can be bought in large quantity at low cost. *S. cerevisiae* can remove toxic metals from aqueous solutions to various levels. This low-cost biosorbent will make the process cost-effective and competitive particularly for environmental applications in detoxifying effluents. Langmuir's and Freundlichs isotherms were also plotted to observe the maximum biosorption of heavy metal chromium (VI).

Keywords: heavy metals, biosorption, yeast, wastewater, chromium (VI)

Introduction

From last few years heavy metal pollution has become most serious environmental threat and discharge of heavy metals into ecosystems has become a matter of concern. These pollutants are introduced into the aquatic systems significantly as a result of various industrial operations (Ahalya *et al*., 2005). Biosorption is defined as the removal of metal from synthetic solution by biological material (Gadd, 1993). Today heavy metal pollution is one of the most important environmental problems. Many industries produce and discharge wastewater containing different heavy metals into the environment. Thus, metal brings serious environmental pollution, threatening to human health and ecosystem.

Toxic metals such Hg, Cr, Pb, Cu, Ni, Cd, (Volesky, 2007) are produced not only by industrial activities, but also mining activities, transport, as well as the spreading of fertilizer and sewage sludge discharge heavy metals into the environment. Methods for removing metal ions from aqueous solutions consist of physical, chemical and biological technologies. Conventional methods for removing metals are reverse osmosis, chemical precipitation, ion exchange, electrodyalysis, ultrafiltration, adsorption on activated carbon, evaporation, etc., (Sarabjeet, 2014; Suryan and Ahluawalia, 2012; Farshid *et al*., 2008; Sarabjeet and Dinesh, 2007). Hence, the disadvantages like incomplete metal removing, high reagent and energy requirements, generation of sludge or other waste products that require careful disposal has made it imperative for a cost-effective treatment method that is capable of removing heavy metals. Ahalya *et al*. (2003) summarized the advantages and disadvantages of those conventional metal removal technologies.

In recent years, applying biotechnologies in controlling and removing metal pollution became hot topic in this field because of its potential application. Alternative process is biosorption, which utilizes various natural materials, including bacteria, fungi, yeast, and algae (Kotrba *et al*., 2011). These biosorbents have metal-sequestering property and can be used to decrease the concentration of heavy metal ions in solution. These natural compounds can sequester dissolved metal ions out of dilute complex solutions with high efficiency (Xie *et al*., 2003). The major advantages of biosorption over conventional treatment methods include low-cost, high efficiency, minimization of chemical or biological sludge, and regeneration of bio sorbent and possibility of metal recovery (Norton *et al*., 2004; Volesky, 2001). The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms are various and not fully understood.

According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into: a) metabolism dependent and b) non metabolism dependent. According to the location where the metal is removed from solution, biosorption can be classified as a) extracellular accumulation, b) cell surface sorption/precipitation) intracellular accumulation (Volesky, 2001). Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This kind of biosorption may take place only with viable cells. During non-metabolism biosorption, metal uptake

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is by physicochemical interaction between the metal and the functional groups present on the microbial cell surface (Ahluwalia and Goyal, 2007). This type of mechanism is relatively rapid and can be reversible (Das *et al*., 2008).

Investigations conducted by several researchers demonstrated that *Saccharomyces cerevisiae* is capable of accumulating heavy metals such as chromium (VI) (Huang *et al*., 1990). Yeasts although some species are through the formation of strings of connected budding cells the term "yeast" is often taken as for *Saccharomyces cerevisiae* (Kotrba *et al*., 2011). *Monascus purpureus*, is the most widely used model organisms for genetics and cell biology. Some yeast can find potential application in the field of bioremediation. One such yeast, *Yarrowia lipolytica*, is known to degrade palm oil mill effluent (Kurtz-man and Fell, 2005; Kurtz-man, 1994) and other hydrocarbons, such as alkanes, fatty acids, fats and oils (Klieger, 2004). It can also tolerate high concentrations of salt (Rao *et al*., 2004) and is being investigated for its potential as a heavy metal removal (Botstein and Fink, 2011). The procedure of metal removal from aqueous solutions is studied by many researchers (José *et al*., 2014; Ghasemi *et al*., 2014; Sage, 2013; Linda and Stanley, 2007). The metabolically independent biosorption of metals by yeast cells occur within several minutes (Babel and Kurniawan, 2003). Heavy metal poisoning could result, and cause allergic skin irritations, dermatitis, irritation to mucous membranes, lung cancer, liver and kidney damage and chrome holes i.e., penetrating ulcers which occur around the fingernails, finger joints, eyelids and occasionally on forearms (Anagnostopoulos *et al*., 2010).

The aim of this study was to test and compare treated and untreated waste baker's yeast cells (*S. cerevisiae*) for their capacity to adsorb heavy metals in synthetic solution.

Materials and Methods

Spectroscopy. The interaction of electromagnetic radiation with matter is known as spectroscopy. Chromium was estimated by Atomic Absorption spectrophotometer model Spectra, 40 Australia.

Chemicals. Merck standard solution, double distilled water was used. All the reagents were of analytical grade and were prepared in double distilled water. For each sample fresh dilutions were used.

Yeast biomass collection. Yeast biomass *Saccharomyces cerevisiae* was selected for the research work. It was collected from the localities of the PCSIR Laboratories, Lahore. The yeast biomass was initially dried from the moisture, spread on a steel tray, by keeping in an oven at 95 °C for 30 min. Then this was taken and kept in the 'carbolite furnace' at 200 °C for about 50 min. After cooling this material was subjected to the sieve analysis.

Chromium standard solution. 25 mL of stock solution was taken in 250 mL volumetric flask and volume was raised up to the mark with distilled water. This was 100 ppm chromium solution. From this 100 ppm solution 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 mL was taken in 10 different 100 mL volumetric flasks through pipettes and then the volume was made up with distilled water 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 ppm standard solutions of chromium. These solutions for treatment and calibration were made using the same stock solutions prepared from the 1000 ppm Merck standard solution. Quality control checks were made in this study. The removal of chromium (VI) concentration due to the adsorption was determined by the AA spectra- 40. The percentage of chromium removal due to the bio-adsorption was calculated as:

$$\% \text{ Cr removal} = [(C_0 - C_e) / C_0] \times 100 \%$$

where:

C_e = initial concentration of Cr (VI) solution,
C_0 = equilibrium concentration of Cr(VI) solution.

Adsorption isotherm procedure. The optimum conditions obtained earlier from the study of metal, i.e. effect of pH, effect of time, effect of biomass quantity and effect of agitation speed at 40 °C were maintained as constant and only concentration of metal was changed. Some factors which are used in the tables having data of Langmuir and Freundlichs adsorption isotherms, such as:

V = volume of metal solution used in the experiment (L)
m = mass of biomass used in the experiment (g)
C_e = initial concentration (ppm) ; C_0 = final concentration (ppm)

$$q_e = v(C_0\text{-}C_e)/m$$

Calibration curve. The instrument was calibrated before analysis and calibration curve was presented in Fig. 1.

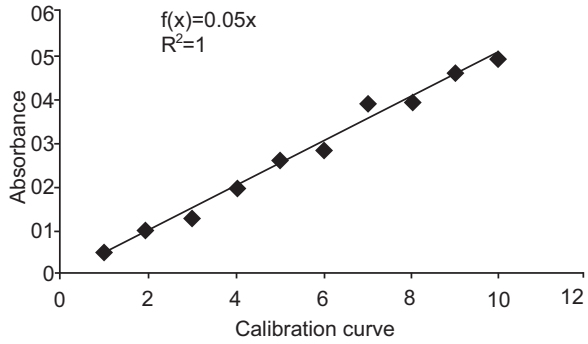

Fig. 1. Cr concentration (mg/L).

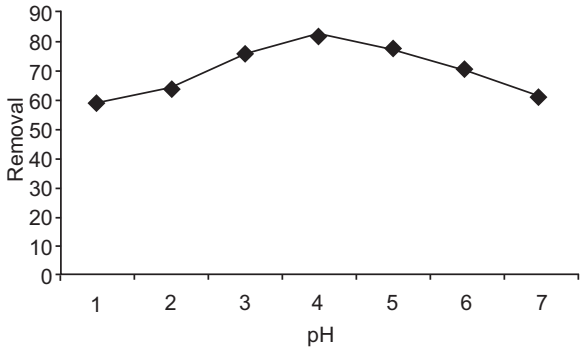

Fig. 2. Different pH values.

General procedure of biosorption. Yeast biomass (*Saccharomyces cerevisiae*) 0.2 g was taken in a 100 mL conical flask. Then 50 mL of 5 ppm solution of chromium metal was added. The flask was then shaken at 10-90 min. After shaking, the sample was filtered using Whatmann filter paper No.1 and the filtrate containing residual concentration of chromium (VI) was analysed with AA spectra 40 by comparing these values with the absorption values of standard solutions. Different parameters i.e., effect of pH, effect of time, effect of biomass quantity and effect of agitation speed were studied at room temperature.

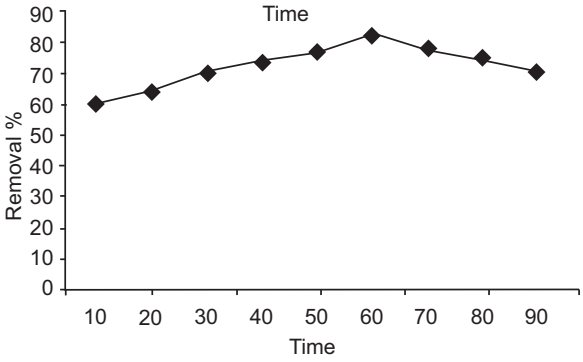

Fig. 3. Time (min).

Results and Discussion

Effect of pH. Absorbance was carried out at pH range of 1-7 and maximum adsorption was in acidic medium at pH 4. The sorption binding phenomenon was found to be pH dependent. It was found that the sorption process increases with increasing pH and is maximum at pH 4.0. The biosorption in acidic media is greater than the basic media as results shown in Fig. 2. At very low pH (below 4.0), active sites of biomass for binding of metal ions become less available, so the removal efficiency decreases. The removal efficiency reduced from pH above 4.0 due to the formation of insoluble chromium hydroxide precipitates that make true sorption studies difficult.

Effect of time. Absorbance was carried out from 10-90 min and maximum adsorption was at 60 min as shown in Fig. 3. It means that after 60 min shaking, maximum sites for biosorption are exposed in the solution to bind the metal. Initial removal occurs immediately as metal solution and biomass came in contact, but after 60 min active sites become unavailable and metal needs time to find out more active sites for binding.

Effect of biomass quantity. Absorbance was carried out at biomass quantity range of 0.2-1.4 g and maximum adsorption was at 1.0 g. The sorption of metal increases with increasing biomass quantity which is maximum 1.0 g as indicated in Fig. 4. The increase in biosorption with the increase in biomass quantity is due to the fact that more active sites are available for metal binding.

