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Editorial Address

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Pakistan Journal of Scientific and Industrial Research, PCSIR Scientific Information Centre,
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Antioxidant Activity of Date Palm Fruit (*Phoenix dactylifera* L.) Extract for Oxidative Stabilisation of Butter Oil at Ambient Temperature

Fazal Rahman[a], Muhammad Nadeem[a], Sabir Khan[a], Shakeel Ahmad[a]* and Yasir Zahoor[b]

[a]Department of Dairy Technology, University of Veterinary and Animal Sciences, Lahore, Pakistan

[b]Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan

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Abstract. In this study, long term preservation of butter oil was achieved through ethanolic extract of date palm fruit (*Phoenix dactylifera* L.). Butter oil was supplemented with date palm fruit extract (DPFE) at three different concentrations i.e. 250, 500 and 750 ppm (T_1, T_2 and T_3) and compared with a control. Total phenolic content, DPPH free radical scavenging activity and inhibition of linoleic acid peroxidation of the DPFE was 5.19 GAE, 74.2 and 81%, respectively. IC_{50} value of date extract for the inhibition of DPPH and linoleic acid peroxidation was 2.45 and 0.82 mg/mL, respectively. The loss of oleic acid and linoleic acid in control after six months of storage was 16 and 52% as compared to T_3 which was 4% and 14%. T_3 yielded the lowest concentration of primary and secondary oxidation products with no effect on sensory attributes. DPFE can be used to enhance the shelf life of butter oil at ambient temperature.

Keywords: date palm, oxidative stability, butter oil

Introduction

Uncontrolled free radical mechanism in the human body leads to a large number of biochemical complications. Free radicals and reactive oxygen species have been implicated in the oxidative breakdown of vital biochemical molecules such as DNA, proteins, lipids (Madhujit and Shahidi, 2008). Phenolic compounds protect the body from the continuous threats of reactive oxygen species (Silva *et al*., 2009; Yazdanparast and Ardestani, 2007). The antioxidant, anti-inflammatory, antiallergic, anticancer and antiviral activities of phenolic compounds of plant origin and their role as a protector in hepatic and cardio-vascular diseases have been well established (Shahidi, 1997). Autoxidation of fats in food systems is a result of free radical mechanism leading to the destruction of essential fatty acids, vitamins and induction of objectionable flavours (Mc Sweeney and Fox, 2003). Perceived carcinogenicity of synthetic antioxidants, safety and efficacy of natural antioxidants in the inhibition of reactive oxygen species has necessitated broadening their array of application (Anwar *et al*., 2007). Studies have shown that most of the natural antioxidants of plant origin are better soluble in methanol (Anwar *et al*., 2010). The application of methanol based antioxidants for the preservation of food systems has a health concern due to the toxicity of methanol, although most of the solvent is evaporated yet the residues can have a potential health concern. Therefore, it is the need of hour to find out the sources of natural antioxidants which are soluble in water rather than polar organic solvents. Autoxidation of fats deteriorates sensory characteristics and limits the shelf life (Gonzalez *et al*., 2003; Shiota *et al*., 2002). The effect of (DPFE) date palm fruit extract for the stabilization of fats and oils has not been studied previously. This study aimed to investigate the antioxidant activity of date palm fruit extract on oxidative stability of butter oil on the basis of selected chemical and sensory techniques.

*Author for correspondence; E-mail: shakeel.1@live.com

Materials and Methods

Materials. Dates (*Zahidi*, Iranian variety) were procured from local market and cream was purchased from Haleeb Foods Multan Road, Lahore. All the chemicals used in this study were HPLC grade and obtained from Sigma Aldrich, USA.

Preparation of antioxidant extract. After removing the stones, dates were washed with distilled water, cut into small chunks, 20 g date was weighed in the flask, 80% ethanol was added into the flask and shaken with magnetic stirrer at 100 rpm for 8 h, the contents of the flask were filtered over filter paper (Whatman 41), the residue was extracted twice following the similar conditions and concentrated with rotary evaporator (Buchi, Switzerland).

Determination of total phenolic content. 125 µL sample, 500 µL deionised water and 125 µL Folin-Ciocalteu were mixed together, followed by the addition of 1.25 mL 7% solution of sodium carbonate and then 1 mL deionised water was added. Absorbance was measured on a double beam spectrophotometer (Shimadzu, Japan) at 760 nm. The concentration of total phenolic contents in the ethanolic date extract was determined by constructing a calibration curve using 10 standards of different concentration of Gallic acid (R^2= 0.9921) as prescribed by Negi *et al*. (2003).

DPPH free radical scavenging activity. DPPH free radical scavenging activity was determined by following the method of Mansouri *et al*. (2005).

Linoleic acid oxidation. Linoleic acid 0.13 mL was mixed with 10 mL (99.8% ethanol) and 10 mL of sodium phosphate buffer (0.2 M, pH 7). The contents were diluted to 25 mL with distilled water in the volumetric flask, sealed and incubated at 40 ºC in an oven for 15 days. The oxidation status was evaluated by the determination of peroxide value (thiocyanate method). 10 mL ethanol (75%) and 30% solution of ammonium thiocyanate prepared in distilled water and 0.2 mL extract and 0.2 mL ferrous chloride (20 mM prepared in 3.5% HCl) were added, contents were stirred for 3 min, absorbance was measured on 500 nm in visible region of spectra on a spectrophotometer using butylated hydroxytoluene (BHT; 100 ppm) as a control according to the method described by Anwar *et al*. (2010).

Experimental plan. Date palm fruit extract (DPFE) was incorporated into butter oil at three different concentrations i.e. 250, 500 and 750 ppm (T_1, T_2 and T_3), filled in PET bottles and stored at ambient temperature for six months and sampled at 60 days intervals for studying various characteristics.

Analysis. For the determination of fatty acid composition, 50 µL representative sample was taken in 11 mL screw capped test tube and 2 mL *n*-hexane was added to dissolve the sample. Methylation was performed by adding 2 mL, 0.5 N methanolic sodium methylate and tubes were vortexed for 3 min at 2200 rpm, after 5 min of settling time the supernatant was dried over anhydrous sodium sulphate, transferred to GC vials and injected into gas chromatograph model Shimadzu, Japan 17-A, fitted with a methyl lignoserate-coated (film thickness 0.25 µm), SP-2330 (SUPELCO Inc. USA) polar capillary column (30 m × 0.32 mm) using flame ionisation detector as per standard IUPAC method (1987) 2301. Fatty acids were identified and quantified by using FAME-37 internal standards (Sigma Aldrich, UK). Peroxide and anisidine values were measured by following the standard method of AOCS (1995). The sensory evaluation of butter oil supplemented with various concentrations of the date extract was performed by a panel of 10 trained judges who were selected and training sessions were conducted for them for standarisation of sensory language and familiarisation of flavour evaluation process. The flavour evaluation was performed on a 9-point scale in the sensory evaluation booths at 20±3 ºC as suggested by Larmond (1986). Each treatment was run in triplicate, the data were analysed by using analysis of variance technique (one way and two way). For the determination of significance difference among the treatments, Duncan Multiple Range Test was used. P-values ($P \leq 0.05$) were used to express the significant difference (Steel *et al*., 1997).

Results and Discussion

Total phenolic content. Total phenolic content of DPFE was 5.19% GAE. The higher concentration of phenols was due to the better solubility of antioxidants of date palm fruit in the ethanolic system, it makes date palm fruit extract superior to other natural antioxidants which are better soluble in methanol and other organic solvents. The application of natural antioxidants extracted by organic solvents for the preservation of food stuffs is questionable due to a great deal of potential health hazards associated with them. For the extraction of natural antioxidants, methanol has been considered to be a better solvent over others (Anwar *et al.,* 2010) but from commercial point of view methanolic based natural antioxidants have limited application due to high toxicity of methanol to humans. The aqueous date palm fruit extract showed higher concentration of phenolic substances and can provide better stabilisation of food systems without putting a question mark on the food safety. The total phenolic contents in this study were even higher than in methanolic extract of barley seeds (*Hordeum vulgare* L.) reported by Anwar *et al*. (2010). The higher total phenolic content of date palm fruit extract has also been reported in some Saudi Arabian date verities (Saleh *et al*., 2011).

DPPH free radical scavenging activity. The DPPH free radical scavenging activity of butter oil supplemented with DPFE increased in a concentration dependent manner and were in the order of $T_3 < T_2 < T_1 <$ control. The DPFE also exhibited good antioxidant activity in the

butter oil (Fig. 1). The DPPH free radical scavenging activity of the extract was 74.2% as compared to the BHT 94% (100 ppm) (1 mL concentration). IC_{50} value of the extract was 2.45 mL; the supplementation of butter oil with DPFE significantly decreased the IC_{50} value of butter oil which was shown in fresh and stored butter oil, the lower doses of the extract exhibited higher IC_{50} value. A longer storage period also had a negative influence on IC_{50} value. The disappearance of DPPH radicals is most likely due to presence of phenolic compounds in the extract. Strong DPPH free radical scavenging activity of date fruit extract has been described in literature (Singh *et al*., 2002).

Linoleic acid oxidation. In this study, the % inhibition of oxidation in linoleic acid system was also determined, the percentage inhibition of lipid peroxidation of DFPE was 81% (R^2=0.9934) as compared to BHT 100 ppm (91.5%) which was used as positive control. IC_{50} value of date fruit extract and butter oil supplemented with extract was also determined at different storage intervals (Fig. 2). The IC_{50} value of ethanolic date fruit extract was 0.82 mg/mL. IC_{50} value of butter oil cream (mL of butter oil to decrease 50% lipid peroxidation) decreased in a dose dependent manner and were in the order of $T_3 < T_2 < T_1 <$ control at zero day and all the determination frequencies. The lower the IC_{50} value, better is the antioxidant activity. The IC_{50} value of butter oil (mL of butter oil causing 50% decrease in lipid peroxidation) in T_2 and T_3 was significantly less than in the control and increased for all butter oil samples during storage period

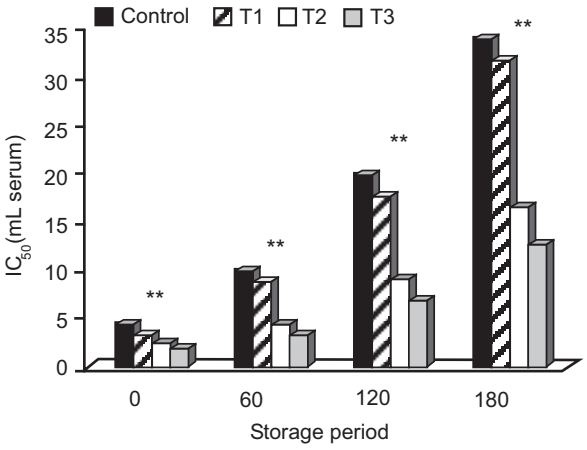

Fig. 1. IC_{50} Value for the inhibition of linoleic acid peroxidation **Highly significant ($p<0.01$) Refer Table 2 for the detail of treatment.

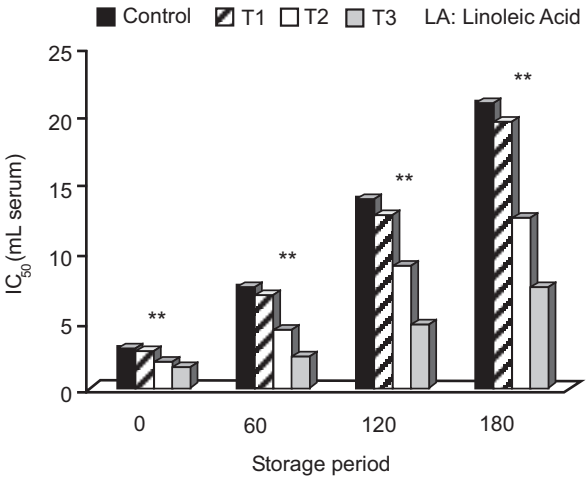

Fig. 2. IC_{50} Value for the elimination of DPPH free radicals. **Highly significant ($p<0.01$) Refer Table 2 for the detail of treatment.

of 180 days. The strong antioxidant activity of the extract could be attributed to the solubility of natural antioxidants in the ethanolic phase. The results regarding lower IC_{50} value of ethanolic date fruit extract in this study are also supported by the findings of Al-Farsi *et al*. (2005) howerver, little information is available on IC_{50} value of foodstuffs supplemented with natural antioxidants.

Changes in the fatty acid composition. The changes in the fatty acid composition of butter oil supplemented with DPFE and control are presented in Table 1. At 250 ppm supplementation level DPFE was virtually inactive to inhibit the lipid peroxidation. The lipid peroxidation inhibition of DPFE was in the order of $T_3 > T_2 > T_1$. Some difference was observed in the fatty acid composition of fresh and six months stored butter oil. The extent of difference was dependent upon three factors; storage period, supplementation and the supplementation level of ethanolic date fruit extract. DPFE in T_2 and T_3 significantly inhibited the free radical mechanism in the stored butter oil, better antioxidant activity was seen in T_3. Unsaturated fatty acids decreased during storage due to their breakdown into primary and secondary oxidation products and saturated fatty acids increased on percentage basis. The loss of oleic acid and linoleic acid in control was 16 and 52% as compared to T_3, 4 and 14% after six months of storage. DPFE significantly retarded the autoxidation process in T_2 and T_3. The strong antioxidant activity of DPFE could be attributed to the higher concentration of polyphenolic compounds. The concentration of unsaturated fatty acids decreased during storage of 42-days when flax seed oil

was added in the formulation of ice cream (Lim *et al*., 2010). The fatty acid composition of fresh and stored butter was slightly different (Mallia *et al*., 2008). The strong antioxidant activity of date palm has been shown in literature (Mansouri *et al*., 2005) but little information is available regarding the application of DPFE for the stabilisation of fat rich dairy products.

Peroxide value. The results regarding increase of peroxide value in the DPFE supplemented and control are presented in Table 2. Supplemented and control butter oils showed varying degree of rise in peroxide value. The magnitude of rise in peroxide value during storage period was in the order of control > T_1 > T_2 > T_3. The peroxide value and concentration of DPFE were highly correlated (R^2=0.9749). The addition of 750 ppm date extract was more effective in the stabilisation of butter oil over other treatments. Peroxides are the products of free radical mechanism, and phenolic compounds can terminate the free radical mechanism by donating protons, the lower peroxide value of T_3 could be attributed to the better proton donating capability of DPFE. Shiota *et al*. (2004) used peroxide value as important parameter to characterise the photochemical oxidation of butter oil. Supplementation of chicken meat mince with date palm extract significantly retarded the lipid peroxidation for 20-days at 4 ºC (Biglari *et al*., 2009). Peroxide value of ice cream prepared from modified and unmodified milk fat increased during storage (Shiota *et al*., 2004; Gonzalez *et al*., 2003). The stabilisation of butter fat through the application of natural antioxidant has been reported by Nadeem *et al*. (2013).

Anisidine value. The data of anisidine value of supplemented and control butter oil is given in Table 3. Anisidine value numerically increased throughout the storage period of 180 days, the classical rise in anisidine value varied considerably among the treatments and control, T_3 revealed the lowest concentration of secondary oxidation products at all the determination intervals followed by T_2. Determination of anisidine value reflects the concentration of aldehydes produced as a course of free radical mechanism (McGinely, 1991). The antioxidant activity of natural antioxidants for the stabilisation of edible oils has been extensively studied by the researchers. In other studies, supplementation of barley extract and wheat bran extract significantly inhibited the generation of secondary oxidation products in sunflower and canola oils (Chatha *et al*., 2011; Anwar *et al*., 2010).