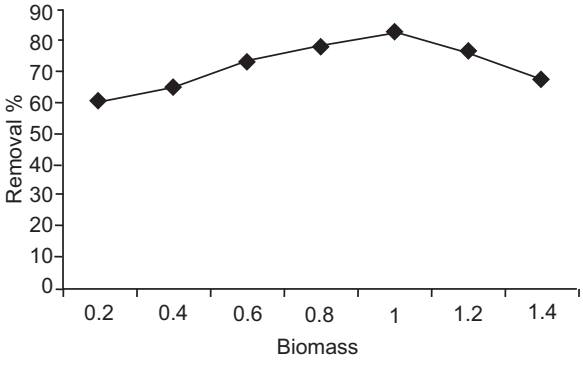

Fig. 4. Biomass (mg).

Effect of agitation speed. Sorption increases as agitation speed increases. Absorbance was carried out at agitation speed of 50-300 rpm and maximum absorption was at 200 rpm. The results of absorbance of various concentrations of chromium are presented in (Fig. 5).

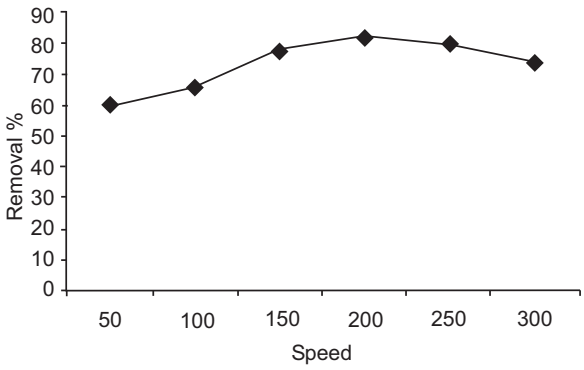

Fig. 5. Agitation speed (rpm).

Biosorption equilibrium isotherms. The sorption of chromium ions were carried out at different initial chromium ion concentrations ranging from 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 ppm, at optimum pH 4, at 200 rpm with the optimum agitation speed of 200 rpm and at optimum time 60 min while maintaining the adsorbent dose at 1.0 g of yeast. There are two types of simple sorption isotherm models that are most frequently applied i.e., Langmuir and Freundlichs models.

Langmuir isotherm. The adsorption isotherm, which describes quantitatively the buildup of a layer of molecules on a biosorbent surface as a function of the concentration of the adsorbed material. In a modified form it can also describe a bi-layer deposition and is often expressed as:

$$q_e = q_{max}\, b\, C_e / (1+b\, C_e)$$

where:

q = milligram of metal accumulated per gram of bio sorbent,

C_e = is the residual metal concentration in solution,
q_{max} = is the maximum uptake of specific metal cross pounding to specific site saturation and
b = the ratio of absorption and de absorption rates. This is a theoretical model of monolayer model for adsorption.

$$1/q_e = 1/b\, q_{max}\, C_e + 1/q_{max}$$

The Langmuir constants q_{max} and b can be evaluated from the slope (y) and intercept (x) linear equation.

Freundlichs isotherm. Freundlichs presented an empirical adsorption isotherm for non-ideal systems. The Freundlichs isotherm is exponential in the form and often represents an initial surface adsorption followed by a condensation effect resulting from extremely strong solute-solute interaction. It is expressed as:

$$\text{Freundlichs isotherm: } q_e = K_f\, C_e^{\,1/n}$$

where:

k and n are constants. These models can be applied at constant pH and can be used for modeling of biosorption of equilibrium at the presence of one metal. This equation is conveniently used in the linear form by taking the logarithmic of both sides which can be used for the linearization of experimental data:

$$\text{Log } q_e = \log K_f + 1/n \log C_e$$

The constants of Freundlichs isotherm K_f and n can be determined from the slope (y) and the intercept (x) of linear equation.

Results of Langmuir and Freundlichs isotherms. The constants of Langmuir and Freundlichs isotherms for chromium metal are given below in Table 1-2.

Yeast is a promising biosorbent for heavy metals and has come into notice of researchers due to its unique nature and average uptake capacity of metals. A comparative study of biosorbent was carried out by

Table 1. Constants of the isotherms

Metal	Langmuir constants		Freundlichs constants	
	q_{max} (mg/g)	b (L/mg)	K_f	n
Cr (VI)	2.014	0.119	1.127	1.090

Table 2. Linear regression data for Langmuir and Freundlichs isotherms

Metal	Langmuir data		Freundlichs data	
	Linear equation	R^2	Linear equation	R^2
Cr (VI)	y=4.181x + 0.4964	0.9128	y=0.917x - 0.7646	0.8874

R^2 = shows correlation or linear relationship while y and x indicate the slope and intercept of the linear equations, respectively.

Bakkaloglu *et al*. (1998). They compared the removal efficiency of Zn, Cu and Ni ions on sedimentation, biosorption and desorption. The results showed that *S. cerevisiae* has average uptake capacity of one or multi metals bio absorption system. The results of this study show that maximum absorption of Cr (VI) was at pH4, with 200 rpm and 60 min time at one gram of biomass material *S. cerevisiae*. The adsorption equibiliuram results were modeled using Langmuir and Freundlichs isotherm and experimental data were well fitted to the Freundlichs equation. Experiments were made in batches to study the effects of Cr (VI). Experimental and kinetics data was expressed by Langmuir effect on the adsorption rate. The potential to remove Cr (VI) from synthetic solution Bengal gram was investigated by Ahalya *et al*. (2005). The Cr (VI) removal from synthetic solution was 82%. The Freundlichs isotherm and Langmuir isotherm showed the similar results as presented in Fig. 6-7.

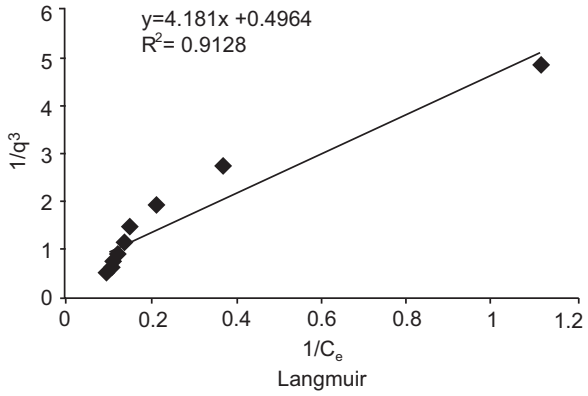

Fig. 6. Initial concentration of chromium (Langmuir isotherm).

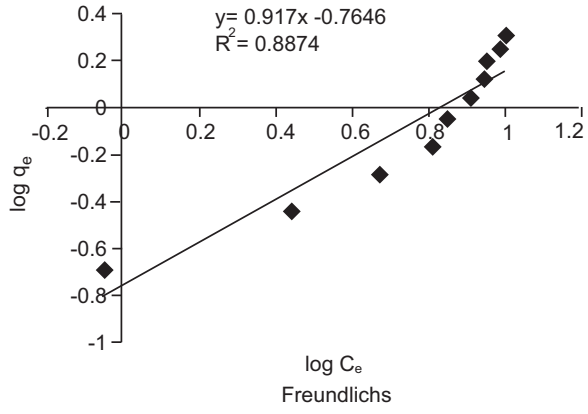

Fig. 7. Chromium concentration after treatment (Freundlichs isotherm).

Conclusion

The cost effective technology that can be applied on large scale in decontamination of industrial effluents was studied by using yeast, *Saccaromyces cerevisiae* as a biosorbent. After removal of metals from wastewater, metals can be recovered and matter can be regenerated for further use. Two adsorption models, the Langmuir model and the Freundlichs equation were applied to the experimental data obtained from yeast biomass and correlations results were found similar for these models. New low-cost technologies are necessary to remove heavy metals in the environment to limit values.

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Extraction of Ecofriendly Leather Dyes from Plants Bark

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(received June 6, 2016; revised January 10, 2017; accepted January 20, 2017)

Abstract. Present study is focused on the isolation of ecofriendly dyes from the bark of different plants. Aqueous extracts of the bark of *Mangifera indica* L.*, Syzygium cumini* L. and *Eucalyptus camaldulensis* Dehn were used to dye the crust blue leather of goat. Four different types of mordents $CuSO_4$, $FeSO_4$, $KMnO_4$ and Potash Alum were used. The fastness properties (washing, heating, sunlight and rubbing) were also evaluated by grey scale. *Syzygium cumini* L extract showed more variation in colours. *M. indica* L. showed good fastness properties as compared to others. The formation of light and soft colours with different mordants was observed.

Keywords: ecofriendly, natural dye, leather, mordants, plant bark

Introduction

Synthetic dyes have harmful impact on environment as well as on human beings. These dyes cause many diseases in human beings like liver tumor, kidney and heart damage (Kumar and Sinha, 2004). Therefore natural dyes are gradually replacing the synthetic dyes in the textile industry. Natural dyes are non-toxic, non-carcinogenic and biodegradable in nature. Natural dyes are not only derived from plants but also can be derived from insects like Cochineal and Kerriclacca and can be used for colouring food products (Sundari, 2015; Bhuyan and Saikia, 2008). Shellfish and lichen are also used for the extraction of dyes. Natural dyes derived from plants are ecofriendly and this makes them a priority for use in the textile industry (Bhuyan and Saikia, 2008). All parts of plants like fruit, seed, flowers, wood and bark can be used for the production of dyes. They are used as colouring compound in textile, ink and cosmetics industries (Siva, 2007). Natural dyes show antimicrobial activity and have medicinal applications (Samanta and Agarwal, 2009) and gained economic advantage over synthetic dyes. Kamal *et al*. (2005) described natural dyes extracted by solid- liquid extraction process and applied on cotton and silk. The dyes were extracted from *Terminalia arjuna, Punica granatum* and *Rheum emidi* and applied on cotton and silk. These dyes were developed with enzyme complexes (protease, amylase and lipase) and addition of tannic acid. The samples which were treated with enzymes gave rapid dye adsorption than those which were untreated. Tannic acid enzyme is used as alternative to metal mordents (Vanker *et al*., 2007). Fungal species like *Monascus purpureus, Isaria* spp, *Emericella* spp, *Fusarium* spp and *Penicillium* spp were also used for the extraction of dyes and applied on leather. Different parameters like pH, temperature, colour, brightness, exhaustion of colour, time duration, fastness and colour intensity were used for quality evaluationof dyes. Best results were obtained when pigments were used 6% on leather. Optimized conditions for dyeing were 70 °C, pH, 5 and 120 min (Palanivel *et al*., 2013). But the use of fungal species is difficult to handle because of its rapid growth. The aim of this study was to extract ecofriendly dyes from bark of *Mangifera indica, Syzygium cumini* and *Eucalyptus camaldulensis* and applied on leather. The process for the formation of crust leather from wet- blue leather is also described.

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Materials and Methods

Collection of plant material: After literature survey three different plants *Mangifera indica* L., *Syzygium cumini* L. and *Eucalyptus camaldulensis* Dehn were selected. Plant materials were collected from Lahore College for Women University, Lahore. Voucher specimens were submitted in Herbarium of LCWU for identification of plants.

Extraction of dyes from plants. The plant barks were weighed and washed to remove the dust particles. After washing, barks were cut into small pieces and soaked in water for dye extraction. The ratio of plant material and solvent was 150:800 mL, boiled on water bath approximately for 6-8 h until the final volume of the

extract was 500 mL. Then solution was filtered and stored at 4 °C in the refrigerator for further processing.

Formation of crust leather. The wet-blue leather sample was collected from New Age Tanneries, Lahore for the formation of crust leather described under the following steps:

Wet back. This method was applied to retain moisture and to remove fatty contents of the leather by adding 150% water and 0.5% detergent then rotated in the electric drum (12-15 rpm, 400×200) for 30 min. Moisture retention is necessary for chemical application.

Rechroming of leather. The whole process was done in an electric rotary drum. Rechroming of leather was carried out by adding 150% water v/w and 0.5% formic acid then rotated the drum for 5 min until pH reached at 3.5. After that 6% chrome powder was added and rotated the drum for 1 h. The cut section was checked and added 0.5% sodium formate and rotated the drum for 10 min. Then 0.5% sodium bicarbonate was added and rotated the drum for 1 h, pH at this point was maintained at 4.1-4.2. Chrome (6%) was added for tanning. Finally sodium formate was added to raise the pH a little bit.

Neutralization of leather. This was done to attain an isoelectric point at which pH is 7. Added 150% water, 0.5% sodium formate and 2.0% Taningan PAKS were taken in the drum, rotated it for 30 min. After that 0.5% sodium bicarbonate was added to increase the pH of leather till 7. Again drum was rotated for 45 min. The pH of leather was checked by two liquid indicators, Bromo Cresol Green (BCG) and Bromo Cresol Purple (BCP).

Retaining of leather. The next step was retaining, dyeing and fat liquoring but overall it is called as retanning. In this process 50% water was added at 25 °C, then 2% Yourmer NATE™ was added as acrylic polymer and ledersyn QMF™ as strinmalic and run the reaction for 30 min. Finally two other syntans (5% Tanigan OS and 5% Retanning R7) were added and rotated the drum for further 60 min.

Fat liquoring of leather. The last step was fat liquoring of leather. It was done for the softening of leather. Softening and lubrication are carried out to increase leather shelf life. In this procedure 7% Plentol (BA) and 7% Sultosol (SK_3) were added and set the rotary for 60 min. Then 100% water was added at 70 °C. Later on 3.0% formic acid in the ratio of 1:10/1:20 (for this 1g formic acid mixed in 20 mL distilled water) was added. Formic acid was added gradually in intervals of 10,10 and 25 min for the fixation of pH.

Dyeing of leather. For dying of leather 50g piece of crust leather was placed in the rotary drum containing 150 mL of aqueous dye and run the drum for 30 min. Formic acid was then slowly added in the aqueous dye after 10-25 min for the fixation of dye at pH 3.5. Four mordents like $CuSO_4$, $KMnO_4$, $FeSO_4$ and Pot. Alum were used for fixation and to improve the colour of the leather. 0.5g mordant was added in the drum to fix the dye and run the drum for 30 min. The extract was drained and the leather strip was rinsed and dried and the colour was checked.

Fastness properties of dye. Fastness properties of dye were checked by different parameters like transfer of colour from leather to cotton, change in colour by sunlight, heating, washing and rubbing. These properties were checked with the help of grey scale reading. The grey scale ranges from 1-5.

Washing. Transfer of colour from leather to the cotton fabric was checked by washing technique using soap solution.

Preparation of soap solution. Soap solution was prepared by dissolving 5g soap in 1 liter water in a flask having magnetic stirrer and put on hot plate for 30 min. Magnetic stirrer was used for complete dispersion and to prevent the settling of soap. The ratio of specimen and soap solution used was 1g of specimen and 50mL of soap solution. A piece of cotton binded with dyed specimen of leather, then dipped in beaker having soap solution and stirred continuously under specific condition (45 °C) for 45 min. The leather was then washed to check any change and migration of colour on cotton by comparing with the original leather and cotton. The fastness property of colour was evaluated with the help of grey scale.

Rubbing. Rubbing was checked by following two techniques.

Dry rubbing. Dry rubbing of leather was carried out with the help of instrument FELT under pressure with to and fro movement on standard cloth. After 15min invert the felt and the colour migration from leather to standard cloth was checked. The fastness of colour was than evaluated with the help of grey scale.

Wet rubbing: The wet rubbing was also done under the same conditions as dry rubbing except that the

standard rubbing cloth was soaked into 100% de-ionized water.

Sunlight. For this purpose a half covered wooden box was taken, specimens was kept in the box and placed it in sunlight for 24 h. The portion of leather which was exposed to sunlight was compared with the covered portion and the colour variation and fastness property of colour was evaluated with the help of grey scale.

Heating. The specimen was heated with the iron and compared its colour with the original colour .The fastness property of colour was evaluated with help of grey scale.

Results and Discussion

Synthetic dyes are widely used not only for the dyeing of leather but wool, cotton and silk too. They are not ecofriendly and show environmental hazards. European Union banned the use of azo dyes because of its harmful effects (Saravanan *et al*., 2013; Sivakumar *et al*., 2011). To overcome hazards of synthetic dyes plants extracts are used for dyeing purpose. Plants dyes are eco-friendly (Purwar, 2016; Bechtold and Mussak, 2009). The present study is concerned with dyeing of leather by using the extracts of plants *M. indica*, *S. cumini* and *E. camaldulensis* for dyeing purpose. The barks of these plants were selected for extraction of dye (Fig. 1). Four different types of mordants ($CuSO_4$, $KMnO_4$, $FeSO_4$ and Pot- Alum) were used for the fixation of dye. The

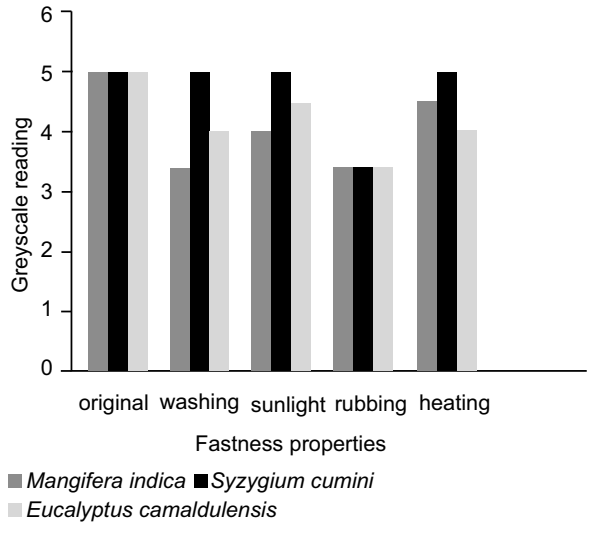

Fig.1. Fastness properties of *E.camaldulensis*, *S.cumini* and *M. indica* without mordant.

colour of extract of *E. camaldulensis* bark was brown. It gave four different colours with the four mordants. $CuSO_4$ gave chocolate brown colour, $FeSO_4$ gave dark blue shade, $KMnO_4$ gave dark brown and Pot. Alum gave greyish brown colour (Table 1). Its fastness properties after washing ranged between 3/4 to 4/5, a sunlight range between 4 to 5, rubbing ranged between

Table 1. Colour variation of leather dyed with aqueous extract *E. Camaldulensis S.cumini and M. indica*

Plant materials	Mordants	Leather colours
Eucalyptus camaldulensis	No mordant	
	$CuSO_4$	
	$FeSO_4$	
	$KMnO_4$	
	$KAl(SO_4)_2$	
Syzygium cumini	No mordant	
	$CuSO_4$	
	$FeSO_4$	
	$KMnO_4$	
	$KAl(SO_4)_2$	
Magnifera indica	No mordant	
	$CuSO_4$	
	$FeSO_4$	
	$KMnO_4$	
	$KAl(SO_4)_2$	

3/4 to 4 and after heating 4/5 to 5 in greyscale reading. (Fig. 2-5)

The colour of *S. cumini* extract was brown. By using mordents different colours were obtained. $CuSO_4$ gave brown colour, $FeSO_4$ gave blue colour, with $KMnO_4$ dark brown was obtaind and Pot. Alum gave light brown colours (Table 1). Its fastness properties after washing ranged between 3/4 to 4/5, sunlight ranged between 4 to 5, rubbing range between3/4 to 4 and heating showed 4/5 to 5 in grey scale reading.(Fig. 3)

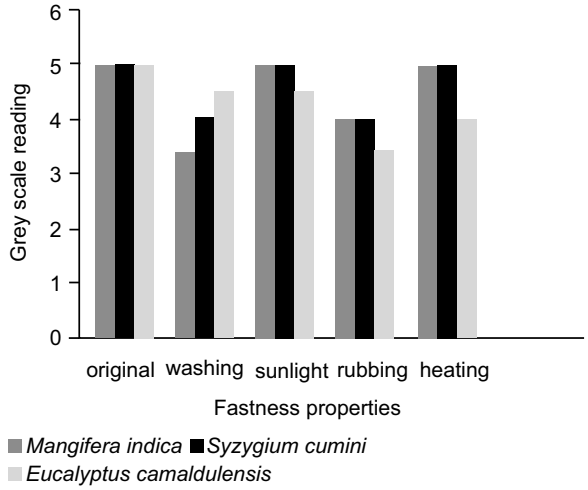

Fig. 2. Fastness properties of *M.indica*, *S. cumini* and *E. camaldulensis* with $CuSO_4$ mordant.

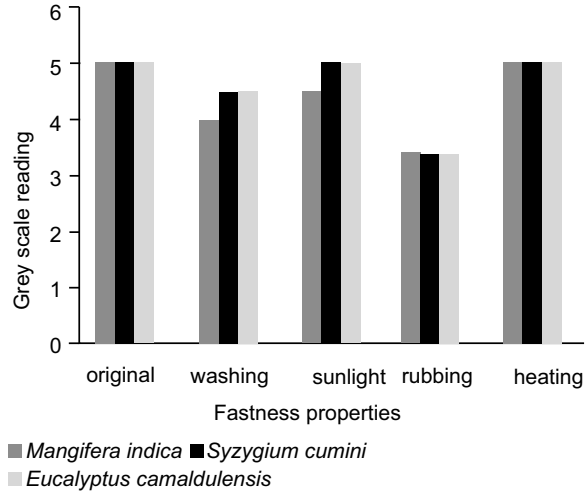

Fig. 3. Fastness properties of *M.indica*, *S. cumini* & *E. camaldulensis* with $FeSO_4$ mordant.