Table 1. Effect of DPFE on fatty acid composition of fresh and six months stored butter oil

Fatty acid	Fresh%	Control-6M%	T_1-6M%	T_2-6M%	T_3-6M%
C4:0	4.68±0.13^c	5.36±0.09^a	5.27±0.06	4.93±0.04^b	4.72±0.013^c
C6:0	2.89±0.14^b	3.27±0.03^a	3.18±0.06^a	2.97±0.07^b	2.92±0.02^b
C8:0	1.75±0.05^c	2.41±0.04^a	2.33±0.06^a	2.04±0.02^b	1.82±0.04^c
C10:0	4.17±0.11^c	4.83±0.08^a	4.71±0.07^a	4.53±0.03^b	4.24±0.12^c
C12:0	5.33±0.19^c	5.79±0.015^a	5.73±0.09^a	5.58±0.07^b	5.41±0.16^c
C14:0	12.19±0.12^d	12.72±0.48^a	12.59±0.22^a	12.43±0.31^b	12.32±0.19^c
C16:0	20.47±0.35^a	20.98±0.84^a	20.84±0.49^a	20.61±0.17^a	20.53±0.28^a
C18:0	5.43±0.10^c	5.87±0.61^a	5.80±0.16^a	5.69±0.21^b	5.40±0.36^c
C18:1	31.68±1.24^a	26.64±0.44^e	27.37±0.38^d	28.66±0.13^c	30.48±0.46^b
C18:2	3.22±0.15^a	1.54±0.05^e	1.99±0.04^d	2.43±0.12^c	2.79±0.02^b

Within a row, means represented by the same letter are not statistically different; T_1 = ethanolic date palm fruit extract 250-ppm; T_2 = ethanolic date palm fruit extract 500-ppm; T_3 = ethanolic date palm fruit extract 750-ppm; 6M = six months stored butter oil

Table 2. Effect of ethanolic date extract on peroxide value of butter oil ($M_{eq}O_2$/kg)

Treatments	0-D	60-D	120-D	180-D	Increase
Control	0.24±0.02^d	0.68±0.05^c	1.85±0.06^b	3.77±0.19^a	3.53
T_1	0.24±0.02^d	0.51±0.09^c	1.43±0.11^b	2.92±0.25^a	2.68
T_2	0.24±0.02^d	0.44±0.10^c	1.15±0.16^b	2.48±0.15^a	2.24
T_3	0.24±0.02^d	0.35±0.04^c	0.82±0.14^b	1.37±0.12^a	1.13

Within the rows and columns, means carrying different letter are statistically different; Increase = increase in PV from the start; D = storage days

Table 3. Effect of ethanolic date extract on anisidine value of butter oil

Treatments	0-D	60-D	120-D	180-D	Increase
Control	4.52±0.13^{d}	7.35±0.15^{c}	13.62±0.38^{b}	21.64±0.45^{a}	17.12
T_1	4.52±0.13^{d}	5.42±0.22^{d}	10.58±0.29^{b}	15.76±0.51^{a}	11.24
T_2	4.52±0.13^{d}	6.75±0.17^{d}	8.49±0.18^{b}	12.92±0.34^{a}	8.40
T_3	4.52±0.13^{d}	5.29±0.13^{d}	7.36±0.15^{b}	9.53±0.26^{a}	5.01

Within the rows and columns, means carrying different letter are statistically different; Increase = increase in PV from the start; D = storage days

Table 4. Effect of ethanolic date extract on flavour score of butter oil

Treatments	0-D	60-D	120-D	180-D	Decrease
Control	8.2±0.2^{a}	7.7±0.24^{b}	7±016^{d}	5.9±0.10^{d}	2.3
T_1	8.1±0.2	7.7±031^{b}	7.2±0.25^{c}	6.3±0.15^{c}	1.8
T_2	8±0.1^{a}	7.9±0.19^{a}	7.7±0.27^{b}	6.8±0.12^{b}	1.2
T_3	8±0.15^{a}	8±0.11^{a}	7.9±0.35^{a}	7.4±0.24^{a}	0.6

Within the rows and columns, means carrying different letter are statistically different; D = storage days

Sensory evaluation. The results of sensory evaluation of butter oils supplemented with DPFE and correlation between peroxide value flavour score are given in Table 4. When fresh, flavour score of the treatments and control were not different from each other (P>0.05). Flavour score decreased during storage period of 180-days, the decline in the flavour score of butter oil was observed in the order of control > T_1 > T_2 > T_3. The decline in the flavour score was not due to the addition of DPFE but due to the development of oxidised flavour. Peroxide value and flavour score were highly correlated (R^2=0.9803), the smallest drop in flavour score of the control, T_1, T_2 and T_3 was 2.3, 1.8, 1.2, and 0.6, respectively from the initial value. The lowest drop in the flavour score of T_3 was due to the generation of considerably lower amounts of primary and secondary oxidation products by the strong antioxidant potential of ethanolic DPFE. Shiota *et al*. (2004) reported a strong correlation between peroxide value and flavour score of butter oil. Nadeem *et al*. (2013) also reported a decline in the flavour score of butter stored for three months.

Conclusion

Characterisation of ethanolic date palm extract revealed strong antioxidant activity; the addition of aqueous date palm fruit extract at 750 ppm concentration significantly inhibited the changes in the fatty acid composition, generation of primary and secondary oxidation products with minimum decline in the flavour score during storage period of 6 months. Date palm extract at 750 ppm can be added for better storage stability of butter oil with acceptable flavour characteristics.

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Cadmium Tolerance and Bioremediation Potential of Bacteria Isolated from Soils Irrigated with Untreated Industrial Effluent

Rizwan Ahmad*, Muhammad Mahmood-ul-Hassan, Muhammad Yasin and Vishandas Suthor
Land Resources Research Institute, National Agricultural Research Centre, Islamabad 45500, Pakistan

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Abstract. The present study was aimed to investigate the Cd tolerance of bacteria isolated from municipal effluent irrigated soils. Thirty bacterial strains were isolated and screened for their Cd^+ tolerance by growing on nutrient agar plates amended with varying amount of Cd^+. Out of them four bacteria (GS_2, GS_5, GS_{10} and GS_{20}) were found highly Cd tolerant (600 ppm Cd). The minimum inhibitory concentration of Cd^+ was found 200 ppm. The isolates showed optimum growth at 30 °C and pH 7.5-8.5. Growth curve study against different concentrations of Cd (0-600 ppm) revealed that GS_2 was more tolerant among selected strains showing only 33% reduction in growth compared to 64% by GS_5 and 77% by both GS_{10} and GS_{20} at 600 ppm Cd. Inoculation of maize seeds with Cd tolerant bacteria for root elongation demonstrated up to 1.7 fold increase in root elongation (in the absence of Cd) and up to 1.5 fold (in the presence of 50 ppm Cd) compared to the un-inoculated plants. The results of the study revealed that the bacterial isolates exhibiting great Cd tolerance and growth promoting activity can be potential candidates for bioremediation of metal contaminated soils and wastewaters.

Keywords: soil contamination, Cd tolerance, tolerance index, bioremediation

Introduction

The contamination of the environment with toxic heavy metals is a serious problem because it is associated with heavy metal accumulation in the food chain which later has an impact towards human health (Hamzah *et al.,* 2009). Municipal/industrial effluents contain considerable amounts of different metals as: chromium (Cr), cadmium (Cd), lead (Pb), nickel (Ni) and copper (Cu) in various combinations depending upon their source and nature (Khan *et al.,* 2013; Mahmood-ul-Hassan *et al.,* 2012). Release of untreated municipal/industrial effluents to agricultural lands and water bodies is a common practice in big cities of developing countries like Pakistan (Khan *et al.,* 2013; Mahmood-ul-Hassan *et al.,* 2012). Its long-term application can adversely affect soil and ecosystem health, ultimately human health (Singh and Bhati, 2005). Contamination of soil with heavy metals negatively affects biodiversity and the activity of soil microbial communities (McGrath *et al.,* 1995). Continuous application of untreated wastewater elevates the metal concentrations in surface soil to toxic levels. As soil is a rich habitat of all major groups of microorganisms (bacteria, actinomycetes, fungi and algae), long-term exposure of microorganism to high metal concentration develop the immunity in the microorganisms (Akhtar *et al.,* 2013; Ezzouhri *et al.,* 2009).

Among metal pollutants of the surface soil, cadmium is one of the most toxic elements. Cadmium is used in industries like Ni-Cd battery manufacturing, electroplating, pigments manufacturing and stabilizers manufacturing. In plants, Cd affects nutrient uptake and homeostasis, inhibits root and shoot growth and frequently accumulated by agriculturally important crops (Sanita di Toppi and Gabrielli, 1999). Cadmium is the most dangerous heavy metal both to human and animal health as it is carcinogenic, embryo toxic, teratogenic and mutagenic (Hussain *et al.,* 2006). Excess Cd can damage kidney and lungs (Dhaliwal and Kukal, 2005). It may cause hyperglycemia, reduced immune potency and anemia, due to its interference with iron metabolism (Bueno *et al.,* 2008).

Several techniques (chemical and physical) are used for remediation of polluted soil and water. Chemical (precipitation and neutralisation) and physical (ion exchange, membrane separation and electro dialysis) techniques are applied to remove heavy metals from contaminated soils and waste water (Atkinson *et al.,* 1998). Such techniques have disadvantages like unpredictable metal ion removal, high reagent requirements, destruction of beneficial micro fauna and generation of toxic sludge (Ciba *et al.,*1999).

*Author for correspondence; E-mail: rizwan_narc@yahoo.co.in

Biological approaches have been considered as an alternative remediation for heavy metal contamination (Khan *et al.,* 2009). Recent advances have been made in understanding metal-microbe interaction and their application for bioremediation of metal contaminated soils (Chibuike and Obiora, 2014). Bioremediation is the use of microbes like fungi and bacteria for removal of heavy metals and have been successfully used (Congeevaram *et al.,* 2007). The metal tolerant microorganisms are helpful to alter the chemical status of the metal ions and in turn metal ion mobility. They act through processes such as reduction, bioaccumulation, mobilisation and biotransformation (Khan *et al.,* 2009). Bioremediation is an efficient strategy due to its low cost, high efficiency and eco-friendly nature. It can be applied without removing and transporting contaminated soils. As soil matrix is not disturbed, soil micro flora and fauna are preserved.

Bacterial surfaces have several types of functional groups that can react with dissolved metals. Bacteria are important microorganisms to be used for biosorption and bioaccumulation of metals and hence are an important factor in controlling the mobility and distribution of metals in contaminated soil and water (Burnett *et al.,* 2007). Biosorption is removal of heavy metals using passive binding process of living and dead biomass while bioaccumulation is referred as metal uptake into the cell across the membrane using active cell metabolism (Kotrba *et al.,* 2011). Considering the importance of bacteria in bioremediation, this study was designed to isolate indigenous bacteria from polluted sites and assess their cadmium tolerance potential to use in bioremediation.

Materials and Methods

Samples collection. Six composite surface soil samples (0-15 cm) were collected from heavy metal polluted peri-urban areas of Gujranwala and Sialkot, Pakistan for this research work. Sampling sites have been continuously (more than 10 years) irrigated with untreated industrial/ municipal effluent with high metal contents. The surface soil samples were collected in sterilised plastic bottles and were transported to Soil Environment Laboratory, NARC, Islamabad, Pakistan in sealed containers. These containers were stored at 4 °C to ensure minimal biological activity till further process.

Isolation for cadmium tolerant bacteria. Dilution plate technique was used for isolation of microbes (Pepper and Gerba, 2004). Bacteria from soil were cultured with 10^{-4}- 10^{-7} dilutions on nutrient agar medium at 28±2 ºC for three days. Thirty prominent isolates (twenty from Gujranwala and ten from Sialkot sites) with some distinguished morphological characters (colony colour, size, shape etc.,) were further cultured and purified through repeated streaking on the same medium. The cultured strains were preserved on slants for Cd tolerance test at 4 ºC and refreshed within three months regularly.

Minimum inhibitory concentrations (MIC) of Cd. To determine MIC for Cd, the growth of isolated bacterial strains was tested on nutrient agar medium amended with ascending concentration of Cd starting from 50 ppm (Kalantri, 2008). Stock solution (1000 ppm) of Cd salt ($CdCl_2$) was prepared with sterile water and added to the nutrient agar in varying concentrations (50-600 ppm). The process was continued with 50 ppm interval till the growth was ceased. Highly tolerant strains (600 ppm Cd) were tested repeatedly for further confirmation.

Morphological and biochemical characterisation. For colony and cell morphology, bacterial strains were grown on nutrient agar medium at 28±2 °C for 36-48 h. Each colony was characterised on the basis of colour, margin, elevation and cell shape with ocular and light microscopy. For gram staining, the slides of tolerant bacterial strains were prepared according to Benson (1994). A small loop of bacterial culture was taken and a thin smear on glass slides was prepared. The smear was air dried and heat fixed, stained with crystal violet stain for one minute and washed with water. Then the smear was flooded with iodine solution for 30 sec. After 30 sec it was washed with water and smear was decolourised with 75% ethanol for 30 sec. After washing, safranin was used for counter staining. The slide was rewashed with water, air dried and observed under light microscope.

Indole acetic acid (plant hormone) production of Cd tolerant bacterial strains was detected by using the method stated by Brick *et al.* (1991). Bacterial cultures were grown in 250 mL conical flasks containing 50 mL nutrient broth (Lab-lemco powder 1.0; Yeast extract 2.0; Peptone 5.0; Sodium chloride 5.0, each was on g/L basis) for 3 days at 28±2 ºC. Flasks were inoculated with different bacterial strains individually. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 mL) was mixed with two drops of orthophosphoric acid and 4 mL of the Salkowski reagent (50 mL, 35% of perchloric acid, 1mL 0.5M $FeCl_3$ solution). Development of pink colour indicates IAA production.

For measurement of phosphate solubilising activity, a single colony of each strain culture was streaked on

Pikovskaya`s medium containing tricalcium phosphate (EL-Komy, 2005) and incubated at 28 ± 2 ºC for 3 days. Qualitative determination was done by optically observing clear P-zone (halo-zone) formation around the colonies.

Screening for acid producing ability was determined by using the bromothymol blue indicator along with nutrient broth. Each strain was grown in 250 mL conical flasks containing 50 mL nutrient broth for 3 days at 28±2 °C. Traces of bromothymol blue indicator were added to each flask at the time of inoculation. Dark green colour appeared at neutral pH. Appearance of bluish green colour indicated the acid producing ability of the bacterial strain (Dupree and Wilcox, 1977).

Determination of optimum growth conditions. For optimum growth of bacterial isolates, two parameters i.e. pH and temperature were considered. To determine optimum pH, 30 mL test tubes having 10 mL nutrient broth were prepared in 5 sets for pH 6.0, 7.0, 8.0, 9.0 and 10 (each containing three test tubes) and autoclaved. These tubes were inoculated with freshly prepared culture of each isolate one by one. The tubes were incubated at 28±2 °C and 70 rpm. After an incubation period of 24 h, their absorbance was taken at 600 nm wavelength on Spectronic Genesys 5 (Milton Roy Company, USA) and then a graph was plotted between pH (along x-axis) and absorbance (along y-axis).

For determination of optimum temperature, test tubes having 10 mL nutrient broth were prepared in 3 sets for 20, 30, and 40 °C. The pH of all the sets, each containing three test tubes was adjusted at 7 by using diluted HCl or NaOH solutions. Test tubes were autoclaved and then inoculated with freshly prepared cultures of different isolates individually. The tubes were incubated at 20, 30, and 40 °C, respectively, with 70 rpm. After an incubation period of 24 h, their absorbance was taken at 600 nm wavelength and then graph was plotted between temperature (along x-axis) and absorbance (along y-axis).

Growth curve and metal tolerance index. Nutrient broth with increasing concentrations of Cd i.e. 200, 400, 600 ppm was prepared and autoclaved. A control (0 ppm Cd) was also made. Growth of the selected heavy metal tolerant strains was studied at standard temperature (30 °C) and pH (7). Test tubes were inoculated and incubated for 24 h in water bath shaker at 70 rpm. After an incubation period of 24 h, their absorbance was taken at 600 nm wavelength and then a graph was plotted between Cd concentration (along x-axis) and absorbance (along y-axis). Metal Tolerance Index (T_i) was calculated as the ratio of the optical density of the treated colony to that of the untreated colony.

$$T_i = \frac{OD_t}{OD_u}$$

Where:

OD_t = optical density of treated colony and
OD_u = optical density of the untreated colony.

Root elongation assay on filter paper culture. The plant root elongation promoting activity of the isolated bacteria was determined using the modified root elongation assay of Belimov *et al.* (2005). The seeds of maize variety ISD-gold were surface sterilised with a mixture of ethanol and 30% H_2O_2 (1:1) for 20 min, washed with sterile water and placed on wetted filter paper. Bacteria were grown in nutrient broth for 48 h at 28 ± 2 °C. Bacterial suspensions 5 mL or sterile water (un-inoculated control) were added to petri dishes containing filter papers, both in the presence and absence of 50 mg/L Cd. Root length of seedlings was measured after incubation of closed petri dishes for 7 days at 28 ± 2 °C in the dark. The assay was repeated twice with three dishes with 10 seeds per dish for each treatment.

Results and Discussion

Total heavy metals in Gujranwala and Sialkot soils. Surface soil samples used in this study were collected from peri-urban area of Gujranwala and Sialkot being irrigated with untreated wastewater (Table 1). The wastewater was a mixture of cottage industries and domestic effluent. Soil organic matter content was 0.7-2.0 %. The soils were alkaline in reaction (pH from 7.0 to 8.5), non-saline (electrical conductivity from 0.3 to 1.2 dS/m) and calcareous in nature (lime contents from 1.5 to 15.2%). The concentrations of different metals in soil of the study areas were; Cd ranged from 2 to 8.4 mg/kg; Cu 60 to 380 mg/kg; Pb 205 to 250 mg/kg; Cr 80-330 mg/kg and Ni from 90 to 130 mg/kg. The total soil Cd, Cu, Cr, Pb and Ni content in almost all the soil samples were higher than the permissible limits, i.e., 3, 100, 100, 100 and 50 mg/kg, respectively, as proposed by FAO/WHO (2001). Heavy accumulation of these metals in the soils are results of their use in different industries like ceramics, sanitary fittings, electrical and gas appliances, detergent manufacturing, dry batteries, plastic-ware, kitchen-ware and tanneries. The elevated concentrations of heavy metals in the soils are most likely due to long-term continuous application of

Table 1. Geographical position of peri-urban sites of sample collection

Site	City	North	East
1	Gujranwala	32° 06'	74° 10'
2	Gujranwala	32° 07'	74° 10'
3	Gujranwala	32° 07'	74° 11'
4	Gujranwala	32° 09'	74° 11'
5	Sialkot	32° 28'	74° 30'
6	Sialkot	32° 29'	74° 32'

untreated municipal/ industrial effluent containing these heavy metals.

Screening and characterisation of Cd tolerant bacterial strains. In this study, a total of 30 soil bacteria were isolated from the heavy metal contaminated soils. Minimum inhibitory concentration of Cd was found 200 ppm. Similar results were also reported by Ansari and Malik (2007), who reported MIC of 200 ppm for Cd. Out of 30, 21 bacterial strains tolerated cadmium (Cd) up to 200 ppm, thirteen strains were moderately tolerant (400 ppm Cd) and only 4 strains (GS_2, GS_5, GS_{10} and GS_{20}) were found highly tolerant (600 ppm Cd).

The selected 4 bacteria were characterised morphologically and bacteria were also observed under microscope for cell shape (Table 2). Most of the strains had phosphorus solublisation and acid producing activity with bacillus cell shape and gram negative staining. None of the tolerant strain had IAA producing ability in the absence of L-tryptophan. IAA producing ability in the presence of L-tryptophan was not studied.

Results have shown that microorganisms in a contaminated environment could have adapted to that environment over a period of time. Piotrowska-Seget *et al*. (2005) also found in his study that, prolonged exposure of soil bacteria to Cd can develop resistance to its toxicity by activating the tolerance mechanism towards Cd. Most of the bacteria they studied were gram negative.

Optimum growth conditions, growth curve and tolerance index. The effect of pH on the growth of selected 4 bacterial isolates is shown in Fig. 1 and the effect of temperature is shown in Fig. 2. Optimum pH varied from 7.5 to 8.5 which was in accordance with the pH of the soils from which the strains were isolated (Mahmood-ul-Hassan *et al*., 2012). Regarding temperature, optimum growth of bacterial isolates was found at 30 °C ± 2. It shows that the selected bacteria are well adapted to soil as well as the climatic conditions of the regions from where they were isolated and can be reused in field conditions of the same ecology.

Growth curves of 4 highly Cd tolerant bacterial strains were made against different concentrations of Cd ranging from 0 to 600 ppm (Fig. 3). It is obvious from the result that at low concentration of Cd (200 ppm) the bacterial growth was high as compared to control; however, at 400 and 600 ppm Cd, the growth of all bacterial strains was also suppressed.

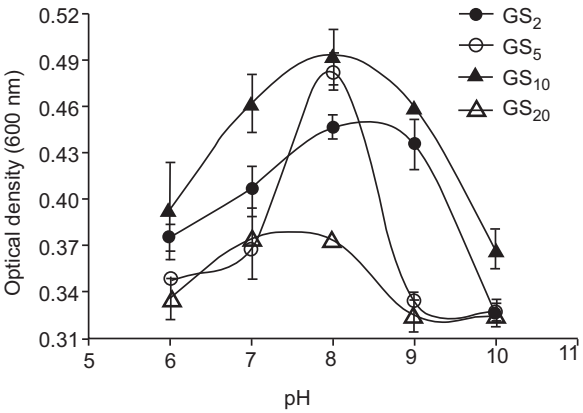

Fig. 1. Growth of Cd-tolerant bacteria as affected by different pH at 30 °C (N=3).

Table 2. Morphological, biochemical and microscopic characteristics of Cd-resistant bacterial strains

Characteristics	Bacterial strain			
	GS_2	GS_5	GS_{10}	GS_{20}
Colony shape	Filamentous	Filamentous	Filamentous	Irregular
Colony colour	Yellowish white	White	Yellowish white	Creamy
Elevation	Umbonate	Concave at centre	Concave at centre	Umbonate
Margin	Undulate	Lobate	Erose	Undulate
Cell shape	Bacillus	Bacillus	Coccus	Bacillus
Gram staining	-	-	-	-
P-solublising	+	-	+	+
Acid production	+	-	+	+
IAA-production	-	-	-	-

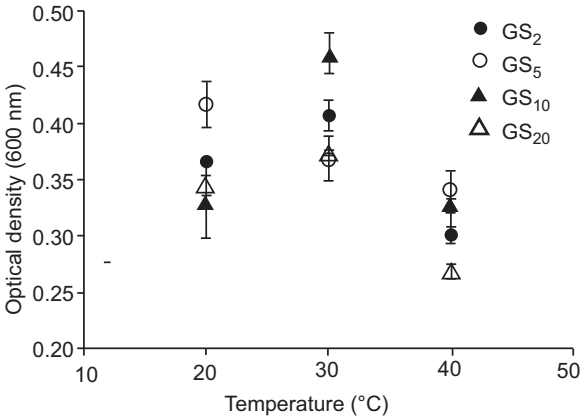

Fig. 2. Growth of Cd-tolerant bacteria as affected by different temperatures at pH 7 (N=3).

At 200 ppm Cd, optical density of GS_2, GS_{10} and GS_{20} showed an increase of 30, 12 and 13%, respectively, over control (0 ppm Cd). However, there was 7% reduction in case of GS_5. The results show that except GS_5 all other bacterial strains have so much adapted to Cd that they have bio-accumulated it to some extent. At 400 ppm Cd, growth of all the bacterial strains was reduced. GS_2 was found more tolerant with 10% reduction in growth and GS_5 least tolerant with 64% reduction. Reduction of growth in case of GS_{10} and GS_{20} was 35 and 55%, respectively, (Fig. 3).

Contrary to other bacterial strains, GS_2 again was found more tolerant at 600 ppm Cd with 33% reduction in growth. The growth of GS_5 at 400 and 600 ppm Cd was almost similar (64% reduction); however there was a sharper decrease in the tolerance indices of GS_{10} and GS_{20} at 600 ppm than at 400 ppm. There was up to 77% decrease in the growth of GS_{10} and GS_{20} at 600 ppm Cd (Fig. 3).

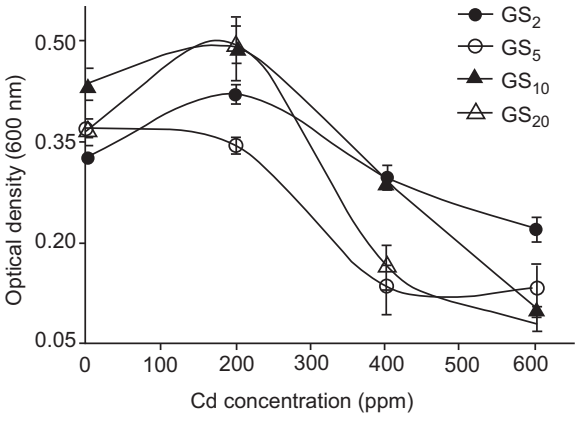

Fig. 3. Growth curve of Cd-tolerant bacteria as affected by different concentration of Cd at pH and temperature 30 °C (N=3).

Cadmium tolerance indices of all tested bacterial strains at different Cd concentrations are presented in Fig. 4. GS_2 being more tolerant among all strains showed highest tolerant index at highest concentration of 600 Cd. Tolerant indices of other bacterial strains revealed the order of tolerance as; $GS_5 > GS_{10} > GS_{20}$.

Findings of other researchers also revealed that, Cd has inhibitory effect on bacterial growth (Kalantri, 2008; Laddaga and Silver, 1985). Higher Cd concentration can reduce the activities of essential enzymes such as protease, urease, arylsulphatase and alkaline phosphatase (Lorenz *et al.,* 2006).

Root length promotion. The effects of 4 Cd-resistant bacterial strains on root elongation of maize variety ISD-gold in the absence of Cd is shown in Table 3. Addition of 50 mg/L Cd to the filter paper culture inhibited root elongation of un-inoculated seedlings by 33%. Inoculations with Cd-resistant bacteria in the absence and presence of Cd significantly increased the root length of maize seedlings over un-inoculated seedlings. The maximum root length promoting effect on Cd-treated plants was observed after inoculation with strains GS_2 (150% over control). It was followed by GS_{10} and GS_{20}; both produced 130% increase over control. The minimum increase in root length where seeds were treated with Cd was observed after inoculation with strains GS_5 (78%). Statistically similar trend was observed where seeds were inoculated in the absence of Cd.

Sheng and Xia (2006) and Belimove *et al*. (2005) also observed root growth promotion of Indian mustard inoculated with Cd resistant bacteria over un-inoculated seedlings in the presence of Cd. Rhizobacteria belonging

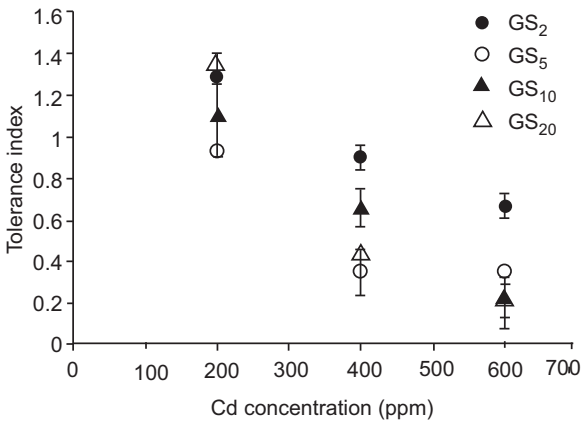

Fig. 4. Tolerance index of Cd-tolerant bacteria as affected by different concentration of Cd (N=3).

Table 3. Root length of maize seedlings inoculated with Cd-resistant bacterial strains grown in absence or presence of Cd in nutrient solution (N=3)

Bacterial strains	Untreated seedlings		Treated with 50 mg/L Cd	
	Root length (mm)	Bacterial effect (%)	Root length (mm)	Bacterial effect (%)
Uninoculated control	33.0[e]	-	22.4[d]	-
GS_2	92.1[a]	+176.6	56.0[a]	+150.0
GS_5	54.5[d]	+ 63.7	40.0[c]	+78.6
GS_{10}	63.0[b]	+ 89.2	51.5[b]	+129.9
GS_{20}	59.8[c]	+ 79.6	51.5[b]	+129.9
LSD	3.3	-	2.7	-

to different genera such as *Pseudomonas*, *Mycobacterium*, *Agro-bacterium* and *Arthrobacter* were found to have plant growth-promoting characteristics that can potentially support heavy metal uptake and reduce stress symptoms in plants (Dell'Amico *et al*., 2005). He *et al*., (2009) observed an increase in root growth and Cd contents in above ground tissues of hyperaccumulator tomato grown in Cd conta-minated soil when inoculated with two metal-resistant bacteria; *Pseudomonas* sp. and *Bacillus* sp. Both the bacteria were indole acetic acid and aminocyclopropane-1-carboxylate deaminase producers. Thus proliferation of root growth in metal contaminated soil either by presence of indole acetic acid or aminocyclopropane-1-carboxylate deaminase enzyme could lead to enhanced uptake of heavy metals in hyperaccumulator plants which could help in bioremediation.

Conclusion

Bacteria isolated from heavy metal-contaminated peri urban areas of Gujranwala have the ability to tolerate higher concentrations of Cd. Cadmium resistance potential and root growth promoting activity of these isolates demonstrated that, these bacteria could be used as a potential candidate in the bioremediation of Cd contaminated wastewater and soil.

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Antibacterial Activities of Aqueous Extracts of *Terminalia catappa*, *Momordica charantia* and *Acalypha wilkesiana* on *Escherichia coli* Isolated from Pediatrics

Ajenifuja Oluwafemi Adeyemi*, Ajibade Victor Adeyinka and Fajilade Temilade Olawande
Department of Science Technology, Microbiology Unit,
Federal Polytechnic, P.M.B. 5351, Ado-Ekiti, Ekiti State, Nigeria

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Abstract: Antibacterial activity of aqueous extract of *Terminalia catappa, Momordica charantia* and *Acalypha wilkesiana* was investigated against *Escherichia coli* isolated from pediatrics with the minimum inhibitory concentration (MIC) of 0.5mg/mL by agar dilution technique. The antibacterial potency of the extracts as evaluated by broth dilution technique, showed diameter of inhibition zone of 22.80 mm, 14.20 mm and 21.00 mm at a concentration of 0.5 mg/mL for *T. catappa, M. charantia* and *A. wilkesiana,* respectively. The antibacterial effect of *T. catappa* was found to be more pronounced with its plausible use for the treatment of infections caused by *E. coli.*

Keywords: *Acalypha wilkesiana, Escherichia coli, Momordica charantia, Terminalia catappa,* pediatrics.

Introduction

The use of plants for therapeutic purposes in Yoruba land in Nigeria dates back to centuries where they first applied the use of plant parts in the cure of different ailments (Sofowora, 1993). Presently, use of modern medicines as antimicrobial agents led to the loss of eminence in the use of perceived healing plants of traditional use which still remains dominant in health care of developing countries especially in rural areas. In Nigerian ethno-medicine extract of different parts of one plant such as stems, leaves, barks and roots are still used for the treatment of a variety of diseases.

The healthcare delivery of the larger proportion of the rural communities in Nigeria, and most part of Africa, today hinge to a large extent on medicinal plants based on traditional health care delivery system and there is a need to identify natural products that could give potent therapy at low or no cost at all. Even today, as many as 80% of the world's population depend on traditional medicines for their primary health care needs (WHO, 2002). The role of plants in health care delivery is even more prominent among rural parts of Nigeria (Osho *et al.,* 2007), and with the relevance of plants in health care of humans, various government and nongovernmental organisations are supporting the development of traditional medicines (Briskin, 2000).

Infectious diseases are one of major health problems in Nigeria, which includes common infectious diseases such as diarrhoea caused by *Escherichia coli.* Limited access to modern drugs has driven the rural Nigerian to rely on medicinal plants including the uses of *Terminalia catappa, Acalypha wilkesiana* and *Momordica charantia.*

T. catappa is commonly called tropical almond in Nigeria (Christian and Ukhun, 2006), and leaves, bark and fruit has been traditionally prepared to treat dysentery, rheumatism, cough and asthma. The fruit is also helpful in the treatment of leprosy and headache and the leaves are specifically used in getting rid of intestinal parasites, treatment of eye problems, wounds, and liver problems, and also for treatment of antifungal infections (Irobi and Adedayo, 1999).

A. wilkesiana is locally named as copper leaf or firedragon, and its ointment is used to treat fungal skin diseases. A previous study revealed that this ointment successfully controlled the mycoses in 73.3% of 32 affected patients (Oyelami *et al*., 2003). It was very effective in treating *Pityriasis versicolor, Tinea pedia* and *Candida intetrigo,* with 100% cure and useful in superficial mycoses (Akinyemi *et al*., 2005).

M. charantia (locally named as bitter melon or ejirin) has been used for a variety of ailments in Nigeria, particularly stomach complaints. Bitter melon (*M. charantia*) is generally, consumed either cooked in the green or early yellowish stage. The young shoots and leaves of the bitter melon may also be eaten as greens (Sofowora, 1993). *M. charantia* seeds possess antimicrobial activity (Braca *et al.,* 2008), antispermatogenic

*Authour for correspondence; E-mail:joseyajenifuja@yahoo.com

activity and androgenic activity (Naseem *et al.,* 1998). They are also used in reproductive health as an abortifacient, birth control agent or to treat painful menstruation and to facilitate child birth (Belion *et al.,* 2005).