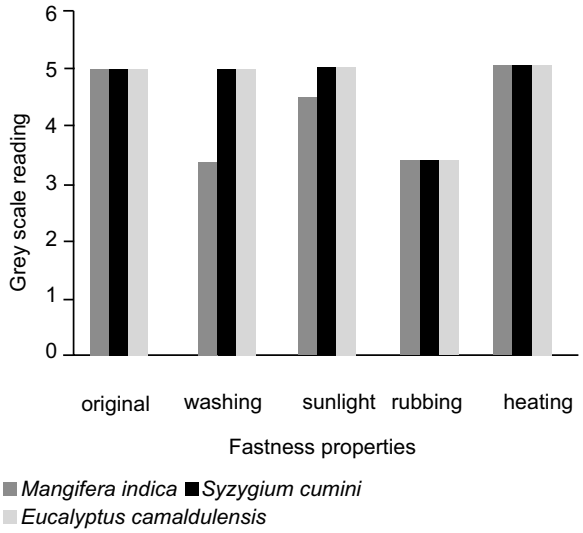

Fig. 4. Fastness properties of *M.indica*, *S. cumini* and *E. camaldulensis* with $KMnO_4$ mordant

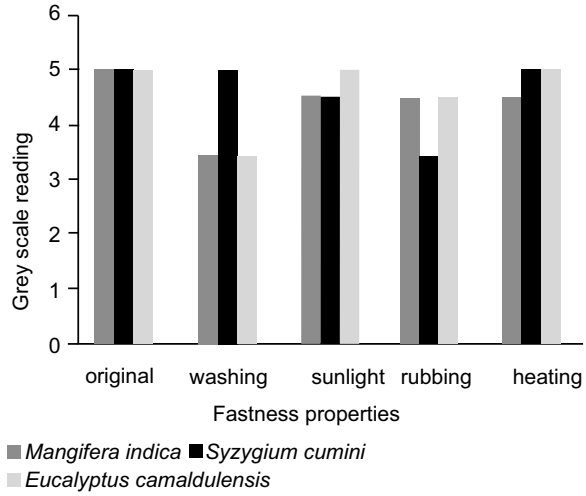

Fig. 5. Fastness properties of *M.indica*, *S. cumini* and *E. camaldulensis* with Pot. alum mordant.

Original colour of *M. indica* extract was brown and $CuSO_4$ mordant gave dark brown, $FeSO_4$ gave black, $KMnO_4$ and Potash Alum gave light brown colours (Table 1). Its fastness properties ranged between good and excellent. After washing ranged between3/4 to 4/5, sunlight ranged between 4 to 5, after rubbing ranged between 3/4 to 4 and after heating 4/5 to 5 in grey scale reading (Fig. 2-5). Selvi *et al*. (2013) used seeds of *Bixa orellana* for the extraction of dye colour and applied on leather for finishing and dyeing. In the present study

barks of *M. indica*, *S. cumini* and *E. camaldulensis* were used which are mostly wasted part of the plant so this work is low in cost than the previous work. The leaves of *Acalypha indica* Linn were also used for the extraction of dyes by post-mordating technique. In the present study metamordating technique is used which is more convenient.

Conclusion

This study conclude that natural dyes extracted from *M.indica*, *S. cumini* and *E. camaldulensis* can be successfully applied on leather and different shades can produced from the same extract by using different mordants. These dyes are ecofriendly and excellent alternate of synthetic dyes.

Acknowledgement

I would like to thank Mr. Anees Sheikh, owner of "New Age Tanneries Lahore" for Providing the wet blue leather of goat and Mr. Arshad Ali Sindhu of PCSIR Laboratories Complex, Lahore for support in this research work.

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Pak. j. sci. ind. res. Ser. A: phys. sci. 2017 **60**(2) 101-105

Quantification of Pesticide Residues in Drinking Water in Different Areas of District Charsadda, Pakistan

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(received August 4, 2015; revised April 7, 2016; accepted April 13, 2016)

Abstract. Pesticides residues were determined quantitatively in drinking water samples collected from district Charsadda of the province Khyber Pakhtunkhwa, Pakistan employing GC-MS technique. The survey was conducted soon after the flood in 2010. Aldicarb (0.003 mg/L) was found only in C7, while residues of acetachlor (0.001 mg/L) was found in C8. Among the pesticides quantified Chlorpyrifos delivered highest amount of residues in C8, C9, C10, C11 and C12. Residues of *o*, *p'*-DDT were found higher in C1 to C6 than the permitted value (0.002 mg/L), while higher concentration of Pyridaben than the allowed value (0.0001 mg/L) was resulted in C1, C5, C6 and C14. Residues of Carbofuran, Atrazine, α-Endosulfan and Dieldrin, β-Endosulfan, Difenoconazole-1 and Difenoconazole-2 were not detected in any of the collected water samples. Concentrations of rest of the pesticides residues detected in water samples were within the permissible limits. The study revealed that water samples collected from district Charsadda are highly contaminated with pesticides, which is a health risk factor for the inhabitant of this areas.

Keywords: drinking water, pesticides residues, GC-MS, district Charsadda, flood 2010

Introduction

Pesticides play a fundamental role in green revolution by producing crops of good quality and quantity through the control of the insectivorous and herbaceous pests. On one side chemistry has proved to be of merit for the life but unfortunately on the other side, it has also brought some demerits. Among these some have threatened the long-term survival of major ecosystems by disruption of predator-prey relationships and loss of biodiversity. Pesticides have also produced numerous health problems (Hayat *et al*., 2010) like neurotoxicity (Karalliedde and Senanayake, 1999; Brown *et al*., 1989) and can result in gastrointestinal, cardiological, dermatological, respiratory, genito-urinary and musculoskeletal problems (Vial *et al*., 1996; Hueser, 1992). It has been shown that these chemicals are injurious to defense and endocrine systems (Luster and Rosenthal, 1993; Arlien-Soberg, 1992; Chambers, 1992).

The most commonly used technique for the extraction of pesticides from the complex sample is liquid-liquid extraction, which is carried out by mixing the aqueous phase with other immiscible organic solvents like ethyl acetate, dichloromethane and hexane. Various analytical procedures are employed for the analysis of pesticides including chromatographic techniques like GC and HPLC coupled to various detection systems. GC-MS is the method of choice which is a robust and routinely employed for pesticides analyses.

Contamination of drinking water after flood is a burning environmental issue and a health threat. Flood water can be contaminated with a variety of substances including pathogenic micro-organisms, automotive fluids, animal wastes, fertilizers, chemicals like pesticides etc. when surface water drained into the ground water carrying these contaminants. Contamination of drinking water with pesticides cause a number of health problems. Therefore, determination of pesticide residues in drinking water sources is important in order to take appropriate measures for the provision of safe drinking water to public and protection of public health.

Surface water contaminated with pathogenic micro-organisms and chemicals like pesticides and herbicides are the most alarming, when the surface water goes into ground water. The surface water drained the pesticides and herbicides into the drinking water resources making it highly contaminated and harmful for the human consumption. Hence, drinking water samples were collected from different areas of district Charsadda after the flood in 2010 for the evaluation of pesticides residues.

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This paper presents the results of pesticides residues determination in drinking water samples from different areas of district Charsadda of the province Khyber Pakhtunkhwa.

Materials and Methods

Chemicals and reagents. Ethyl acetate (GC grade) and dichloromethane (GC grade) were purchased from Fischer Scientific (Leicestershire, UK). Sodium sulphate anhydrous (analytical grade), potassium dihydrogen phosphate, HCl and sodium chloride (analytical grade) were obtained from Merck (Darmstadt, Germany). GC grade pesticide standards acetamiprid (>99%), acetochlor (>99%), atrazine (>99%), cypermethrin (>99%), dichlorvos (>99%) and pyridaben (>99%) were purchased from AccuStandard New Haven, CT, USA. Aldicarb (99.9%), alpha endosulfan (99.6%), beta-endosulfan (99.9%), chlorpyrifos (99.2%), cyhalothrin (99.7%), fenvalerate (99.8%), methamidophos (98.4%) and popachlor (99.5%) were procured from Sigma-Aldrich GmbH, Seelze, Germany. Carbofuran (98.5%), dieldrin (98.3%), methiocarb (98.5%), *o,p′*-DDD (99.6%), *o,p′*-DDT (99.5%) and *p,p′*-DDE (98.5%) were obtained from Dr. Ehrenstorfer GmbH Ausburg, Germany. Helium gas (99.9999%) was procured from Pak Gas (United Arab Emirates). Double distilled water was used through out this experimental work.

Preparation of pesticide standard mixture. The pesticides selected for the study were those which are most commonly sprayed in these areas as insecticides and herbicides, and are easily available in the form of standards. The data of the most commonly used pesticides in the area has been taken from the department of Agriculture Training Institute Peshawar, Khyber Paktunkhwa. For this study total of 21 pesticides were selected as shown in Table 1. Detail of the retention times, areas, concentrations and quantifying ion (m/z value) of each pesticide is tabulated in Table 1.

Stock solutions of the individual pesticides under study were prepared in methanol. For preparing working standard solution, appropriate volume from each individual pesticides solution was mixed together in a vial. 2 L of the standard mixture was injected into the GC column using auto injection system of GC-MS.

Samples collection. Samples from drinking water sources were collected from various places of districts Charsadda, which were under water for many days in flood 2010 in Pakistan. Samples were properly preserved at 4 °C until their use for experimental work.

Extraction of pesticides and preparation of samples. The official methods of analysis of AOAC International with some modifications was employed for the extraction of pesticides. One litre of water sample was adjusted to pH by adding phosphate buffer (pH 7). 100 g of NaCl was dissolved in this solution followed by the addition of 300 mL of ethyl acetate. The mixture was shaken for one hour at 200 rpm through shaker. After one hour, the mixture was poured into separating funnel and the layers were let to separate. The upper organic layer was collected in a round bottom flask. The lower aqueous layer was again extracted with 60 mL of ethyl acetate. The mixture was shaken for 15 min. Then the organic layer was separated and mixed with the first time collected layer. Organic layer was dried with sodium sulphate anhydrous. The solution was filtered and evaporated to dry residue through rotary evaporator. The dried residue was dissolved in 2 mL of dichloromethane. The solution was filtered through 0.45 m membrane filter and 2 L of this solution was injected into the GC column using auto injection system.