E. coli is commonly present in the gastro-intestinal flora of most vertebrates, including humans, and mostly nonpathogenic. Most *E. coli* strains fall into 4 main phylogenetic groups, designated A, B_1, B_2 and D (Arpin *et al*., 2007) with extra intestinal infections derived predominantly from group B_2 and, to a lesser extent, group D whereas, group A and B_1 strains are largely devoid of virulence determinants (Johnson *et al*., 2009). Although strains harboring a robust extra-intestinal virulence factors repertoire cluster predominantly in groups B_2 and D, isolates within each phylogenetic group can be further classified as extra-intestinal pathogenic *E. coli* (EXPEC) or non-EXPEC depending on whether specific virulence traits are present (Johnson *et al*., 2009; Calbo *et al*., 2005).

The aim of this study is to determine the antibacterial potency of aqueous extracts of *A. wilkesiana*, *M. charantia* and *T. catappa* against *E. coli*.

Materials and Methods

Collection, identification and processing of plants. Young leaves of *T. catappa, A. wilkesiana* and *M. charantia* were collected from farmlands at Ado Ekiti, Nigeria. The plant samples were identified at the Department of Science Technology, Federal Polytechnic Ado Ekiti, Nigeria and a voucher specimen was kept in the laboratory No: Med Plant 2011/098. The method described by Osho *et al.* (2007) for extraction of plants active components was used. Samples were air-dried at room temperature of (26 °C ± 1 °C) and milled using a Thomas Willey milling machine. 100 g of the milled samples was soaked with 200 mL of distilled water. The aqueous extract was filtered and evaporated to dryness at 20 °C using a rotary evaporator.

Isolation and identification of *E. coli.* Strains of *E. coli* were isolated from stool samples of pediatrics between 9 months and 2 years of age that were referred to the laboratory of the University Teaching Hospital, Ado- Ekiti, Nigeria. The bacteria were identified using conventional methods and were maintained on nutrient agar slants at 4 °C in the refrigerator until required.

Extraction of bioactive components from the plant materials. Extraction method described by Ajibade and Famurewa (2011) was employed. Fifty grams (50 g) of the powdered plant materials *(T. catappa, A. wilkesiana* and *M. charantia)* were poured into different beakers and 500 mL of distilled water was poured into each beaker, respectively and were boiled on electric cooker at 100 °C. The contents were stirred using a sterile glass rod and allowed to stand for 72 h at room temperature (25 °C ± 1). The contents were filtered through a filter paper (Whatman No. 1) and the filtrate concentrated and evaporated using water-bath at the temperature of +95 °C. Extracts were then kept at 20 °C prior use.

Reactivation of organism. The bacterium was re-suspended in 20 test tubes containing nutrient broth and these test tubes were incubated at 37 °C for 18 - 20 h.

Determination of minimum inhibitory concentration (MIC). This was carried out using the agar dilution method previously described by Odelola and Okorosobo (1996). A colony from stock was sub-cultured into 5 mL of nutrient broth and incubated at 37 °C for 18 h. 0.1mL of the overnight broth of each organism was pipetted into 9.9 mL of the broth to yield a 10^1 dilution. The procedure was continued to obtain a final dilution of 10^3 (Smith *et al*., 2000). Streak of bacterial strains A (2 cm) were made on an oven-dried nutrient agar plates containing increasing concentrations (0.5–2.5 mg/mL) of the extracts. The lowest concentration that gave no visible growth after overnight incubation at 37 °C was taken as the minimum inhibitory concentration (MIC) of each extract.

Determination of the degree of antibacterial potency. The disk diffusion method described by Brady and Katz (1990) was employed. Various concentrations of the extracts were prepared in test tubes (2.5 mg/mL – 0.5mg/mL). Disks obtained from Whatman No. 1 filter paper were sterilised in an oven at 160 °C for 30 min. and soaked in the extracts for 24 h. A loopful of the final dilution (10^3) of the test bacterial suspension was spread on an oven-dried nutrient agar. The disks of different concentrations of the extracts were placed at equidistance on the agar and incubated at 37 °C for 24 h. Zones of inhibition were measured in millimeters (mm) with a meter rule. Whatman No. 1 filter paper disks were placed at the centre of each agar plate as a control.

Phytochemical analysis. *Determination of saponins*. Separately, plant extract (0.5 g) was shaken with distilled water (10 mL) in a test tube and frothing which persisted on warming was taken as evidence for the presence of saponins.

Determination of tannins. Plant extract (5 g) was stirred with 100 mL of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue-black green precipitate indicated the presence of tannins.

Determination of alkaloids. Plant extract (0.5 g) was diluted with acid alcohol (10 mL), boiled and filtered. Diluted ammonia was added (2 mL) to the filtrate (5 mL). Five milliliter of chloroform (5 mL) was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with acetic acid (10 mL). This was divided into two portions. Meryer's reagent (5 mL) was added to one portion and Draggendorff's reagent (5 mL) to the other. The formation of a cream (with Meryer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was taken as positive for the presence of alkaloid.

Determination of steroids. Plant extract (0.1 g) was dissolved in chloroform (2 mL) and sulphuric acid (2 mL) was carefully added to form a lower layer. A reddish brown color at the interphase was indicative of the presence of steroidal ring.

Determination of flavonoids. Powdered sample (2 g) was mixed with acetone (50 mL). The sample was placed on a hot water bath for all traces of acetone to evaporate. Boiling distilled water was added to the sample mixed with acetone. The mixture was filtered while hot. The filtrate was cooled and sodium hydroxide (20% 5 mL) was added to equal volume of the filtrate. A yellow solution indicated the presence of flavonoids.

Determination of terpenoids. This was done using Sakowski test as described by Sofowora (1993). Extract (5 mL) was mixed with chloroform (2 mL) and concentrated H_2SO_4 (3 mL) was added to form a layer. 2:4 formation of a reddish brown colouration at the interphase indicated the presence of terpenoids.

Statistical Analysis. Statistical analysis of P-value was calculated by using Fisher exact test; a test of comparison of 0.50 – 2.50 mg/mL between plant extracts (*T. catappa* and *A. wilkesiana*) was done. Variables with ≥ 1.0 diameter of zones of inhibition and a P-value of ≤ 0.10 in univariate analysis were subsequently analysed in a multivariate model.

Results and Discussion

The susceptibility of different concentrations of the extracts on test microorganism is shown in Table 1. The isolates were susceptible to *T. catappa, M.charantia* and *A. wilkesiana* with *T. catappa* showing the highest potency ranging from the diameter of inhibition of 22.80 ± 0.13 – 27.70 ± 0.20 mm at concentration ranging from 0.5 – 2.50 mg/mL, respectively. Susceptibility was not highly pronounced with *M. charantia* as seen in *T. catappa* and *A. wilkesiana* except at a higher concentration of 2.50 mg/mL. The result showed that *T. catappa* and *A. wilkesiana* have more efficacies in the treatment of both diarrhoea from the diameter of zone of inhibition observed against *E. coli.* This is an indication that their extract could be useful in the therapy of diarrhoea. The potency showed by *T. catappa* is highly significant P ≥ 1.80, 1.20 and 1.30 at the concentration of 0.50 mg/mL, 1.00 mg/mL and 2.00 mg/mL, respectively than other extracts. These showed the relevance of the plant extract (*T. catappa*) compared to other plant extracts. The P-values of only the plant extracts with the highest zones of inhibition were compared, that is, *T. catappa* and *A. wilkesiana*. These plants extracts were more potent against the test organism (*E. coli*).

The qualitative chemical analysis (Table 2) showed that *T. catappa* and *A. wilkesiana* contain saponin and flavonoid while *M. charantia* and *A. wilkesiana*, steroid is also present in *M. charantia*. However, all the plant extracts contained flavonoid. The quantity of the phytochemicals e.g., saponin was higher in *A. wilkesiana* (12.85%); tannin (7.14%) and flavonoid (10.6%) (Table 3).

Minimum inhibitory concentration (MIC) of the extracts on *E. coli* has been presented in Table 4. *T. catappa* having the MIC of 22.80 ± 0.13 mg/mL at the concentration of 0.50 mg/mL; *M. charantia* having the lowest MIC of 16.00 ± 0.92 mg/mL at the concentration of 1.00 mg/mL; and *A. wilkesiana* having the MIC of 21.00 ± 0.01 mg/mL at the concentration of 0.50 g/mL.

The bioactive compounds responsible for the inhibitory effects of the leaf extracts were detected in its phytochemical screening, some of which were reported

Table 1: Antibacterial activity of aqueous extracts of *T. catappa, M. charantia* and *A. wilkesiana* on *E. coli.*

Conc. (mg/mL)	Plant extracts Zones of inhibition (mm)			
	T. catappa	*M. charantia*	*A. wilkesiana*	P-value
0.50	22.80 ± 0.13	14.20 ± 0.58	21.00 ± 0.01	1.80
1.00	24.30 ± 0.30	16.00 ± 0.92	23.10 ± 0.09	1.20
1.50	26.50 ± 0.58	18.10 ± 0.29	25.40 ± 0.40	1.10
2.00	27.30 ± 0.08	19.80 ± 0.14	26.00 ± 0.50	1.30
2.50	27.70 ± 0.20	21.50 ± 0.26	27.60 ± 0.18	0.10

Table 2. Phytochemical (qualitative) analysis of aqueous extracts of *T. catappa, M. charantia* and *A. wilkesiana.*

Bioactive constituent	Plant extracts		
	T. catappa	*M. charantia*	*A. wilkesiana*
Saponin	+	–	+
Tannin	–	+	+
Alkaloid	–	–	+
Steroid	–	+	–
Flavonoid	+	+	+
Terpenoid	–	–	–

+ = present; – = not present

Table 3. Phytochemical (quantitative) analysis of aqueous extracts of *T. catappa, M. charantia* and *A. wilkesiana.*

Bioactive constituent	Plant extracts % composition		
	T. catappa	*M. charantia*	*A. wilkesiana*
Saponin	2.24	–	12.85
Tannin	–	5.6	7.14
Alkaloid	–	–	0.36
Steroid	–	0.47	–
Flavonoid	9.32	7.15	10.6
Terpenoid	–	–	–

– = not present

Table 4. Minimum inhibitory concentration (MIC) of the extracts on *E. coli.*

Plant extracts	Minimum inhibitory concentration (MIC) (mg/mL)
Terminalia catappa	22.80 ± 0.13
Momordica charantia	16.00 ± 0.92
Acalypha wilkesiana	21.00 ± 0.01

in literature as antimicrobial constituents (Oluduro *et al*., 2011). The qualitative and quantitative analysis of the leaves of *T. catappa*, *M. charantia* and *A. wilkesiana* revealed that they contain flavonoid, saponin and tannin in varying proportions with traces of steroid and alkaloid, while terpenoid was absent. The antimicrobial activities observed in this study may be attributed to the presence of these phytochemicals in the leaves (Table 3). Plants such as *Phyllanthus niruri*, *Acalypha hispida*, and *Mormodica charantia* that are rich in a wide variety of secondary metabolites have been found *in vitro* to have antimicrobial properties (Ajibade and Famurewa, 2011; Oluduro *et al*., 2011).

Herbal medicines in developing countries are commonly used for the traditional treatment of health problems (Martinez *et al*., 1996). In recent years multiple drug resistance in human pathogenic microorganisms have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Service, 1995). In addition to this problem, antibiotics are sometimes associated with adverse effects on host including hypersensitivity, immune suppression and allergic reactions (Ahmad *et al*., 1998).

Evaluation of these plants in rural areas of Nigeria is more urgent than ever. Thus, ethnobotanical studies of Africa could provide inputs with the isolation of new phytochemicals and their pharmacological studies. Therefore, scientific documentations of plants with effective use against certain microorganism could lead to the sustainable cultivation of plant resources for the small-scale production of raw phytotherapeuticals. and new findings will help to develop alternative antimicrobial medicines for the treatment of infections using plants (Dulger and Gonuz, 2004).

Conclusion

The results of the present study signify the potentiality of *T. catappa* leaf as a source of therapeutic agent which is encouraging in the ongoing search for antimicrobial botanicals. Thus, there is a need for a continuous search for new effective and affordable antimicrobial drugs.

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Nutrients Dynamics of Co-composting Poultry Litter with Fast Food Wastes

Asim Hayat[a]*, Arshad Nawaz Chaudhary[b], Fayyaz Hussain[a], Tanveer Iqbal[b], Vishandas Suthar[a], Sana Ullah Jalil[c] and Zeshan Ali[d]

[a]Land Resource Research Institute, National Agricultural Research Centre (NARC), Park Road, Islamabad, Pakistan
[b]Department of Soil Science & SWC, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
[c]Rice Research Programme, National Agricultural Research Centre (NARC), Park Road, Islamabad, Pakistan
[d]Ecotoxicology Research Institute, National Agricultural Research Center (NARC), Park Road, Islamabad, Pakistan

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Abstract. Co-composting of poultry litter (PL) and fast food waste (FFW) in different combinations was carried out to explore the nutrient dynamics. The PL and FFW were co-composted in pits of dimensions 2 m×2 m×1.5 m (L×W×D) in ratios of 100:0, 75:25, 50:50, 25:75 and 0:100, respectively, for a period of 105 days. Co-composts of PL and FFW in a 50:50 ratio yielded highest total nitrogen (3.63%), total phosphorus (0.81%), and total potassium (3.40%) levels in the mature compost after 105 days of composting period. Carbon to nitrogen ratio for this combination was 18.33, which is suitable for safe land application. Present study identified PL and FFW co-composting in equal proportions yields maximum N, P and K levels with suitable C:N ratio which may be applied to soils to meet crop nutrient demands and enhanced agricultural productivity.

Keywords: co-composting, poultry litter, fast food waste, total nitrogen, total phosphorus, C:N ratio

Introduction

Agriculture sector in Pakistan is facing an ever increasing pressure to meet food and fibre requirements of rapidly growing population, which currently stands at 180 million (Ali *et al*., 2013a). To ensure food security for continuously expanding population, crop productivity has become the ultimate goal of the farming communities. To achieve desired crop production, application of municipal/industrial effluents, sewage sludge, municipal solid wastes and excess pesticides/fertilisers usage in agricultural soils has become a custom resulting in considerable deterioration of the soil ecosystems (Ali *et al*., 2013b). All these practices are believed to supply essential nutrients for plant growth. However deleterious effects to human and livestock health remain a pressing concern.

Composting of different agricultural and municipal wastes to supply nutrients to growing crops has a long scientific prowess linked with significant agricultural production (Iyengar and Bhave, 2006). Composting is increasingly considered a good way for recycling the surplus manure as a stabilised and sanitized end-product for agriculture (Chaudhry *et al*., 2013; Khan *et al*., 2003). The advantages of composted organic wastes to soil structure, fertility as well as plant growth have been increasingly accentuated in recent literature (Goyal *et al*., 2005; Esse *et al*., 2001). Addition of un-decomposed wastes or non-stabilised compost to agricultural land may lead to immobilisation of plant nutrients and cause phytotoxicity (Cambardella *et al*., 2003). Moreover, the waste physico-chemical characteristics may not always be appropriate for composting. For instance, high moisture contents in food waste, inappropriate C:N ratio, imbalanced amount of plant nutrients, pathogens and foul smelling odours may result in long treatment time or low degradation efficiency (Chaudhry *et al*., 2013). Co-composting of different types of organic products together overcomes the drawbacks of composting a single material (Goyal *et al*., 2005). Co-composting is extensively practiced method for solid waste management, which recovers organic matter from organic wastes (Castaldi *et al*., 2008).

Different agricultural/non-agricultural wastes are generated in excess in rural and urban communities of Pakistan which can be harvested by the composting process to ensure sustainable nutrient supply to growing plants. In current times enormous generation of poultry litter (agricultural waste) and fast food wastes (municipal waste) has caused serious environmental issues in Pakistan. According to Economic Survey of Pakistan (ESP, 2010), poultry sector is growing at the rate of 15-20% per annum, which generates poultry litter in

*Author for correspondence; E-mail: asimsatti94@gmail.com

voluminous amounts. According to Chaudhry *et al*. (2013) and Khan *et al*. (2003) poultry litter is being used as fertiliser by the farmers and is considered a better organic fertiliser than the farmyard manure. On another side, the number of hotels, motels and fast food restaurants has increased many folds in the last decades. These hotels and fast food restaurants are producing tonnes of solid food waste every year, lacking proper disposal and presenting a great challenge to the scientific community.

Present research was therefore, carried out to investigate the co-composting of poultry litter with fast food wastes in different combinations to convert these biological wastes (poultry litter and fast food wastes) into useful nutrient rich composts for supplementing plant growth. Co-composting of poultry litter and fast food wastes is an economical and environment friendly use of these biological wastes (Ranalli *et al*., 2001). Co-composting offers safe disposal coupled with optimal nutrient supply to the growing plants via mature composts. Present research also describes nutrient output in detail in different combinations and best suitable combination that gives better performance at the end of the composting process. Findings of this study will help farmers and researchers in efficient exploitation of the selected wastes for plant production.

Materials and Methods

Composting process, sampling and preparation. Present experiment was carried out at Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan. Co-composting of poultry litter with fast food wastes was carried out in the following five combinations; T_1=poultry litter+fast food waste (100:0), T_2=poultry litter+fast food waste (75:25), T_3=poultry litter+fast food waste (50:50), T_4=poultry litter+fast food waste (25:75) and T_5=poultry litter+fast food waste (0:100). Poultry litter used was in pure guano form. Both PL and FFW were collected from the nearest locations in Rawalpindi city.

Composting was carried out in pits having dimensions of 2×2×1.5 meter (L×W×D) for 105 days. Raw poultry litter and fast food waste was placed in pits for composting under natural conditions. To maintain aerobic conditions, composting material was thoroughly mixed after every 15 days. Approximately 60-70% of the moisture content was sustained in the composting pits to support composting processes optimally. Compost sample collection for nutrient analyses was done at an interval of 15 days i.e. l0, 15, 30, 45, 60, 75, 90 and 105 days (Chaudhry *et al*., 2013; Castaldi *et al*., 2008). Three random subsamples were collected from each pit (top, center and bottom) making a composite sample of 1 kg for physical and chemical characterisation of the compost. Samples were dried at 65 °C in hot air oven for 48 h followed by grinding and passing through 1 mm sieve. Processed samples were stored in labeled plastic bottles at room temperature until further analyses.

Physical and chemical analyses of compost. Total nitrogen in the compost samples was determined by Kjeldahl method (Bremner, 1996). For phosphorus and potassium compost samples were digested using the perchloric acid-nitric acid digestion mixture (Kuo, 1996). Phosphorus in the acid digests was analysed on spectrophotometer at a wavelength of 880 nm whereas, potassium was analysed on flame photometer. Total organic carbon content was determined using $K_2Cr_2O_7$ as an oxidizing agent (Nelson and Sommer, 1982). Temperature and moisture contents were recorded after every 15 days interval. Temperature was randomly recorded from middle and bottom locations of the composting pits and averaged to get a mean temperature value.

Statistical analysis. Analysis of variance (ANOVA) was performed for the studied parameters using Statistix 8.1 and the means were compared using LSD tests at 5% probability level.

Results and Discussion

Temperature changes. Change in temperature at various stages of decomposition of poultry litter with fast food wastes is shown in Fig 1. During composting, the internal temperature of the compost pits remained below 50 °C for period of 45 days that increased to 60 °C at 60-75 days of composting in all treatments. After attaining peak temperature of 60 °C, it began to decline to ambient level (35 °C) in all the treatments. Different combinations of PL and FFW co-composts showed very small difference in temperature at the beginning and at the end of the experiment except 50:50 ratio of PL and FFW co-composts in which temperature was 6-16 °C higher than other treatments. Increase in temperature can be attributed to elevated microbial activity at the thermophilic stage (Chaudhry *et al*., 2013; Zhang *et al*., 2003). This increased temperature is also responsible for killing pathogens in the composting material which otherwise can be harmful for soil and plant health (Chaudhry *et al*., 2013).

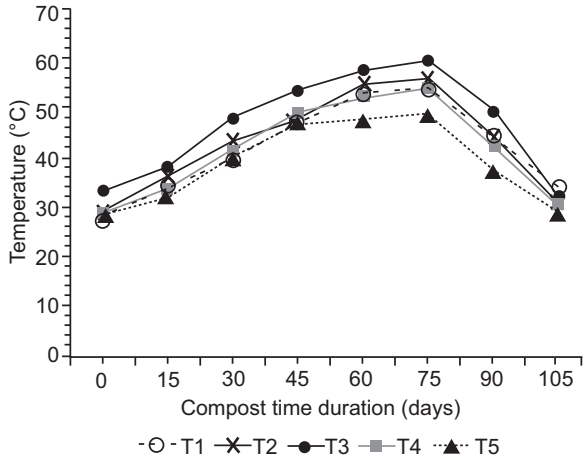

Fig. 1. Comparative effect of co-composting of poultry litter and fast food waste on temperature.

Carbon-nitrogen ratio (C:N). Data regarding carbon to nitrogen (C:N) ratio is shown in Table 1. The C:N ratio declined with the passage of time in all treatments due to the decomposition of carbohydrates which is a rich source of carbon. The availability of nitrogen also decreased with the increase in decomposition rate (Chaudhry *et al*., 2013; Adhikari *et al*., 2008; Benito *et al*., 2006). At the start of composting process the highest C:N ratio (39.81) was observed in the treatment T_5 and lowest was observed in T_1 (25.36). In remaining treatments, at the initial stages, high C:N ratios were observed, alike to T_5. Maximum decreasing trend of C:N ratio i.e. from 38.78 to 18.33 was observed in the T_3. Minimum C:N ratio (14.76) was observed in the treatment T_1. Decrease in C:N ratio with increasing composting time is also supported by the results of Benito *et al*. (2006). Carbon to nitrogen ratio ranging from 12 to 20 is considered suitable for soil fertility and plant growth. From these results it can be inferred that the treatment T_3 gives promising decline in the C:N ratio and proves to be the best combination investigated in this study for better crop production.

Nitrogen concentration increased due to the loss of dry weight as carbon dioxide and water during the mineralisation of organic matter. This correlates with the previous investigations of Alburquerque *et al*. (2006) regarding composting experiments. They concluded that due to the variation in carbon and nitrogen levels, the C:N ratio decreased during the composting process. They achieved a final C:N ratio of 14 at compost maturity.

Total nitrogen concentration. The data regarding nitrogen concentration in the co-composting process is depicted in Table 2. It was observed that the total nitrogen concentration increased with the composting time. Maximum nitrogen concentrations were recorded at 105 day in all the treatments. Maximum nitrogen concentration (3.38%) was observed in treatment T_3 having 50:50 percent poultry litter and fast food restaurant waste, however, minimum concentration (1.70%) was recorded in treatment T_5. High nitrogen levels in T_3 can be attributed to high mineralisation rates of composting material via microbial decomposition process whereas, lower N levels in T_5 were due to slower microbial decomposition rate. In FFW initial carbon contents are generally higher however, initial N content were lower (Chang *et al*., 2006). These results are in accordance with the Rodriguez *et al*. (2003) who investigated co-composting of barley wastes and solid poultry waste revealing 3.56% N concentration at compost maturity. The results for low nitrogen concentrations in the treatment T_5 with 100% fast food restaurant waste were similar with those reported by Chang *et al*. (2006) and Zhang *et al*. (2003).

Total phosphorus concentration. The data pertaining to concentration of total phosphorus is illustrated in

Table 1. Comparative effect of co-composting of poultry litter (PL) and fast food waste (FFW) on C:N ratio

Treatment	C:N ratio (days)								Average
(PL:FFW ratio)	0	15	30	45	60	75	90	105	
~~T_1 (100:0)~~	~~25.36~~	~~25.13~~	~~20.35~~	~~16.95~~	~~16.10~~	~~15.26~~	~~15.34~~	~~14.76~~	~~18.66^{e}~~
T_2 (75:25)	35.44	34.15	25.59	26.05	19.73	17.76	17.15	16.47	24.04^{d}
T_3 (50:50)	38.78	35.61	28.42	27.84	19.42	18.96	18.67	18.33	25.75^{c}
T_4 (25:75)	36.31	33.61	29.89	25.65	24.42	23.60	22.63	22.48	27.32^{b}
T_5 (0:100)	39.81	37.21	36.57	30.84	27.61	27.29	26.43	26.38	31.52^{a}
Days avg.	35.14^{a}	33.146^{b}	28.16^{c}	25.47^{d}	21.47^{e}	20.57^{ef}	20.04^{f}	19.68^{f}	-

LSD value (p = 0.05): day*treatment = 2.741; treatment = 0.969; day = 1.226.

Table 2. Comparative effect of co-composting of PL and FFW on concentration of total nitrogen (%)

Treatment	Total N concentration (days)								Average
(PL:FFW ratio)	0	15	30	45	60	75	90	105	
T_1 (100:0)	3.22	3.26	3.33	3.36	3.36	3.43	3.45	3.46	3.37^{a}
T_2 (75:25)	2.83	2.84	2.96	3.23	3.22	3.27	3.37	3.38	3.167^{b}
T_3 (50:50)	3.23	3.26	3.27	3.34	3.42	3.46	3.47	3.63	3.38^{a}
T_4 (25:75)	2.72	2.73	2.76	2.82	2.85	2.92	2.94	2.95	2.84^{c}
T_5 (0:100)	1.42	1.46	1.51	1.53	1.56	1.61	1.67	1.70	1.56^{d}
Day avg.	2.68^{c}	2.71^{c}	2.77^{bc}	2.86^{abc}	2.88^{abc}	2.94^{ab}	2.98^{a}	3.02^{a}	

LSD value (p = 0.05): day*treatment = 0.449; treatment = 0.159; day = 0.201.

Table 3. Overall results reflected that total phosphorus increased linearly from 0 to 105 days, respectively. Lowest P levels were recorded in all treatments at the start of composting process. Maximum total phosphorus concentration (0.81%) was recorded in treatment T_3 however, minimum concentration (0.51%) was observed in treatment T_5. Rodriguez *et al*. (2000) also reported an increase in total P level from 0.98% to 1.96% while, composting barley waste with solid poultry manure. Increase in the P concentration is also recorded from the vermi-composting of poultry manure (Kwansod, 2003). Cooperband *et al*. (1996) also consistently reported maximum P concentration in mature compost while co-composting poultry litter with different wastes.

Total potassium concentration. The results pertaining to total potassium in co-composting of poultry litter and fast food waste is depicted in Table 4. The results revealed that all the treatments differed significantly from one another with the increasing days of composting. Increase in the potassium concentration was observed from 0 to 105 days of co-composting process. On 105th day, maximum concentration of total K (3.4%) was found in T_3 whereas minimum was found in T_5 (1.67%). Maximum concentration of total K in T_3 was due to higher microbial activity of composting material. Lowest concentration of total K in T_5 was due to presence of high carbohydrate and low nutrient levels. Chaudhry *et al*. (2013) also reported increase in the K concentration

Table 3. Comparative effect of co-composting of PL and FFW on concentration of total phosphorus (%)

Treatment	Total P concentration (days)								Average
(PL:FFW ratio)	0	15	30	45	60	75	90	105	
T_1 (100:0)	0.61	0.62	0.63	0.67	0.67	0.67	0.68	0.70	0.66^{b}
T_2 (75:25)	0.51	0.55	0.60	0.67	0.70	0.71	0.72	0.73	0.65^{b}
T_3 (50:50)	0.55	0.58	0.65	0.66	0.73	0.76	0.78	0.81	0.69^{a}
T_4 (25:75)	0.43	0.48	0.54	0.52	0.57	0.63	0.65	0.70	0.56^{c}
T_5 (0:100)	0.37	0.39	0.42	0.43	0.48	0.47	0.50	0.51	0.45^{d}
Day avg.	0.49^{d}	0.52^{d}	0.57^{c}	0.59^{c}	0.63^{b}	0.65^{b}	0.67^{ab}	0.69^{a}	

LSD value (p = 0.05): day*treatment = 0.086; treatment = 0.030; day = 0.0385.

Table 4. Comparative effect of co-composting of PL and FFW on concentration of total potassium (%)

Treatment	Total K concentration (days)								Average
(PL:FFW ratio)	0	15	30	45	60	75	90	105	
~~T_1 (100:0)~~	~~2.81~~	~~3.00~~	~~3.12~~	~~3.17~~	~~3.21~~	~~3.26~~	~~3.29~~	~~3.28~~	~~3.41^{a}~~
T_2 (75:25)	2.27	2.37	2.62	2.74	2.79	2.88	2.93	2.96	2.69^{b}
T_3 (50:50)	1.34	1.37	1.85	2.50	2.75	2.90	3.33	3.40	2.43^{c}
T_4 (25:75)	1.16	1.22	2.03	1.72	2.03	2.14	2.20	2.22	1.77^{d}
T_5 (0:100)	0.82	0.86	1.00	1.29	1.42	1.58	1.60	1.67	1.28^{e}
Day avg.	1.68^{f}	1.76^{cf}	2.01^{e}	2.30^{e}	2.44^{c}	2.55^{b}	2.67^{a}	2.71^{a}	

LSD value (p = 0.05): day*treatment = 0.194; treatment = 0.069; day = 0.087.

in the mature composts. Clark (2000) found K levels in similar ranges in food waste composting and associated K increase with the microbial activity.

Conclusion

In the present research, co-composting of poultry litter and fast food waste was carried out in different combinations. Among different ratios of composts prepared and analysed for the nutrient dynamics, maximum concentration of total nitrogen (3.63%), total phosphorus (0.81%), and total potassium (3.40%) were found in the mature compost of T_3 (containing equal proportion of poultry litter and fast food restaurant waste). Carbon to nitrogen ratio (18.33%) for this treatment (T_3) was also suitable to promote better plant growth. Temperature remained effectively high in T_3 (6-16 °C higher as compared to the rest of the treatments) which supported strong microbial activity in this treatment leading to enhanced levels of studied nutrients (N, P, K) and lower C:N ratio. This investigation supports the utilization of co-composting of poultry litter and fast food waste to enhance nutrient concentrations in the mature compost as compared to the other combinations studied. Usage of co-compost resulting from the combination (T_3) can effectively improve soil health, fertility and nutrient availability resulting in better plant growth and avoiding phytotoxic effects.

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Application of Extrusion Technology to Prepare Bread Crumb, A Comparison with Oven Method

Imran Pasha, Muhammad Farhan Jahangir Chughtai*, Arsalan Sarwar, Muhammad Asim Shabbir and Shabbir Ahmed

National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

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Abstract. The current research project was designed to conclude the upshot of extrusion cooking temperature on the properties and acceptability of bread crumb. Bread crumbs were obtained by drying the bread, maintaining moisture up to 3-8% and then broken down using hammer mill or crusher which breaks the bread into bread crumbs. Significantly highest moisture contents 7.26% was observed in oven baked bread crumb as compared to 6.25% in bread crumb prepared by extrusion cooking method. The highest bulk density (28.13 g/100 L) was observed in extruded bread crumb whereas, the oven baked bread crumbs showed lower bulk density (7.03 g/100 L). The fat uptake of extruded and oven baked bread crumbs were found 0.516 mg/g and 0.493 mg/g, respectively. The extruded bread crumb showed higher water binding capacity as 34.76 g H_2O/kg as compared to oven baked bread crumb which showed 27.92 g H_2O/kg. Sensory evaluation of extruded and oven baked bread crumbs depicted that bread crumbs prepared from extrusion cooking methods got significantly higher scores for taste, flavour and over all acceptability as compared to those prepared by oven baked method. As far as crispiness is concerned oven baked bread crumbs got comparatively higher scores. Moreover, it was concluded that the treatment T_2 of extruded bread crumbs got more sensory scores than oven baked bread crumbs.

Keywords: bread crumb, extrusion technology, oven method, sensory evaluation

Introduction

Wheat flour bread is the staple food in many countries (Altamirano-Fortoul *et al*., 2012). Bread plays key role in our balanced food due to the presence of starch and other carbohydrates (Rosell, 2009). Bread is directly consumed and is also used for the production of bread crumb which is bread by-product. Bread crumbs are made by drying bread generally at ambient environments and its purpose is prevention from further gelatinization and breakdown of starch. The dried material is then mildly milled to prevent uninvited destruction of starch granules which is then sieved according to desired particle sizes. Bread crumbs have several applications in food products and used as a main ingredient in processing food products, such as the breading fried food and also used as a coating on confectioneries. Bread crumbs increase the stability of food and therefore, permit industries to supply and produce bread crumbs for fish stick or fish finger and also for other fried food (Shittu *et al*., 2007).