Table 1. GC-MS data of pesticides standard mixture

Name	R. Time (min)	Quantification ion (m/z value)
Aldicarb	3.86	68
Methamidophos	4.35	98
Dichlorvos	6.43	109
Carbofuran	7.27	164
Methiocarb	10.02	168
Propachlor	10.69	120
Cyhalothrin	11.76	198
Atrazine	12.24	200
Acetochlor	13.57	59
Chlorpyrifos	14.54	97
α- Endosulfan	16.17	241
p, p′-DDE	16.55	246
Dieldrin	16.69	79
β-Endosulfan	17.30	195
o, p′-DDD	17.36	235
o, p′-DDT	18.06	235
Acetamiprid	18.82	56
Pyridaben	20.92	147
Cypermethrin-1	21.74	181
Fenvalerate-1	22.60	125
Fenvalerate-2	22.85	125

Chromatographic separation of pesticides. A gas chromatograph from Shimadzu hyphenated to a mass spectrometer QP 2010 plus (Tokyo, Japan) equipped with an auto-sampler (AOC-20S) and auto-injector (AOC-20i) was used. Ultra high pure helium was used as carrier gas. All chromatographic separations were performed on a capillary column (DB-5ms; Agilent Technologies, CA, USA) having specifications: length; 30 m, i.d.; 0.25 mm, thickness; 0.25 µm. Other GC-MS conditions are: ion source temperature (EI); 280 °C, interface temperature; 280 °C, solvent cut time; 2 min. 2 µL of samples and standard were injected into the GC column. Injector was operated in a splitless mode. Injection temperature was 250 °C. The column temperature programme started at 50 °C for 1 min and ramped to 125 °C at the rate of 25 °C/min. The temperature was further increased to 220 °C at the rate of 10 °C/min and hold for 15 min. Total elution time was 37.5 min. MS was operated in single ion monitoring (SIM) mode. GC-MS solutions software provided by the supplier was used to control the system and to acquire the data. Identification and quantification of the compounds was carried out by comparing the mass spectra obtained with those of external pesticide mixed standard solution. Qualification of the peaks was further authenticated through standard mass spectra from the NIST Library (NIST 05).

Results and Discussion

Selection of sampling area and choice for pesticides. The samples were collected from different areas of district Charsadda, Pakistan.

Pesticide residues in drinking water samples. Standard maximum permissible values for pesticide residues in drinking water have been shown in Table 2. Quantities of pesticides under study in drinking water samples collected from different areas of district Charsadda are depicted in Table 3. Many pesticides were found in appreciable amounts in these samples. Among the pesticides studies, quantities of methiocarb obtained were 0.012, 0.019 and 0.016 (mg/L) in C5, C7 and C8, respectively. Cyhalothrin were found at the level of 0.013 and 0.016 (mg/L) in C1 and C3, respectively. In sample C8, 1.23 mg/L of chlorpyrifos was found while in C9, C10, C11 and C12 this analyte was obtained at the concentration of 0.030, 0.45, 0.41 and 0.91 (mg/L), respectively. In C1, C2, C3, C4 and C5 the concentration of *o,p′*-DDT found were: 0.028, 0.032, 0.025, 0.019 and 0.017 (mg/L), respectively. Appreciable amount of acetamiprid was detected in C1 and C2 samples as 0.013 and 0.016 (mg/L), respectively, while high amount of propachlor i.e., 0.012 mg/L was found in C11. In rest of the samples the pesticides quantified were either at the level of 0.01 (mg/L) or below. For some pesticides

Table 2. Standard maximum permissible values for pesticide residues in drinking water

Pesticide name	Maximum permissible limits (mg/L)	Reference
Aldicarb	0.01	(Hamilton *et al*., 2003; Jenkins, 1999)
Methamidophos	Data not available	
Dichlorvos	0.012	(Moermond *et al*., 2008)
Carbofuran	0.007	(Hamilton *et al*., 2003)
Methiocarb	0.035	(Jenkins, 1999)
Propachlor	0.09	(Jenkins, 1999)
Cyhalothrin	Data not available	
Atrazine	0.002	(Hamilton *et al*., 2003)
Acetochlor	0.14	(Jenkins, 1999)
Chlorpyrifos	Data not available	
α- Endosulfan	0.042	(Jenkins, 1999)
p, p′-DDE	0.002	(Hamilton *et al*., 2003)
Dieldrin	0.0002	(Hamilton *et al*., 2003)
β-Endosulfan	0.042	(Jenkins, 1999)
o, *p′*-DDD	0.002	(Hamilton *et al*., 2003)
o, *p′*-DDT	0.002	(Hamilton *et al*., 2003)
Acetamiprid	Data not available	
Pyridaben	0.0001	(Moermond *et al*., 2008)
Cypermethrin-1	Data not available	
Fenvalerate-1	Data not available	
Fenvalerate-2	Data not available	

Table 3. Concentration of pesticides in flood water samples collected from district Charsadda (C); LOQ: 0.001 (mg/L)

Name	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16
	(mg/L)															
Aldicarb	ND	ND	ND	ND	ND	ND	0.003	ND	ND	ND	ND	ND	ND	ND	ND	<0.001
Methamidophos	ND	ND	ND	ND	0.007	0.009	0.004	0.008	0.002	0.005	0.010	0.005	0.007	0.004	0.004	0.003
Dichlorvos	ND	ND	ND	ND	ND	ND	0.004	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.001
Methiocarb	ND	ND	ND	ND	0.012	0.008	0.019	0.016	0.007	ND	ND	ND	0.002	0.010	ND	ND
Propachlor	ND	ND	ND	ND	ND	0.003	ND	ND	0.002	0.002	0.012	0.010	ND	ND	<0.001	<0.001
Cyhalothrin	0.013	ND	0.016	0.007	0.009	ND	ND	ND	ND	ND	0.001	ND	ND	ND	ND	ND
Acetochlor	ND	ND	ND	ND	ND	ND	ND	0.001	ND	ND	ND	ND	ND	ND	ND	ND
Chlorpyrifos	ND	ND	ND	ND	ND	ND	ND	1.231	0.030	0.449	ND	ND	ND	ND	ND	ND
p, p′-DDE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.413	0.910	ND	ND	ND	ND
o, p′-DDD	ND	0.001	ND	ND	ND	0.001	0.001	ND	ND	ND	ND	ND	ND	ND	ND	ND
o, p′-DDT	0.028	0.032	0.025	0.019	0.017	0.002	ND	ND	0.001	0.001	ND	ND	0.001	ND	<0.001	<0.001
Acetamiprid	0.013	0.016	ND	0.001	ND	0.001	ND	0.001	0.002	ND	ND	ND	0.001	ND	ND	<0.001
Pyridaben	0.001	ND	ND	ND	0.001	0.001	ND	ND	ND	ND	0.003	0.001	ND	0.001	<0.001	<0.001
Cypermethrin-1	ND	ND	ND	ND	ND	0.002	ND	ND	ND	ND	ND	ND	ND	0.003	<0.001	<0.001
Fenvalerate-1	ND	ND	ND	0.001	ND	0.003	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fenvalerate-2	ND	ND	ND	ND	ND	0.004	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = not detected.

the data for their permissible limit in drinking water is not available as shown in Table 2.

Aldicarb (0.003 mg/L) was found only in C7 while residues of acetachlor (0.001 mg/L) in C8. Among the pesticides quantified chlorpyrifos delivered highest amount of residues in C8, C9, C10, C11 and C12. Residues of *o,p′*-DDT were found high in C1 to C6 than the permitted value (0.002 mg/L), while higher concentration of pyridaben was resulted in C1, C5, C6 and C14 than the allowed value (0.0001 mg/L). Residues of carbofuran, atrazine, α-endosulfan and dieldrin and β-Endosulfan were not detected in any of the water samples collected. Concentrations of rest of the pesticides residues detected in water samples were within the permissible limits.

Conclusion

From the data it is evident that the flood water has contaminated the drinking water sources especially in the severely flood hit KP areas like Charsadda. Therefore, proper measures should be taken to clean the drinking water from such contaminants. It is further suggested that preventive actions should be taken to avoid such occurrence in future.

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Pak. j. sci. ind. res. Ser. A: phys. sci. 2017 **60**(2) 106-113

Effects of Crude Oil Inundated Soils on the Ecosystem – A Case Study

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(received January 24, 2017; revised April 17, 2017; accepted May 5, 2017)

Abstract. Crude oil inundated soils were collected from Agbada field after a recorded incidence of oil spillage to ascertain the effects of the oil spill on the soil status. Soil samples were collected from (0-30 cm) depths, using the grid sampling technique. Total petroleum hydrocarbon content (TPH) of the impacted soils ranged from 1.81541×103-4.8525040×103 mg/kg. The levels of total organic carbon (%TOC), pH, conductivity, sulphates, nitrates and phosphates were enhanced in impacted soil. The concentration of some trace metals such as Cd, Cu, Cr, Pb, Ni, Fe and V were also analysed using atomic absorption spectroscopy. Cd ranged from 0.2-0.38 mg/kg, Cu ranged from 4.20-5.20 mg/kg, Cr ranged from 18.40-44.40 mg/kg, Pb ranged from 1.20-30.40 mg/kg, Ni ranged from 2.40-2.70 mg/kg, Fe ranged from 17581.77-30273.25 mg/kg and V ranged 0.20-0.30 mg/kg. Most of the trace metals were highly enhanced in the impacted soil. Multivariate statistical analysis was carried out on the dataset to unveil the variation and relationship among them. Results showed that the first three principal components with the eigen values greater than one (>1.0) represent 93.4% of the total variability, suggesting that three principal components effectively describe the disparity in the data set. It was concluded that soils impacted with high hydrocarbon content; ultimately affect its physicochemical characteristics, which in turn impinge on the agricultural potentials of the soil.

Keywords: petroleum hydrocarbons; trace metals; impacted soil, depollution, oil spillage

Introduction

In recent times oil spillage has become a global phenomenon. This is because our fragile environment, which is a natural resource has been severely altered and destroyed by the incessant and persistent effect of hydrocarbon contamination. This global and domestic challenge of hydrocarbon contamination caused by oil spillage has made it imperative for more research to be carried out in this area.

In the developing countries such as Nigeria, the Niger Delta is most vulnerable to environmental degradation due to many activities of oil prospecting and exploration companies operating in the area. Oil spillage causes extensive damage to marine and terrestrial life, human health, and natural resources (Wang *et al*., 1999). The environment is critical to human existence for various reasons, especially agriculture, but has been subjected to numerous abuses including spillage of petroleum (crude oil) and petroleum-by products, dumping of waste and other contaminating activities. It is necessary to assess the probable impact of oil spillage on the environment and take effective depollution measures to avert the adverse effect on the flora and fauna.