Coatings can even be applied to the food items to create a good seal off against moisture loss. Although by reheating, coatings will reabsorb moisture associated with nutrition and up to desirable content, crispy external outside together with conserving, with saving all natural flavour and with nutritive valuation within the food nutrition (Yu and Augustine, 2002). Bread crumb is a typical component within the food industry as possibly utilized for coating or filling of stuffed pasta. It may be used in dried layer to produce a good exterior coating more than various food formulations. The quantity of bread crumb may vary and signify as much as 40% from the filling (personal likeness), based on the specific formula of the final item resulting in high quality as well as have high features. Primarily, the actual assimilation is associated with absorption of water as well as fat elements during cooking food by the crumbs and also contribute final volume of the filling. Therefore, it is cheaper and economical as compared to costly elements, for example cheese, and other raw materials, etc. Definitely, an essential technical role of bread crumbs may be the accomplishment of the practical filling with a commercial forming as well as filling device (Pajak *et al*., 2012).

Appealing textural qualities associated with bread tends to be mainly based on their specific cellular morphology (porosity, size of air tissue, interconnectivity, thickness of beam, thickness of brown crust area, and so on.),

*Author for correspondence;
E-mail: m.farhan.chughtai@hotmail.com

elements as well as water content. Qualities associated with brown crust area and crumb will vary, brown crust area is dryer, tougher, darker as well as denser and may end up from the crumb depending on these types of requirements (Vanin *et al*., 2009). Clusters of amylose as well as amylopectin made an appearance within the crumb after baking. These clusters had been ghosts of starch granules by which amylose offers focused in the centre as well as amylopectin is situated in the border from the ghosts of starch granules (Hug-Iten *et al*., 1999).

Extrusion cooking food is really a continual cooking, mixing, as well as developing procedure. The extrusion is inexpensive, flexible as well as efficient technologies (Guy, 2001). The raw material go through numerous reactions as well as structural changes throughout extrusion cooking food, for example starch gelatinization, amylose as well as proteins denaturation, retain nutritional vitamins and colours, etc. (Ilo and Berghofer, 1999). Using extrusion cooking food may be manufacturing associated with meals for example breakfast every day cereals, modified starches, fiat bread as well as cheese analogues and infant meals.

Customers usually love the foods having a fried-like flavour as well as consistency (Yu and Augustine, 2002). As a result, the food business offers replied through building items, that on traditional oven reheating lead to food having a fried-like consistency as well as flavour. Breads crumb-like items in many cases are utilised in the food business to improve fried-like consistency associated with food. In the view of these facts, the present project was carried out to determine the outcome of extrusion cooking temperature on the properties and acceptability of bread crumb prepared by oven baking and extrusion cooking method.

Materials and Methods

Materials. Wheat flour and defatted soy flour, baking powder, sugar, salt and emulsifier, yeast used in this project were purchased from the local market.

Preparation of bread crumb by oven. The breads were prepared according to the AACC (2000) straight dough method No. 10-10B. The ingredients were mixed for 5-10 min in a Hobart A-200 Mixer to form dough and allowed to ferment at 30 °C and 75% R.H. for 180 min. First and second punches were made after 120 and 150 min, respectively. The dough was molded and panned into 100 g test pans, and final proofing was done for 45 min at 95 °F (35 °C) and 85% R.H. The bread was baked at 232 °C for 13 min. The bread was dried and the moisture content at the drying step was about 3-8%. The bread was then placed into a hammer mill or crusher which breaks the bread into crumbs. Treatments from T_0 to T_4 contain different moisture contents. Moisture contents from different treatments are presented in Table 1.

Table 1. Treatment plan for oven baked bread crumb (bread crumb will be prepared in oven at 200±20 °C temperature by using following formulation)

Treatment	Flour	Sugar	Salt	Yeast	Emulsifier	Moisture of crumb (%)
	(g)					
T_0	100	3	1	3	5	3.55
T_1	100	3	1	3	5	4.76
T_2	100	3	1	3	5	6.17
T_3	100	3	1	3	5	6.52
T_4	100	3	1	3	5	7.26

Preparation of bread crumb by extrusion. The extruder used in this project was an experimental extruder model SYSLG30.VI co-rotating twin screw equipped with two barrel sections. Extrusion was done at optimised conditions of temperature and pressure and die configuration. The screws were 5.55 cm in diameter and had an overall active length of 50 cm. The extruder was operated at 150 and 155 rpm. A 14.7 KW DC motor was used to drive the extruder. Moisture content of the feed was controlled at 27-30% by injecting water. An adjustable cutter with four blades facing the die was operated at 300 rpm to cut the extrudate as it emerges from the extruder. Extruder temperatures measured by a PC computer were 120 °C for the extruded samples, respectively. Glycerol solution (20%) was injected into the barrel during extrusion in some cases. The extrudates (pellet) were dried in an oven at 100 °C for 35 min (Yu and Augustine, 2002). Formulations of different treatments are presented in Table 2.

Analysis of bread crumbs. The moisture content was determined by oven drying the bread crumb samples at 100 °C to constant weight and loss in weight will be expressed as moisture as described in Method No. 926-08. AACC (2000). The bulk density was calculated by dividing the weight of extrudates by its volume presented by method of Hwang and Hayakawa (1980).

Table 2. Treatment plan for the preparation of extruded bread crumb (bread crumbs were prepared by using following formulation)

Treatment	Temperature (°C)	Flour (%)	Sugar (%)	Salt (%)	Baking powder	Emulsifier (%)	Soy flour (%)
T_0	120	88.7	-	2	4	0.3	5
T_1	120	90.7	-	2	2	0.3	5
T_2	110	85.3	3.4	2	4	0.3	5
T_3	120	85.3	3.4	2	4	0.3	5
T_4	130	85.3	3.4	2	4	0.3	5

Colonna *et al*. (1983) has shown that the value obtained by dividing the cross sectional area of the rod shaped extrudate by the cross sectional area of the diameter of the die is termed as expansion of extrudate. Fat uptake by the bread crumb was measured as described by Yu and Augustine (2002). While for the determination of water binding capacity of bread crumbs, the method devised by Lucisano *et al*. (2010) was adopted.

Sensory evaluation. The extruded and oven baked bread crumb samples were coated on cutlasses and served for sensory evaluations to a panel of judges from the staff and postgraduate students of National Institute of Food Science And Technology (NIFSAT). A 09 point hedonic scale (from 1=extremely dislike to 9=extremely like) was used to determine the preference in flavour, taste, crispiness and overall acceptability according to the procedure described by Lawless and Heymann (1998).

Statistical analysis. The data obtained was analysed statistically as described by Steel *et al*. (1997). The data was analysed by Complete Randomized Design (CRD) and the mean values of replications of different treatments were calculated by LSD at 0.05.

Results and Discussion

This research was aimed to establish the standardised recipe of bread crumb by oven and extrusion methods. The extrusion parameter studied includes extrusion temperature. This is the major factor known that affect extruder performance, product density, expansion ratio, colour, textural and sensory characteristics of extrudates.

Analysis of bread crumb. ***Moisture content.*** An important parameter for consumer acceptability is frying and affected by moisture content of the bread crumbs. According to results, the moisture content of extruded and oven baked bread crumb have highly significant effect. The moisture content of extruded and oven baked bread crumb are given in Table 3 and graphically expressed in Fig. 1. The moisture content of extruded bread crumb varied from 4.12 to 6.25%, while that of oven baked bread crumb, it varied from 3.55-7.26% among different

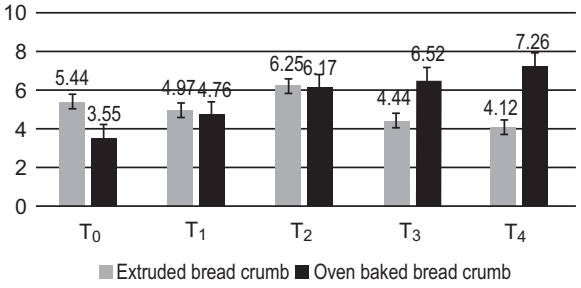

Fig. 1. Moisture content (%) in extruded and oven baked bread crumb.

Table 3. Means table regarding moisture, bulk density, fat uptake and water binding capacity in extruded and oven baked bread crumb

Treatment	Moisture content		Bulk density		Fat uptake		Water binding capacity	
	Extruded bread crumb	Oven baked bread crumb	Extruded bread crumb	Oven baked bread crumb	Extruded bread crumb	Oven baked bread crumb	Extruded bread crumb	Oven baked bread crumb
T_0	5.44 ± 0.34^{b}	3.55 ± 0.15^{d}	6.36 ± 0.44^{c}	27.46 ± 0.98^{ab}	0.423 ± 0.003^{c}	0.403 ± 0.0019^{ab}	32.23 ± 1.1^{c}	27.76 ± 1.6^{a}
T_1	4.97 ± 0.25^{bc}	4.76 ± 0.25^{c}	6.46 ± 0.49^{bc}	26.90 ± 0.74^{abc}	0.403 ± 0.0012^{c}	0.423 ± 0.0015^{b}	30.50 ± 1.9^{bc}	27.63 ± 1.01^{a}
T_2	6.25 ± 0.43^{a}	6.17 ± 0.49^{b}	6.90 ± 0.24^{ab}	28.13 ± 0.81^{a}	0.516 ± 0.0024^{a}	0.476 ± 0.0024^{a}	28.96 ± 0.89^{b}	27.15 ± 1.06^{b}
T_3	4.44 ± 0.22^{cd}	6.52 ± 0.51^{b}	6.66 ± 0.31^{abc}	26.16 ± 0.47^{bc}	0.470 ± 0.0032^{ab}	0.470 ± 0.0033^{ab}	34.53 ± 2.3^{a}	27.92 ± 0.98^{a}
T_4	4.12 ± 0.19^{d}	7.26 ± 0.61^{a}	7.03 ± 0.42^{a}	25.13 ± 0.70^{c}	0.450 ± 0.0021^{bc}	0.493 ± 0.0013^{ab}	34.76 ± 1.9^{a}	27.75 ± 0.77^{a}

treatments. Results showed that the highest moisture content were present in extruded bread crumb in T_2 and in oven baked bread crumb the highest moisture content was found in T_4.

These results were in accordance with study of Yu and Augustine (2002) and Lucisano *et al*. (2010), who proposed that increased or decreased moisture content overall effect the texture and taste of bread crumb. If the moisture content is high then the bread crumb would be denser. The loss of moisture occurs rapidly during frying and the water replace with fat during frying and the products becomes too oily to taste and touch (Yu and Augustine, 2002).

The moisture content is affected as the temperature of barrel is changed. Moisture content (the quantitative determination of total water content) of final product determines the stability and quality of food material as moisture content of final product affects different nutritional as well as organoleptic properties of food and most importantly it determines the texture of product.

In baked products, the softness and tenderness is due to the moisture content and it helps in the chewing, enhancing palatability which is favourable for consumer. If the moisture is more than the required limit then promotes microbial growth and product is prone to spoilage however, much less quantity of final product makes the texture harder and less appealing and greatly affects the sensory properties of product.

Bulk density (g/100 L). Bulk density is defined as the "mass of particle occupied in a unit volume". Bulk density is not only useful descriptor of food texture but also describes the quality of extrudates. Bulk density is not an intrinsic property of a material it can change depending on how the material is handled. Screw speed and temperature during extrusion also affects the bulk density.

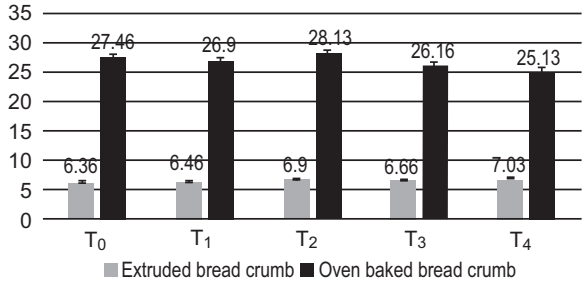

Fig. 2. Bulk density of extruded and oven baked bread crumb (G/100 L).

It is evident from the results that bulk density of extruded and oven baked bread crumb varied significantly (Fig. 2). The bulk density of extruded bread crumb ranged from 6.36 to 7.03 g/100 mL while the bulk density of oven baked bread crumb varied from 25.13 to 28.13 g/100 mL (Table 3). Significantly, the highest bulk density (28.13 g/100 mL) of extruded bread crumbs was observed at lowest screw speed and at high moisture content while the highest bulk density (7.03 g/100 mL) of oven baked bread crumbs was observed in T_4. Bulk density is affected by the change of moisture content of the extruded and oven bread crumb which also affect the taste of bread crumb. The results are in accordance with findings of Lucisano *et al*. (2010) and Yu and Augustine (2002).

There was a specific interaction, between barrel temperature and expansion ratio keeping moisture content and screw speed constant. Overall expansion increased linearly with increasing temperature and screw speed up to 100 °C. An increase in temperature resulted in an increase in expansion and decrease in bulk density. Temperature was a dominant variable affecting macroscopic characteristics of extrudates. The different levels of temperature affected all macroscopic (expansion) properties of extrudates (Gautam and Choudhury, 1999). During extrusion, bulk density (BD) was influenced by temperature and it decreased with increasing temperature. If expansion increased it would be logical to assume that BD would decrease under similar conditions; but BD increased abruptly when temperature increased from 130 to 140 °C. This could be due to the effect of high temperatures on viscosity and starch degradation resulting in less expansion (Grenus *et al*., 1993).

There is close relationship between bulk density and expansion ratio. When temperature of barrel is increased the expansion ratio rises to certain degree of temperature while the bulk density of extrudates show negative course of action in this regard (Altan *et al*., 2008). Increased process temperature, up to a certain point, increased expansion ratio and decreased BD, but further increases in temperature decreased expansion ratio and increased BD. Bulk density decreased steadily as process temperature increased to a certain high temperature. Increasing process temperature from the lowest to the highest resulted in 46% increase in expansion ratio, 47% decrease in BD (Breen *et al*., 1977).

Fat uptake. Lipids are present in variable amount in many different foods. One of the components of lipids

is glycerides that are mostly common. Lipids are richest source of energy and maintains the body temperature by providing heat energy by their oxidation but their excess usage can be harmful as they can lead to the chronic illness, such as heart disease, cancer and obesity (Sharma *et al*., 2004). It is evident from the results that fat uptake of extruded bread crumb has significant effect while fat uptake of extruded bread crumb also have significant effect.

The fat uptake of extruded bread crumb varied from 0.410 mg/g to 0.523 mg/g while that of oven baked bread crumb, it varied from 0.403 mg/g to 0.470 mg/g among different treatments (Table 3). The highest fat uptake of extruded bread crumb was observed in T_2 while highest fat uptake of oven baked bread crumb was observed in T_4. It is clear from the results that fat uptake decreases slightly with gradually increase in temperature.

It shows that by increasing barrel temperature there is a little bit difference in degree of fat absorption of extrudates because at higher temperature and more low temperature extruder does not give proper product. At low temperature there is less water or moisture absorption and hence less disruption of bonding which results in less proper extrusion. At higher temperatures more than desired, there is burning of product and loss of nutrients including fat level and other minerals. The results are in accordance with findings of Lucisano *et al*. (2010) and Yu and Augustine (2002).

Fat addition lowers the barrel temperature of extruder due to the lubricating effect of fat and it ultimately decreases the starch gelatinisation during the extrusion process (Bredie *et al*., 2002). During extrusion, starch conversion can be reduced by the addition of fat as lubricator and shortening which ultimately prevent the severe mechanical breakdown of the starch granules by rotating screw and preventing water from being absorbed by starch. Reduced starch conversion/gelatinisation ultimately results in decreased expansion.

Water binding capacity (g H_2O/kg). It is evident from the results that water binding capacity of extruded bread crumb has highly significant effect, while oven baked bread crumb has significant effect. The water binding capacity of extruded bread crumb varied from 28.96 g H_2O/kg to 34.76 g H_2O/kg while, that of oven baked bread crumb, it varied from 27.15 g H_2O/kg to 27.92 g H_2O/kg among different treatments (Table 3) and expressed in Fig. 3.