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Oil spill in the environment is usually caused by activities such as damage to pipelines conveying crude oils, accidents during the work operation, sabotage, equipment failure etc. These activities inundate arable soils with crude oil which inevitably affects soil nutrients and in turn agricultural output in the oil producing communities (Osuji *et al*., 2006a). Oil spillage affects the aquatic soil ecosystem and has a deleterious effect on soil biota and crop growth beyond 3% concentration, soil macronutrients, microorganism and the ecosystem in general (Osuji, 2002). Oil spillage may also, sometimes, cause inferno that may destroy several acres of arable agricultural land.

Crude oil inundation of the terrestrial ecosystem is prevalent in the Niger Delta geographical region as a result of numerous oil exploration and exploitation activities, extensive networks of oil producing wells, flow stations and ageing pipelines which cut across the

oil producing communities and the mangrove swamp within the region. These make the region synonymous to crude oil pollution.

The aim of this study was to assess the level of impact of crude oil pollution on the ecosystem and recommend probable depollution measures to mitigate the adverse effect on the flora and fauna.

Materials and Methods

Study site. The study site, Agbada oil field falls within longitude 6° 490'E to 7° 530'E and latitude 4° 470'N to 5° 1430'N (Fig. 1). It is a moderately populated sub-urban environment in Ikwerre Local Government Area of Rivers State, Nigeria. Agbada is susceptible to crude oil contamination as a result of network of pipelines linking Rumuekpe and Ibaa communities located on the outskirts of Port Harcourt city in Rivers State. This is a cause of crude oil leakage into the environment. At the time of the sampling, the total volume of crude oil spill was not known.

Sample collection and analyses. A hand auger was used to collect oil-impacted soil after a field reconnaissance was carried out to define the area to be sampled. The grid sampling method was used as reported by Osuji and Onojake (2004). Six replicate soil samples were collected from the impacted area at a depth of up to 30 cm. The contaminated soil samples were put into appropriate sample bags and transported to the laboratory for analysis.

Physicochemical analysis. Soil samples were air dried at a room temperature of 25 °C. The dried samples were crushed and sieved with a 1.5 mm steel sieve attached to an electronic shaker to obtain a uniform particle size. Ten grams (10 g) of test sample was weighed and put in a beaker that was rinsed with deionized water, dried, and then properly homogenised. The physicochemical

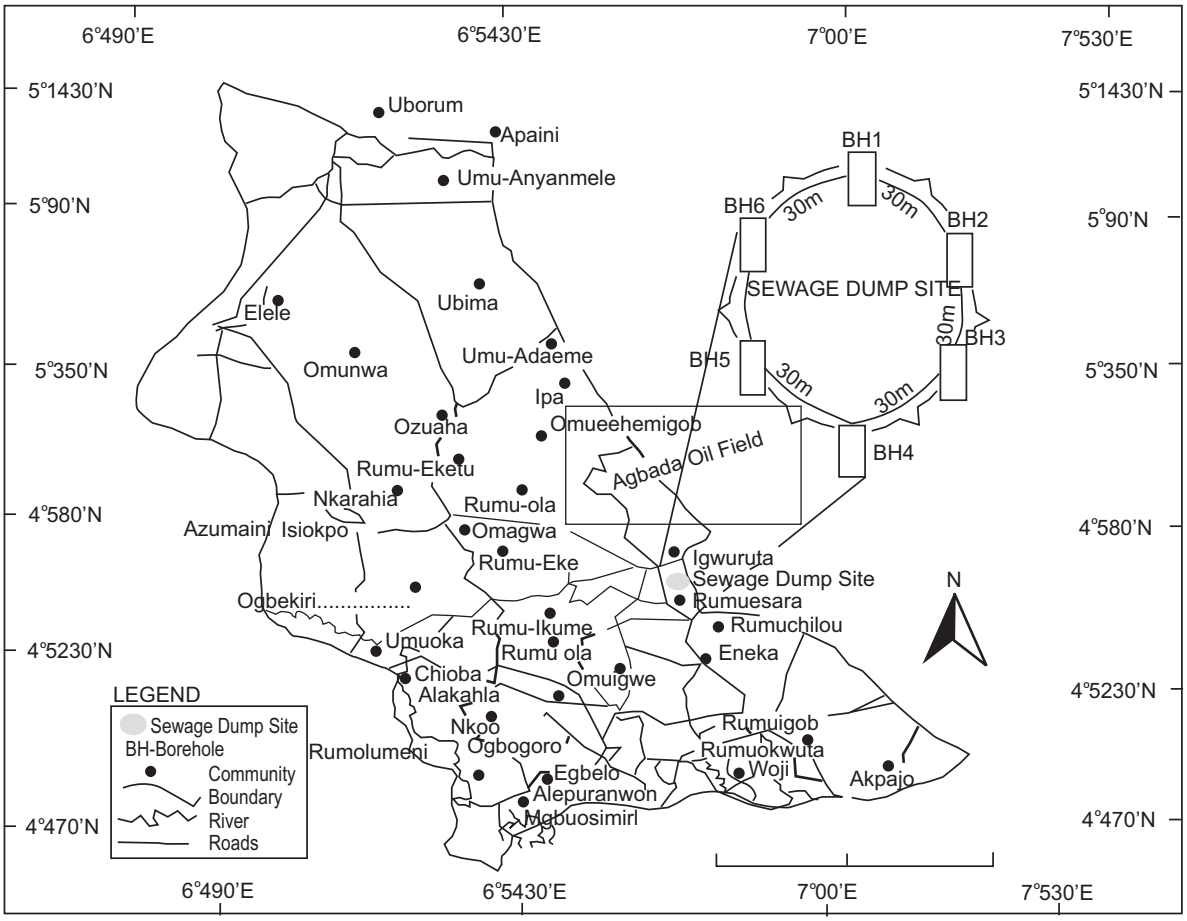

Fig. 1. Map of study area showing Agbada oil field.

parameters of soil samples such as pH, conductivity, sulphate, nitrate, phosphate were analysed using the Horiba U-10 (Horiba, LA-920, Kyoto, Japan), a state-of-the-art instrument for simultaneous multiparameter measurement.

Walkey Black wet oxidation method as modified by Nelson and Sommers (1982) was adopted for the determination of Total Organic Carbon (TOC) as follows: 1 g of soil sample was weighed into a 500 mL flask, and then 10 mL of $K_2Cr_2O_7$ followed by 20 mL of conc. H_2SO_4 were added. 200 mL of distilled H_2O, 10 mL of H_3PO_4, and five drops of diphenylamine indicator were further added to the mixture before titrating with $0.5N(NH_4)_2SO_4Fe$. A blank titration was thereafter carried out, and percent TOC was calculated using the formula below:

%TOC = blank titer - sample titer × 0.003 × 100/sample weight

Determination of chloride ions. The sample to be analysed was put into a 250 mL Erlenmeyer flask. Mixed indicator was added to it and swirled. This was followed by the addition of nitric acid and the solution titrated against mercuric nitrate. Distilled water was titrated with the same reagent and used as blank sample.

Spectrophotometric analyses. The cations in the oil-impacted soil samples were extracted with dithionite-citrate carbonate according to Hessler method as described by Osuji and Onojake (2004). Concentrations of Cd, Cu, Cr, Pd, Ni, Fe, V in the extract were determined using Perkin–Elmer model 2280/2380 atomic- absorption spectrophotometer.

Sample preparation for gas chromatographic analysis. Five grams (5 g) of oil spill impacted soil samples were weighed into a clean, dry beaker and extracted with 10 mL of hexane. Whatman filter paper was used to filter the sample and then sent to the laboratory for gas chromatographic analysis. The total petroleum hydrocarbon carbon content of the soil was analysed on a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a flame-ionization detector (FID) and an HP 7683 autosampler. One microlitre (1 μL) of the sample was injected in the splitless mode using syringe through a rubber septum into the column. The oven temperature was programmed from 60 to 280 °C at 4 °C/min with an initial hold time of 1 min and final hold time of 15 mins while detector (FID) and injector temperatures were kept at 250 and 280 °C, respectively. Helium was used as carrier gas at a linear velocity of 2 mL/min. The data were collected from retention time: 0-71 min (Peters *et al*., 1993).

Results and Discussion

pH and electrical conductivity. Results for total petroleum hydrocarbons on some physicochemical characteristics of the crude oil inundated soil and the unpolluted samples used as the control are shown in Table 1. The analysed physicochemical properties are pH, electrical conductivity, total organic carbon, sulphates, nitrates, phosphates, and chlorides.

The pH ranges from 4.80-5.20 for oil spill sites and 4.50 for the control site. Perhaps before the spill incident, the soil of Agbada 1 was a little on acidic level. Researchers such as Wang *et al*. (2010) had shown from previous studies that oil pollution increase soil pH which can adversely affect the availability of plant nutrients. Generally, pH values, organic matter and mineral elements of crude oil polluted soils are higher than the non-polluted soils (Maddela *et al*., 2016). This is supported by our results in Table 1. The pH values in the impacted soil were increased as a result of oil spillage when compared to the control soils. The degree of acidity or alkalinity of soils is considered as a major variable that affects nearly all soil properties (Osuji and Ezebuiro, 2006). A low pH levels below 5.0 causes some soluble ions such as aluminium and manganese to be more available in soils thereby increasing their concentrations, which discourage plant growth, hinder microbial metabolism and in turn reduce the hydrocarbon utilizing capacity that helps in degrading these hydrocarbons (Manahan, 2011; Odokuma and Okpokwasili, 1993). The electrical conductivity (EC) is the ability of soils to conduct electric current. The electrical conductivity (EC) in the affected soils was observed to increase slightly with a range of 31.30-38.30 μS/cm when compared to the control sample which has 25.40 μS/cm. The alterations in EC signify a changing composition of soil, the slight increase in the electrical conductivity may not be unconnected with the high values of salt and mineral content in crude oils (Onojake and Okonkwo, 2011).

The total organic carbon (TOC) contents increased as shown in Table 1. This is due to the addition of carbonaceous substances into the crude oil inundated soil. Organic carbon in the soil is derived from biota, such as peat formation with time, plant fine roots turnover,

microbial biomass and others (Wang *et al*., 2013). However, crude oil contamination or oil spillage can also contribute to the TOC in the soil because of the much higher TPH concentration (Wang *et al*., 2010; 2009). Oil usually causes anaerobic environment in soil by asphyxiating soil particles and obstructing air diffusion in the soil pores thus impinging on the soil microbial communities (Sutton *et al*., 2013; Labud *et al*., 2007; Townsend *et al*., 2003).