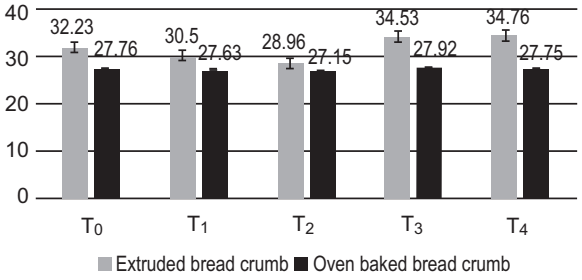

Fig. 3. Water binding capacity of extruded and oven baked bread crumb (g H_2O/kg).

The highest water binding capacity (34.76 g H_2O/g) was observed in extruded bread crumb for highest temperature (130 °C) while, lowest extrusion temperature (110 °C) gave lower value of water binding capacity (28.96 g H_2O/g). It is also clear from results that water binding capacity increases with gradually increase in extrusion temperature. The highest water binding capacity (27.92 g H_2O/g) was observed in oven baked bread crumb. These results matched with Lucisano *et al*. (2010) and Tireki *et al*. (2006).

Water binding characteristics represent the ability of a product to gel formation of firmness when water has been added. Better taste, delay staling, softer crumb and prevention of water binding additives like corn, gums coating and for long time keep ability are the advantages of more water binding capacity. Water binding capacity increase with the increase in porosity (Tireki *et al*., 2006).

Expansion ratio of extruded bread crumb. It is evident from the results that the expansion ratio of bread crumb of extrudates has highly significant effect on temperature. The expansion ratio of bread crumb extrudates varied from 2.04, 2.210, 1.64, 2.30 and 2.53 for T_0 to T_4, respectively (Table 4). It is evident from the results that extrusion temperature significantly affects the expansion ratio of the bread crumb extrudates. The highest expansion ratio (2.53) was observed for highest temperature (130 °C) while at lower extrusion temperature (110 °C) gave lower value for bread crumb extrudates expansion ratio (1.64). It is also clear from these results that expansion ratio increase with gradually increase in temperature. The results were in accordance with findings of Yu and Augustine (2002).

By keeping constant the screw speed and moisture content, an interaction can be observed between expansion ratio and barrel temperature. Overall expansion increased

Table 4. Means table regarding sensory attributes of extruded and oven baked bread crumb

Treatment	Expansion ratio		Flavour		Taste		Crispiness		Over all acceptability	
	Extruded bread crumb	Oven baked bread crumb	Extruded bread crumb	Oven baked bread crumb	Extruded bread crumb	Oven baked bread crumb	Extruded bread crumb	Oven baked bread crumb	Extruded bread crumb	Oven baked bread crumb
T_0	2.04±0.012^{c}	n.d.	6.92±0.52^{a}	6.96±0.61	6.50±0.19^{b}	5.93±0.24^{a}	5.36±0.43^{b}	6.96±0.55^{a}	6.80±0.53^{b}	6.07±0.45^{a}
T_1	2.21±0.009^{bc}	n.d.	7.00±0.67^{ab}	6.80±0.31	6.97±0.54^{a}	5.73±0.46^{a}	5.31±0.21^{ab}	6.76±0.42^{a}	6.67±0.19^{b}	5.80±0.31^{ab}
T_2	1.64±0.014^{d}	n.d.	6.87±0.45^{abc}	6.65±0.48	6.00±0.41^{c}	5.47±0.14^{b}	5.26±0.37^{abc}	6.50±0.31^{b}	6.36±0.42^{c}	5.53±0.24^{bc}
T_3	2.30±0.004^{b}	n.d.	6.93±0.33^{bc}	6.96±0.39	6.33±0.6^{b}	5.30±0.41^{bc}	5.43±0.49^{bc}	6.33±0.51^{b}	6.42±0.31^{c}	5.37±0.41^{c}
T_4	2.53±0.023^{a}	n.d.	7.26±0.21^{c}	6.75±0.17	7.00±0.34^{a}	5.10±0.33^{c}	5.70±0.17^{a}	6.25±0.26^{c}	6.99±0.36^{a}	5.57±0.36^{bc}

n.d. = not determined

linearly with increasing temperature and screw speed up to 130 °C. An increase in temperature resulted in an increase in expansion and decrease in bulk density. Temperature was a dominant variable affecting macroscopic characteristics of extrudates. The different levels of temperature affected all macroscopic (expansion) properties of extrudates (Gautam and Choudhury, 1999). Starch can be able to expand in a better way as the temperature is increased and ultimately become fully cooked (Linko *et al*., 1982). They proposed that, increase in barrel temperature show a positive linear effect on expansion ratio of the final product. Also by gradual rise in temperature there is gradual rise in expansion ration of the extrudates and this occurs to a certain level of temperature which is 168 °C. They suggested the existence of temperature plateau for expansion, between 150 and 170 °C depending on the type of food material. This phenomenon may be caused by excessive structure breakdown and starch degradation under high temperature which weakened the extrudate structure and therefore, caused it to collapse. But at 160 °C there was gradual increase in expansion ratio of the extruded product due to gelatinisation of starch content of raw material.

Sensory evaluation. ***Flavour.*** Quality is perceived by the consumer's attitude and liking. Flavour is one of the important attributes in the purchasing ability of consumer. As our taste buds are encountered with any of the food commodity being composed of either sweet, salty, sour, and bitter or umami, the receptors will automatically detect the respective flavour sensation. A panel of trained sensory analysts determined the flavour of the food stuffs. It is evident from the results that the extruded bread crumb has significant effect on sensory evaluated flavour while the oven baked bread crumb has non-significant effect on flavour.

The sensory evaluation values for flavour of cutlass coated with extruded bread crumb varied from 6.87 to 7.26 while, that of coating of oven baked bread crumb, it varied from 6.65 to 6.96 among different treatments (Table 4) and expressed in Fig. 4. It is evident from results that extrusion temperature affects the sensory evaluated flavour of the extruded bread crumb. The values of coated bread crumb on cutlass are in the range of liking flavour attributes.

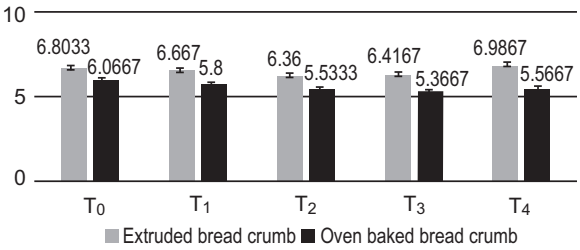

Fig. 4. Overall acceptability scores of extruded and oven baked bread crumb.

The highest sensory evaluated flavour (7.26) was observed at highest temperature (130 °C) while at lower extrusion temperature (110 °C) gave lower value for bread crumb extrudates sensory evaluated flavour (6.87). It is also clear from these results that sensory evaluated flavour increases with gradually increase in temperature.

The results of oven baked bread crumb match with the results given by Al-Abdullah *et al*. (2011). The results of extruded bread crumb match with the results given by Yu and Augustine (2002). He reported that by increasing temperature of extrusion, flavour is developed in extruded product due to activation of flavour producing compounds at higher temperature.

Bhandari *et al*. (2001) defines flavour as the "sensory manifestation" for the perception of products in terms of their:

(1) Reaction to taste by the kinesthetic sense in the muscles of the hand, fingers, tongue, jaw, or lips (e.g. adhesiveness, cohesiveness, hardness, etc.), and
(2) Tactile feel properties measured by the tactile nerves in the surface of the skin of the hand, lips, or tongue (e.g. oiliness, tenderness, moistness, etc.).

***Taste*.** The taste is the most important factor that, consumers consider when shopping for food. It is evident from the results that the extruded bread crumb has highly significant effect on sensory evaluated taste while the oven baked bread crumb also has highly significant effect. The mean values of sensory evaluated taste of cutlass coated with extruded bread crumb varied from 6.00 to 7.00 while in cutlass with coating of oven baked bread crumb, it varied from 5.10 to 5.9 among different treatments (Table 4). It is evident from results that extrusion temperature affects the sensory evaluated taste of the extruded bread crumb. The values of coated bread crumb on cutlass are in the range of liking and fairly liking taste attributes.

The highest score for taste (7.00) was observed for highest temperature (130 °C) while lower extrusion temperature (110 °C) gave lower value for bread crumb extrudates sensory evaluated taste (6.00). It is also clear from the results that sensory evaluated taste of extruded bread crumb increases with gradually increase in temperature.

The results of oven baked bread crumb match with the results given by Al-Abdullah *et al*. (2011). The results of extruded bread crumb match with the results given by Yu and Augustine (2002). He reported that by increasing temperature of extrusion taste is developed in extruded product due to lowering the moisture of product. He proposed that when temperature of processing technique is increased to a certain degree it produce a desirable taste in the product and when temperature was low then the cooked food has not desirable taste characteristics. This is because when temperature is raised in extrusion cooking it results in cooking of product and removing and disruption of certain components which affect the taste of product in negative way. In this way in present study, application of 130 °C in T_4 showed most acceptable taste.

***Crispiness*.** The crispiness plays a crucial role in the enjoyment of eating foods. Crunchy in an otherwise smooth dish, may increase the appeal of eating it. Crispiness is also important parameter of the product to determine its quality with respect to its sensory characteristics. And also an important characteristic in consumer's perception of food and purchasing decisions. The results regarding the analysis of variance of extruded bread crumb was significant while the oven baked bread crumb of sensory evaluated crispiness was highly significant. The values of sensory evaluated crispiness of cutlass coated extruded bread crumb varied from 5.26 to 5.70 while that of coating of oven baked bread crumb, it varied from 6.25 to 6.96 among different treatments (Table 4). It is evident from the results that the extruded bread crumb has highly significant effect on sensory evaluated crispness while the oven baked bread crumb also has highly significant crispiness. It is evident from results that extrusion temperature affects the sensory evaluated crispiness of the extruded bread crumb. The values of coated bread crumb on cutlass are in the range of liking and fairly liking taste attributes.

The highest sensory evaluated crispiness (5.70) was observed for highest temperature (130 °C) while lower extrusion temperature (110 °C) gave lower value for bread crumb extrudates sensory evaluated crispiness (5.26). It is also clear from results that sensory evaluated crispiness of extruded bread crumb increases with gradually increase in temperature. Low moisture in bread crumb give lower density which causes the bread crumb to uptake low fat and create white crystal spot which enhance the look of the product and also make the product crispy.

The results of oven baked bread crumb match with the results given by Salvador *et al*. (2008). The results of extruded bread crumb match with the results given by Yu and Augustine (2002). He proposed that when temperature of processing technique is increased to a certain degree it produce a desirable crispiness in the product as its human perception by senses to observe the taste of product. But if temperature is low, the cooked food has not given desirable crispiness texture.

The results were in accordance of Yu and Augustine (2002), according to him by increasing the barrel temperature the maximum bond disruption occurs results in formation of more homogeneous laminate along barrel, hence imparting better textural characteristics.

Overall acceptability. The overall acceptability is also important parameter of the product to determine its quality with respect to its sensory characteristics. It is evident from the results that high significant effect on overall acceptability of extruded and oven baked bread crumb.

The values of sensory evaluated over all acceptability of cutlass coated extruded bread crumb varied from 6.36 to 6.98 while, that of coating of oven baked bread crumb, it varied from 5.36 to 6.06 among different treatments (Table 4) and graphically expressed in Fig. 5.

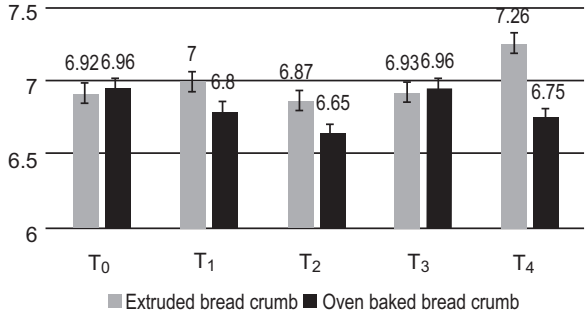

Fig. 5. Flavour scores of extruded and oven baked bread crumb.

The highest overall acceptability (6.98) was observed for highest temperature (130 °C) while, lower extrusion temperature (110 °C) gave lower value for bread crumb extrudates overall acceptability (6.36). It is also clear from results that overall acceptability increases with gradually increase in temperature. The highest overall acceptability of oven baked bread coating is (6.06). The values of coated bread crumb on cutlass are in the range of liking and fairly liking overall acceptable. The results of oven baked bread crumb match with the results given by Al-Abdullah *et al*. (2011). The results of extruded bread crumb match with the results given by Yu and Augustine (2002).

Conclusion

The characteristics of the sample extruded at 110 °C showed bulk density and fat uptake properties that were more acceptable. The study clearly showed that the good functionality of extruded bread crumb was highly dependent on the extrusion processing conditions. Comparing to the oven baked bread crumb, the extruded bread crumb showed good functionality in relation to the control and considering further, the effect of ingredient on the texture of extruded bread crumb, some ingredient can be excluded without adverse effect thus the crisp texture and maximum expansion of extruded bread crumb may be obtained by selecting the following extrusion processing conditions; barrel moisture content 27%; screw speed 150 rpm; barrel temperature 110 °C; flow rate 55-61 kg/h; cut at die face and a cost effective formulation comprising of wheat flour (85.3%); soy flour (5%); emulsifier (0.3%); salt (2%); baking powder (4%). On the basis of this study, extrusion cooking for the production of bread crumb is recommended because with extrusion, some of the ingredients can be removed from the formulation without adverse effect probably due to the interplay between extrusion conditions during cooking. Extrusion thus reduces the cost of input than oven baked. Similarly, less time is required for the process.

Hence, it is concluded that T_2 treatment of extruded bread crumb is efficient, cheap, and good sensory, and also perform overall well then oven baked bread crumb.

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Accumulation of Heavy Metals in Edible Organs of Different Meat Products Available in the Markets of Lahore, Pakistan

Nadia Jamil[a], Mujtaba Baqar[a], Maryam Manzoor[a], Naeem Abbas[b]*, Abdul Qadir[a], Naveed Ahsan[a], Irfan Ahmad Shaikh[a], Soniya Munir[a], Muhammad Arslan[c] and Hina Zahid[a]

[a]College of Earth and Environmental Sciences, University of the Punjab, Lahore, Pakistan

[b]Centre for Environment Protection Studies, PCSIR Laboratories Complex, Ferozepure Road, Lahore 54600, Pakistan

[c]Department of Earth Sciences, King Fahd University of Petroleum and Minerals (KFUPM), Dhahran, Dammam, Saudi Arabia

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Abstract. The present study assessed the accumulation of selected heavy metals (Cd, Cr, Pb and Cu) in different organs including brain, heart, lungs, liver, stomach, kidney and flesh (muscles) of several animals commercially available in the market of Lahore, Pakistan. The concentrations found in different organs of chicken, goat and cow ranged between 0.132-2.165 µg/g for Cd, 0.768-2.335 µg/g for Cr, 0.260-1.411 µg/g for Pb and 0.092-1.195 µg/g for Cu. In the absence of national safety standards in respect to the content of heavy metals in foodstuffs, the results obtained were compared with international guidelines and found concentrations considerably higher than the prescribed safe limits. Therefore, immediate attention must be paid to prevent public health risks associated with the presence of toxic heavy metals in the commercially available meat products.

Keywords: meat products, toxicity, heavy metals, bioaccumulation

Introduction

Meat and meat products are extensively consumed all over the world as they are substantial source of proteins, amino acids, and essential minerals, required for proper tissue formation, growth and repair (Alturiqi and Albedair, 2012; Chowdhury *et al.,* 2011). With population increase worldwide, the consumption of the meat products has also been increased. According to Worldwatch Institute (WWI, 2014), the global meat consumption has been increased 3 fold over last four decades and by 20% only in last decade, which is significantly more than the population rise. However, in recent times, the food security is considered a significant global concern due to the direct public health risks associated with it. In this context, heavy metals contamination of food products, especially the meat products has been broadly investigated worldwide because of their direct toxic effects on human health (Asegbeloyin *et al.,* 2012; Oforka *et al.,* 2012; Mariam *et al.,* 2004). It is evident that human intake is the most common source of potentially deleterious heavy metals (Bennet, 1984).

Meat contamination with heavy metals is a serious threat because of their toxicity, bioaccumulation and bio-magnification in the food chain being transferred to humans (Demirezen and Uruc, 2006; Demirezen and Aksoy, 2004; Abou-Arab, 2001). These heavy metals are stored in body tissues and often have direct physiological toxic effects (Mariam *et al.,* 2004). The accumulation of toxic heavy metals may lead to organ failure, retarded mental development, and cancer (Asegbeloyin *et al.,* 2012). Although trace amount of heavy metals occur due to natural geological activities including such as ore formation, weathering of rocks and leaching may occur. Heavy metals are transferred to the meat's source animals via polluted water, grazing crops on irrigated sewage and industrial wastewater and contaminated feed (Sabir *et al.,* 2003). Moreover, the contaminated soil ingested by animals, upto 18% during grazing in some domestic ungulates is also another source of these toxic elements (Thornton and Abrahams, 1983).