Sulphate and chloride ions (SO_4^{2-} and Cl^-). Crude oil contamination in the area under investigation increased the concentration of sulphate in the soil. This ranges from 3.00-4.00 mg/kg against the 1.00 mg/kg in the uncontaminated soil (Table 1). The enhanced concentration of sulphate in soil can be ascribed to the fact that as sulphates are being depleted from the soil, some sulphur oxidizing bacteria which belong to the species of Thiobacillus (*T. thioparus, T. novellas*) oxidizes hydrogen sulphide (H_2S) and other sulphur containing compounds and deposit elemental sulphur rather than produce tetraoxosulphate (vi) acid as product of their oxidation. The production of sulphate follows this by the genus *Thiobacillus* from the oxidation of elemental sulphur and other organic sulphur compounds (Atlas and Bartha, 1995; Kuenem *et al*., 1985).

Oil and gas production in the environment are often associated with the release of Cl^- in the form of salt to soil. These ions can be very mobile and may easily move with water over the surface or down through the soil. Some of the environmental impacts associated with these Cl^- in salt form in soil are degradation of soil physical and chemical properties usually caused by excess concentrations of these ions in soil which eventually impaired vegetation growth. The effects on plant are reductions in plant growth; thickened leaves with a dark green colour and appreciable changes in plant appearance.

Phosphates and nitrates. Phosphates and nitrates are essential nutrients required by plants and animals to maintain their growth, metabolism, and reproduction. Phosphorus is one of the essential macronutrients for plants and microorganisms in the soil. It was observed from our study that the concentration of phosphorus in the crude oil impacted soil was reduced significantly as compared with unimpacted soil used as control (Table 1). This observation is in agreement with the results of previous studies on crude oil contaminated soil which showed that the concentration of phosphorus in crude oil contaminated soil decreases by various degrees (Wang *et al*., 2013; Eneje *et al*., 2012; Wang *et al*., 2010; 2009). The lower concentration of phosphorus could be due to various reasons. The increase concentration of total petroleum hydrocarbons (TPH) may increase the population of soil microbes that are hydrocarbon degraders, and their adaptation to hydrocarbons. Microbes in the process of degrading hydrocarbons, utilize considerable amounts of phosphorus causing the amount to decrease in soil (Wang *et al*.,

Table1. Results of physicochemical and trace metal analyses of crude oil impacted and control samples

Parameter	A1	A2	A3	A4	Mean	Control
pH	5.20	5.40	5.10	5.10	5.20	4.50
Conductivity	38.30	31.30	32.10	35.60	34.33	25.40
Total organic carbon	5.73	4.48	5.15	5.72	5.27	4.16
Sulphate	3.00	3.00	4.00	3.00	3.25	1.00
Nitrate	0.60	0.70	0.71	0.80	0.70	0.10
Phosphate	0.08	0.22	0.24	0.18	0.18	0.30
Chloride	8.56	10.20	7.54	7.53	8.46	33.20
TPH	4713.66	4725.60	4852.50	1815.41	3264.54	69.84
Cd	0.20	0.60	0.40	0.30	0.38	0.01
Ni	2.40	2.50	2.70	2.50	2.53	<0.01
Cr	18.40	18.40	44.40	35.40	29.15	7.20
Pb	20.20	7.60	1.20	30.40	14.85	<0.01
Cu	4.20	5.20	4.80	4.70	4.73	1.65
Fe	30273.25	17581.77	24831.60	18865.99	22888.15	15122.93
V	0.30	0.20	0.30	0.20	0.25	0.01

A1, A2, A3 and A4 are sample identity; conductivity (µS/cm), TOC (%), sulphate, nitrate, phosphate, chloride, TPH and trace metals are in (mg/kg).

2009; Braddock *et al*., 1997). Consequently, the decrease in the concentrations of phosphorus in soil could affect the vegetation and structure of soil and microorganisms, and adversely affect the ecosystem of the area. Nitrogen fixation, or the conversion of atmospheric N_2 to NO_3^-, occurs mainly due to soil microbes. Inorganic nitrogen in soil are taken up by most microorganisms and plants, as nitrate (NO_3^-) or ammonium (NH_4^+) ions, which makes nitrogen to be depleted from the soil while other species of bacteria convert nitrate to gaseous nitrogen by using nitrate as a metabolic electron acceptor in place of oxygen (Nester *et al*., 2001).

Total petroleum hydrocarbons (THC). The total hydrocarbon content (THC) of oil spill impacted sites is commonly used to assess and establish the degree of hydrocarbon contamination (Osuji *et al*., 2006b). Results obtained show that the oil spill impacted site had elevated hydrocarbon content than the control which presupposes that there was a high level of hydrocarbon contamination on the site (Table 1). The concentration of total hydrocarbons shows a significant difference when compared to the control. This represents a level of hydrocarbon contamination, which is capable of creating an anoxic condition in soils because oil films reduce gaseous exchange. Osuji *et al*. (2004) affirm that high hydrocarbon content levels impinge on ground and subterranean flora and fauna which are crucial element in the biogeochemical cycle that affect availability of plant nutrients and consequently soil productiveness. High hydrocarbon content on the agricultural farmland has been responsible for delay in the decay process of the litters from soil, which normally augments the accumulation of organic matter (Osuji and Ezebuiro, 2006). Raju *et al*. (2017) in their research showed that inoculation of crude oil contaminated soil with microorganisms resulted in nearly or complete removal TPHs from the crude oil-polluted soil. The indigenous microorganisms were also effective in removing TPHs.

Trace metals. The results in Table 1 show increased levels of Cd, Ni, Cr, Pb, Cu, Fe and V in the impacted soils compared with unimpacted soil. This may result in better absorption by plants, which in turn leads to possible bioaccumulation in plants and the animals hence, leading to noxious feedback down the food chain (Osuji and Onojake, 2004). It was established that toxic metals and PAHs detected in used oil subdued the microbial degradation of hydrocarbons in contaminated soils (Adesodun and Mbagwu, 2008). Cadmium (Cd) is a known environmental pollutant which easily dissolves in water and adheres to soil particles. It does not break down in the environment but can change forms making them available to fish, plants and animals to take up from the environment. Cadmium can bioaccumulate and stays in the body for a very long time and can build up from many years even at low level of exposure causing human health hazard (Jarup and Akesson, 2009; Nordberg, 2009). The level of Ni was also higher in the impacted than the unimpacted soils. Though it is an essential micronutrient, its excessive levels in soil can be toxic to some soil fauna (Osuji and Onojake, 2004). The presence of Ni in the environments can lead to damage to tissues, genotoxicity and decreased growth. Organisms like mollusks and crustaceans are sensitive to the high concentration of Ni than other organisms (USDHHS, 1999). Chromium is an essential nutrient present in soil which is needed by plants and animals only in slight amount. High level of Cr can be toxic to plants and animals that depend on these plants. Pb is one of the environmental toxicants. Eating food grown on Pb contaminated soil and exposure to Pb^{2+} at low concentrations of 0.1 mg/kg reduces heterotrophic activities of microflora in soil and can be detrimental to plants and animals (Smith *et al*., 1975). Cu is one of the essential elements and can be toxic to some aerobic microbes in soil at the parts per billion (ppb) levels. High concentration of Cu is known to inhibit root growth and the germination of lettuce (Smith *et al*., 1975). It was reported by Maddela *et al*. (2015) that biomass of fungi was effective in the biosorption of the copper metal, and can be a suitable bioremediator for bioremediation in copper (II)-contaminated soil. The two novel fungi can be used for crude oil biodegradation where the soil is polluted with high concentrations of the copper.

Iron is essential for crop growth and food production, though plants require only small amounts. Increased soil pH reduces the level of Fe available for plants. The high level of Fe in the soils may result in enhanced absorption and likely bioaccumulation in plants and the animals that depend on them for continued existence, thus leading to deleterious response along the food chain. Bioaccumulation in humans can lead to increased plasma iron levels, suppresses immune cell function, allows increased growth rates of infectious organisms and increases the risks of morbidity and mortality due to infectious disease (Collins, 2003; Failla, 2003; Ward *et al*., 1996). Similarly, V when present in a small amount can be essential for green algae and

stimulate the growth of higher green but at a concentration higher than 10 mg/kg can be fatal (Onojake and Frank, 2013).

Multivariate statistical analyses. Principal component analysis (PCA) is valuable in reducing the number of variables in a dataset to a small number of components. These components (factors) may well represent most of the variation in the original data that simplifies the interpretation of multiple variables. The essence of performing PCA plot is to obtain a better-quality resolution of discriminant analysis with a better understanding of the relationship from the correlation of sets of variables from the fields (Onojake *et al*., 2015; Sappa *et al*., 2014). The basis of PCA is on the Eigen analysis of the correlation matrix, and the Varimax rotation which is usually adopted to maximize the variation of different components. The significance of the factor is determined by the Eigen value: values of 1.0 or greater are considered significant (Everitt *et al*., 2011; Onojake *et al*., 2011). In this study, the multivariate statistical approach which comprises of the principal component analysis (PCA) is used in the classification of oils into genetic types.

Principal component analysis was performed on the physicochemical properties and trace metals of the soil samples from the study area. The variables that were used in PCA, the obtained loadings and Eigen values are shown in Table 2. Notable among the four (4) PC sets, PC1 has a variance of 10.35 (Eigen value) which represents 69.0% of the whole variation and loaded positively with all variables except phosphate and chloride. The PC2 has a variance of 2.44 which accounts for 16.3% of the whole variation, has a positive loading on conductivity, TOC, Cl^-, Pb, Fe and V. The PC3, with variance 1.22 accounts for 8.1% of the entire data variation, has a positive loading with SO_4^{2-}, Cl^-, TPH, Fe and V. The first three principal components with the Eigen values greater than 1 correspond to 93.4% of the whole data variation, signifying that three principal components effectively explain the variation in the dataset (Table 3, Fig. 2). The high correlation between each variable and the principal component is very significant which probably signify that soil impacted by high hydrocarbon content; ultimately affect soil physicochemical properties, which sequentially have an effect on the agricultural prospects of such soils.

Table 2. Eigen analysis of the correlation matrix for soil samples

Eigen value	10.35	2.44	1.22	0.99
Proportion	0.69	0.16	0.08	0.07
Cumulative	0.69	0.85	0.93	1.00

Table 3. Loading table of variables on principal components for soil samples

Variable	PC1	PC2	PC3	PC4
pH	0.276	-0.158	-0.005	-0.387
Conductivity	0.269	0.315	-0.018	-0.097
Total organic carbon	0.237	0.377	-0.134	0.221
Sulphate	0.288	-0.165	0.071	0.262
Nitrate	0.292	-0.103	-0.271	0.017
Phosphate	-0.221	-0.395	-0.139	0.298
Chloride	-0.309	0.035	0.084	-0.018
TPH	0.254	-0.184	0.435	-0.145
Cd	0.203	-0.445	-0.106	-0.272
Cu	0.288	-0.205	-0.139	-0.113
Cr	0.210	-0.141	-0.203	0.672
Pb	0.161	0.396	-0.511	-0.182
Ni	0.306	-0.100	-0.060	0.004
Fe	0.207	0.277	0.534	0.153
V	0.295	0.021	0.257	0.138

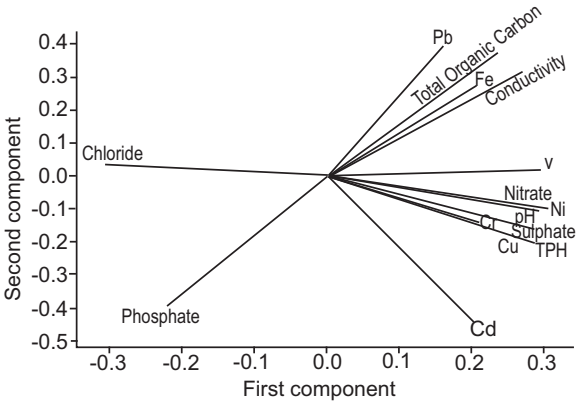

Fig. 2. Loading plot of the PCA for physicochemical and trace metals of crude oil impacted soil.

Conclusion

The enhanced level of the hydrocarbons, physicochemical parameters, and trace metals undoubtedly will adversely affect the soil nutrient status which in turn affects soil flora and fauna causing growth reduction in plants, delayed seed germination, availability of plant nutrients and in general, affect the soil fertility. The results of

present research do not provide enough scientifically conclusive evidence that the Agbada 1 crude oil spill was solely responsible for the enhanced level of trace metals but these results established the fact that their presence in crude oil may be a contributory factor. The use of principal components analysis (PCA) in elucidation of the data also shows a high similarity of 93.4%. This infers the likelihood that soil impacted by high hydrocarbon content; eventually influence soil physicochemical properties, which have an effect on the agricultural prospects of such soils. The presence of spilled oil on agricultural farmland should be followed up with adequate de-pollution measures such as excavation, liming and addition of nutrient in the form of inorganic fertilizer on the impacted soil which immediately restore the fertility of the soil and avert the adverse effects on the plants and animals.

Acknowledgement

The authors gratefully acknowledge the Department of Petroleum Resource (DPR) for granting us access to samples used for this research and the Management and staff of INDORAMA-Eleme Petrochemical Company Ltd. for the use of their laboratory in the analyses of soil samples. We sincerely thank to Prof. Leo C. Osuji for his useful comments which helped to improve the quality of this manuscript.

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Pak. j. sci. ind. res. Ser. A: phys. sci. 2017 **60**(2) 114-116

Short Communication

Effect of pH During Composting of Municipal Solid Waste

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(received August 25, 2016; revised March 14, 2017; accepted March 16, 2017)

Abstract. This study was designed to monitor the pH during process of composting by using organic waste segregated from municipal solid waste. pH was measured by preparing sample in laboratory by mixing compost with distilled water (1:10). and monitored in laboratory for the authentic results. The main objective of this study was to monitor the effect of pH during aerobic composting process that do not release harmful gases. It concluded that the pH value end up with alkalinity in degradation process but initially it was acidic.

Keywords: pH, composting, C: N, moisture %

There are many issues related to waste management system in an under developed country like Pakistan. One of the main issue is the non-availability of proper landfill sites for waste. Managing this waste is the need of society now a days. There are two requirements that need to be fulfilled when deal with waste, (i) less waste and (ii) proper system to manage this waste. Several models that predict environmental burden caused by municipal solid waste (MSW) are developed. The production of biogas during incineration from incinerator emission could cause harmful effects. Anaerobic composting at industrial level could emit many harmful gases that can badly pollute environment. The life cycle model for MSW are still lacking these areas. The objectives of municipal solid waste degradation can be achieved by using this composting approach. It includes resource recovery, bulk and mass reduction (McDougall *et al.,* 2008)

There are many applications of biodegradable MSW. One important application is to sort MSW and degrade it with microbial activity to get compost. This compost has several benefits for plants. This compost can be combined with N, P, K to get better quality crops (Dees and Ghiorse, 2001). Compost improves organic matter status in soil. The grain, rice and wheat crops are grown with addition of compost with fertilizer as it increases the chemical properties of soil and result in better quality of crop (Sarwar *et al*., 2007)

The mature compost play unique role in specialized practices, including gardening which require self-heating organic matter and also it is used as substrate for edible mushroom cultivation. Compost is used as a soil conditioner for high value crops such as flowers and vegetable grown out of season.

LCL is operating an aerobic composting plant at Lahore that has been purchased from and installed by the Menart Composting Company, Belgium. The LCL is a part of Saif Group of Companies and is operating a composting plant by using organic waste, the municipal solid waste transported to Mahmood booti landfill place, Lahore. LCL utilizing 1,000 tonnes in a day. The LCL imports inoculum from Belgium. The main focus of this research was to produce an inoculum for decomposing organic waste that could give economic benefits to Pakistan.

Pakistan has good economic growth in agriculture sector. The efficient preparation of compost with addition of inoculum can be a better initiative to further increase the economic growth at agriculture side, because it can improve soil structure and help to grow healthy crops and plants.

pH is an important parameter need to be checked because it decide the level of maturity of end product. pH should be alkaline as the soil in Pakistan is also alkaline. The changes of the composition during the biodegradation process and the final waste composition were strictly dependent on the process conditions (Liwarska- Bizukojc and Ledakowicz, 2003)

There are many important parameters that need to be checked during the process of biodegradation of MSW to get the good quality of compost. These parameters

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include porosity, temperature, oxygen, C: N, moisture content, windrow weight, pH, EC and CEC (Wakchaure *et al.,* 2013)

The MSW was taken to landfill site and sorted by using machine at LCL to remove any inert material. The sorted waste was composed of screening matter from MSW, cow dung and saw dust. Cow dung and saw dust was added to manage C: N. The screening matter was the organic waste which include biodegradable kitchen waste, food waste and plant waste. LCL took only organic waste at landfill site. The windrow of 50 tonnes were prepared by mixing screening matter, cow dung and saw dust. It was divided in to 4 equal parts of 12 feet width and 5 feet height. The prepared windrow was evaluated for various parameters including C: N, moisture content, temperature and oxygen. The C: N was adjusted below the value of 30:1 as initial C: N was measured 34:1. The moisture content in all the four treatments were adjusted to about 50% for bacterial metabolic activity. Initially it was below 50% and the temperature was maintained at 65-700C by adding microbial inoculum because microbial activity increase temperature and rapid degradation. Proper turning of the windrow was provided to give aeration. The temperature of windrow was monitored by using OT meter. The four divided parts of windrow were treated differently

Treatment A: microbial inoculum combined with molasses

Treatment B: microbial inoculum

Treatment C: commercially available inoculum

Treatment D: without any microbial inoculum

The designed microbial inoculum used in treatment A and B contained two strains of *Bacillus* bacteria. Treatment A, B, C and D were compared weekly for pH value.

Determination of pH . The samples were taken from four divided differently treated parts of 50 tonne windrow. The compost solution was made by adding distilled water in 1:10 and the solution was left for 2 h so that the maximum salts were dissolved. The electrode of pH meter was dipped in the compost solution. Reading was noted on pH meter when it was stabilized. The electrode was washed with distilled water and dried with tissue paper (Sánchez Monedero *et al*., 2001)

pH profile of compost. There were varied pH values for samples obtained from each treatment windrow as summarized in Table 1-2 and displayed in Fig. 1 and 2 with the majority showing alkaline pH. The lowest pH obtained was 5.25 in treatment D and the highest 7.98 in treatment A at the first week of composting. The increase in the pH value was observed in each treatment with the interval of time.

The pH of all treatments was increased with the time interval. All the treatments at the end of degradation process showed alkaline pH. The pH of mature compost of all treatments was also alkaline. Low pH effects the rate of respiration in a compost pile (Sánchez-Monedera *et al.,* 2001). It reduces the rate of respiration and slow down the process of composting. Wang *et al*. (2015) recommended a range of pH from 6.9-8.3 at the end of

Table 1. pH profile of compost during 1st month

Treat-ments	pH			
	Week 1	Week 2	Week 3	Week 4
A	6.08±0.09	7.21±0.13	7.38±0.14	7.24±0.22
B	5.89±0.60	7.58±0.89	7.11±0.12	7.54±0.90
C	5.36±0.37	6.72±0.44	6.75±0.38	7.05±0.10
D	5.25±0.27	6.90±0.35	6.59±0.44	6.91±0.11

Table 2. pH profile of compost during 2nd month

Treatments	pH			
	Week 5	Week 6	Week 7	Week 8
A	7.68±0.85	7.15±0.78	7.45±0.21	7.98±0.31
B	6.34±0.33	7.56±0.47	7.67±0.11	7.62±0.11
C	6.42±0.38	6.45±0.26	6.89±0.39	6.86±0.56
D	6.74±0.10	6.58±0.17	6.88±0.10	7.58±0.11

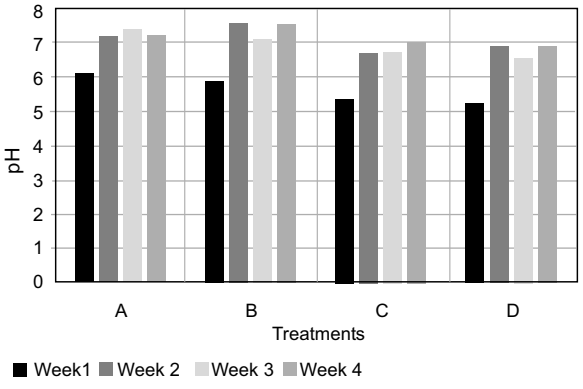

Fig. 1. pH variation of compost heap during 1st month of composting. (Treatment A, B, C and D were compared weekly for pH value.)

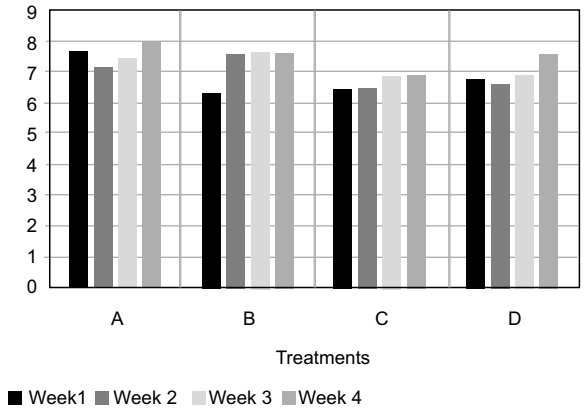

Fig. 2. pH variation of compost heap during 2nd month of composting. (Treatment A, B, C and D were compared for pH value weekly.)

composting and the results show this range of pH in all treatments. The pH of treated waste was alkaline at the end and these results are in line with the earlier findings of Sundberg *et al*. (2004) that the pH of the end product compost should be alkaline (Nakasaki *et al.,* 1993).

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