Since heavy metals are bio-accumulative and in less developed countries, less preference is given on existence of these toxic metals in the food products due to limited resources and lack of proper legislative framework that leads to frequent prevalence of the fatal epidemics outbreaks. The heavy metals' contamination like Pb can effects the animals present in its surrounding that can be risky for

*Author for correspondence; E-mail: naeemchemist@gmail.com

human meat consumers (Pareja-Carrera *et al.* 2014). Therefore, determination of heavy metals associated with the consumption of the meat products, commercially available in local markets of less developed countries has become necessary. The aim of the present study was to assess the concentration of Cd, Cr, Cu and Pb in different organs of chicken, mutton (goat), and beef (cow) samples, commercially available in the markets of Lahore, (Pakistan) and to evaluate the public potential health risk. These results were compared with different available international safety standards, since no national food safety standards have been formulated to date for comparison.

Materials and Methods

Sample collection. A total of 30 fresh samples of different organs (brain, heart, lungs, liver, gizzard, kidney and muscle tissues) of chicken, goat and cow were collected from June to September in 2013. Age of these animals was 3-4 months, 2-3 years and 3-4 years old for chicken, goat and cow, respectively. For sampling five markets of Lahore city, including Tollinton Market, Icchra Market, Mozang Bazar, Gulberg Main Market, and Township Main Market were visited. All samples were collected in polyethylene bags, sealed, stored in ice box and transported to the laboratory and wet digestions were performed on the same day.

Sample preparation. The samples were prepared for heavy metals determination in the laboratory using a wet digestion method. One gram of chicken, meat and beef samples was dried in oven at 105 ^{o}C for 1h. The digestion was then carried out using 5 mL of conc. HNO_3 and 1 mL of $HClO_4$ in a digestion flask. The flask was then heated at 200-250 ^{o}C on a hot plate untill the digest became colourless and volume was raised up to 50 mL. The digest was then filtered through Whattman Filter Paper No. 42, preserved in polyethylene bottles to avoid contamination and stored at 4 ^{o}C until analysis.

Standards preparation. Six working standards of 0.2, 0.5, 1, 1.5, 2, and 5 ppm for Cd, Cr, Pb, and Cu were prepared using stock solutions of 1000 ppm, prepared in accordance to the ASTM Standards. Stock solutions (1000 ppm) of selected metals were prepared by dissolving appropriate amounts of $Cd(NO_3)_2$, $K_2Cr_2O_7$, $Pb(NO_3)_2$ and $CuSO_4.5H_2O$ in 1000 mL of doubly distilled deionised water. Further dilutions were made from these stock solutions when required.

Analysis of metals. The digests were analysed for trace metal (Cd, Cr, Cu, and Pb) in chicken, mutton and beef using a Perkin Elmer Analyst 800 Atomic Absorption Spectrophotometer using air-acetylene flame. Concentrations were determined by WinLab32 software operated.

Statistical analysis. Statistical Package for Social Sciences (SPSS) 16.0 was used for the data analyses. Descriptive statistics $\bar{x} \pm SD$ was of main concern. Student's t-test was applied to study the significance ($p<0.05$) of mean values with the permissible limits of the selected heavy metal in the organs of mutton (goat), chicken and beef (cow).

Results and Discussion

Concentration of Cd, Cr, Pb and Cu in different organs of mutton (goat), chicken and beef (cow) are presented in Table 1. Among the selective heavy metals of the study, the highest and lowest mean concentrations were recorded for Cd in liver (2.165 μg/g) and Cu in brain (0.241 μg/g) in mutton (goat). Moreover, it was revealed that the mean heavy metals concentrations in different organs of chicken and beef (cow) samples, ranges from 0.097 μg/g (Cu) to 2.335 μg/g (Cr) and 0.092 μg/g (Cu) to 1.421 μg/g (Cr), respectively.

Cadmium (Cd). Cadmium is a non-essential, toxic element for human and food is reported to be an important source of human exposure to Cd (Baykov *et al.,* 1996). The Agency for Toxic Substances and Disease Registry (ATSDR, 2013) reported Cd as seventh most toxic substance. The high dose of Cd may lead to kidney dysfunction, liver and testicles damage, hypertension, lung damage and hepatic injury (John and Jeanne, 1994). Among different organs of mutton, chicken and beef samples, the highest mean concentration was recorded in kidney of mutton (2.165 ± 0.070 μg/g) and lowest in heart of chicken (0.132 ± 0.088 μg/g) (Table 1). The concentration of Cd in the samples showed significant variability ($p<0.05$) among the brain, liver, gizzard, kidney and flesh of mutton; and lungs, liver, kidney and flesh of beef. This indicates the high Cd exposure risk associated with their consumption. It has no statistical significance in the meat of chicken ($p>0.05$). Cadmium mean concentration in all the chicken organs was found to be within the permissible limit of 0.5 ppm set by FAO/WHO (2000). However, the mean concentrations in brain, liver, kidney, gizzard, and flesh samples of goat, and lungs, liver, kidney and flesh samples of cow exceeded this limit, indicating high risk associated with their consumption. Compared with other studies, the Cd concentrations in beef were found lower than in some previously reported studies (Abd EI-Salam *et al.*,

Table 1. Mean concentration ± SD (µg/g) of the selective heavy metals in different organs of mutton (goat)(n=210), chicken (n=210) and beef (cow)(n=210) samples

	Organ (n = 30)	Cd	Cr	Pb	Cu
Mutton	Brain	1.784 ± 0.088**	1 ± 0.135**	1.281 ± 0.087*	0.241 ± 0.071
	Heart	0.266 ± 0.032	1.053 ± 0.275*	0.559 ± 0.216	0.315 ± 0.066
	Lungs	0.341 ± 0.100	0.967 ± 0.160*	0.728 ± 0.093	0.319 ± 0.039
	Liver	2.074 ± 0.212**	1.029 ± 0.149**	1.411 ± 0.139*	1.195 ± 0.077
	Gizzard	1.023 ± 0.162*	1.342 ± 0.067**	0.974 ± 0.197	0.898 ± 0.059
	Kidney	2.165 ± 0.070**	0.884 ± 0.096**	0.788 ± 0.069	0.524 ± 0.024
	Flesh	0.741 ± 0.064*	1.148 ± 0.260*	0.683 ± 0.122	0.491 ± 0.055
Chicken	Brain	0.337 ± 0.070	0.768 ± 0.069**	0.504 ± 0.224	0.154 ± 0.179
	Heart	0.132 ± 0.088	0.769 ± 0.143*	0.697 ± 0.297	0.174 ± 0.115
	Lungs	0.137 ± 0.191	0.902 ± 0.278*	0.261 ± 0.188	0.088 ± 0.049
	Liver	0.156 ± 0.119	1.048 ± 0.229*	0.705 ± 0.072	0.168 ± 0.069
	Gizzard	0.178 ± 0.083	2.335 ± 0.697*	0.783 ± 0.324	0.097 ± 0.063
	Kidney	0.182 ± 0.092	1.211 ± 0.699*	0.846 ± 0.367	0.354 ± 0.208
	Flesh	0.211 ± 0.149	1.912 ± 0.458*	0.962 ± 0.417	0.409 ± 0.201
Beef	Brain	0.407 ± 0.083	1.421 ± 0.060**	0.698 ± 0.023	0.453 ± 0.074
	Heart	0.398 ± 0.089	1.206 ± 0.351*	0.504 ± 0.139	0.354 ± 0.106
	Lungs	0.574 ± 0.105*	1.411 ± 0.635*	0.688 ± 0.059	0.211 ± 0.082
	Liver	0.619 ± 0.060*	1.086 ± 0.326*	0.634 ± 0.185	0.181 ± 0.089
	Gizzard	0.367 ± 0.092	1.212 ± 0.314*	0.773 ± 0.279	0.092 ± 0.039
	Kidney	0.634 ± 0.191*	1.219 ± 0.332*	0.714 ± 0.088	0.191 ± 0.105
	Flesh	0.597 ± 0.140**	0.898 ± 0.256*	1.122 ± 0.250*	0.125 ± 0.060

*and ** shows statistically significant and highly significant mean values ($p<0.05$), respectively.

2013; Alturiqi and Albedair, 2012; Chowdhury *et al.*, 2011; Asegbeloyin *et al.*, 2010; Mariam *et al.,* 2004), except in the study by Akan *et al.* (2010) which reported higher Cd concentration in beef than in this study. In chicken samples, Cd levels were reported lower than some previous studies (Abd EI-Salam *et al.*, 2013; Alturiqi and Albedair, 2012; Chowdhury *et al.*, 2011; Mariam *et al.*, 2004) but were higher than those reported by Mohammed *et al.* (2013) and Akan *et al.* (2010). However, the Cd levels detected in mutton were found higher than those previously reported (Table 2). Therefore, consumption of local meat with these high Cd content may cause serious public health concerns such as kidney dysfunction, liver and testicles damage, hypertension, lung damage and hepatic injury in the consumers (Maurice *et al.,* 1994). Moreover, Cd accumulates in the liver and kidney where it interacts with essential minerals such as Zn, Cu, Fe, and Se and competes for binding sites (McLaughlin *et al.,* 1999) and also affects the calcium and phosphorus metabolism in human (Jarup *et al.,* 1998).

Chromium (Cr). Chromium (VI) has been ranked as 17th most toxic substance (ATSDR, 2013) and has been classified into the group A: "Human Carcinogen" by USEPA (1999), due to its carcinogenic impacts. The mean concentration of Cr (VI) determined in different organs of mutton, chicken and beef were ranged from 0.768 ± 0.069 µg/g in brain samples of chicken to 2.335 ± 0.697 µg/g in gizzard samples of chicken (Table 1). The concentration of Cr was statistically significant ($p<0.05$) in all the organs of mutton, chicken and beef, particularly a high significance was reported in beef samples.

The mean Cr concentrations in all the studied organs of chicken, mutton, and beef samples exceeded the permissible limit of 0.1 ppm set by ANZFA (2008). These high concentrations of Cr (VI) in meat samples are probably due to its uncontrolled release from industrial discharges; where it has been used in leather tanning, mining, cement and construction industries, electroplating, dyeing, paints and pigments, rust inhibitors, fungicides, alloys manufacturing and glass manufacturing industries (Fahim *et al.,* 2006). Moreover, fascinatingly, Mahmud *et al.* (2011) reported a fact that in Pakistan to meet the high chicken consumption demand, the poultry chicken is fed with the feed containing small leather pieces from leather tanneries, contaminated with Cr (VI) during chrome tanning process. In the present study, the Cr concentrations in the meat products were higher as compared to previous

studies (Abd EI-Salam *et al.*, 2013; Chowdhury *et al.*, 2011; Mahmud *et al.*, 2011), except for beef samples in comparison with previously reported data by Abd EI-Salam *et al.* (2013) (Table 2).

Lead (Pb). Lead concentrations assessed in different organs of mutton, chicken and beef varied from lowest in lungs samples of chicken (i.e. 0.261 ± 0.188 µg/g) and highest in liver samples of mutton (i.e. 1.411 ± 0.139 µg/g) (Table 1). On comparison with permissible limit of 1 ppm set by ANZFA (2008), it was revealed that the mean Pb concentration in brain and liver samples of mutton and flesh samples of beef exceeded the limit, indicating the potential risk to human from the second most hazardous substance worldwide (ATSDR, 2013). In comparison with previously reported data, the Pb concentrations in this study were lower than previous reported data (Abd EI-Salam *et al.*, 2013; Alturiqi and Albedair, 2012; Chowdhury *et al.*, 2011; Mariam *et al.*, 2004), and higher than those reported by Akan *et al.* (2010). However, results were comparable with data reported by Asegbeloyin *et al.* (2010). The Pb exposure to the meat consumer in the less developed country may impart toxic impacts on haemopoietic, nervous, renal and gastrointestinal systems (Baykov *et al.,* 1996).

Copper (Cu). Copper is an essential element in trace amount for the production of heamoglobin and haemocyanin in the vertebrates. It also plays a vital role in bone formation, integrity of the connective tissues, and skeletal mineralisation (Akan *et al.,* 2010). However, its concentration in excess to the permissible limits may cause adverse impacts such as liver and kidney damage (Brito *et al.,* 1990). Macrae *et al.* (1993) reported that dizziness, intestinal discomfort and headaches, hepatitis or cirrhosis, and/or hemolytic crisis in human were associated with ingestion of copper in food. The highest Cu level in this study was determined in liver samples of mutton (1.195± 0.077 µg/g), while the lowest mean Cu concentration was recorded in lungs tissues of chicken samples (0.088 ± 0.049 µg/g) (Table 1). As Cu is an essential nutrient, a recommended dietary allowance (RDA) of 0.9 mg/day (0.013 mg/kg/day) has been set by ATSDR (2004). Thus, the Cu content in all the meat samples were in excess to recommended nutrient requirements by human through diet. Moreover, the Cu concentration in all the studied organs of the mutton, chicken and beef was found to be well within the permissible limits of 200 ppm set by ANZFA (2008). The copper concentrations were also found lower than those reported previously in other publications

Table 2. Concentrations (ppm) of heavy metals in meat samples reported in other studies

Meat sample	Metal	Concentration	Region	Reference
Chicken	Cd	1.36-1.68	Saudi Arabia	Alturiqi & Albedair (2012)
	Pb	7.61-10.49		
	Cu	2.31-7.88		
Beef	Cd	1.56-2.02		
	Pb	5.85-7.93		
	Cu	9.59-13.10		
Chicken	Cd	0.03-0.019	Nigeria	Mohammed *et al.* (2013)
	Pb	0		
Beef	Cu	25-30	Azad	Sabir *et al.* (2003)
Mutton	Cu	68-71	Kashmir,	
Chicken	Cu	11-13	Pakistan	
Beef	Cd	0.17-0.22	Nigeria	Akan *et al.* (2010)
	Pb	0.15-0.25		
	Cu	0.54-0.87		
Mutton	Cd	0.34-0.76		
	Pb	0.08-0.16		
	Cu	0.34-0.98		
Chicken	Cd	0.16-0.27		
	Pb	0.16-0.22		
	Cu	0.01-1.44		
Beef	Cd	0.33-0.909	Lahore,	Mariam *et al.* (2004)
	Pb	2.02-2.19	Pakistan	
	Cu	5.42-93.24		
Mutton	Cd	0.37-0.45		
	Pb	3.85-4.25		
	Cu	5.01-318.82		
Chicken	Cd	0.31-0.49		
	Pb	3.1-3.15		
	Cu	6.91-12.86		
Beef	Cd	0.03-8.04	Bangladesh	Chowdhury *et al.* (2011)
	Cr	0.06-1.22		
	Pb	0.67-24.9		
	Cu	0.15-11.51		
Goat	Cd	0.15		
	Cr	0.08		
	Pb	1.35		
	Cu	3.92		
Chicken	Cd	5.20		
	Cr	0.69		
	Pb	41.94		
	Cu	10.33		
Beef	Cd	0.28-1.50	Nigeria	Asegbeloyin *et al.* (2010)
	Pb	0.80-1.42		
Mutton	Cd	0.04-0.93		
	Pb	0.02-1.36		
Beef	Cd	0.3-1.23	Kohat,	Abd EI-Salam *et al.* (2013)
	Cr	0.3-15.76	Pakistan	
	Pb	2.5-11.83		
	Cu	4.6-8.58		
Goat	Cd	0.37-1.58		
	Cr	0.41-0.46		
	Pb	1.85-2.7		
	Cu	3.22-82.83		
Chicken	Cd	0.86-1.51		
	Cr	0.07-0.53		
	Pb	1.95-3.25		
	Cu	0.41-20.86		
Chicken	Cr	0.233-1.266	Lahore, Pakistan	Mahmud *et al.* (2011)

(Abd EI-Salam *et al.*, 2013; Alturiqi and Albedair, 2012; Chowdhury *et al.*, 2011; Mariam *et al.*, 2004; Sabir *et al.*, 2003) (Table 2). Therefore, Cu contents in the local meat samples could be considered with insignificant risk to public.

Conclusion

This study revealed that the concentrations of Cd, Cr and Pb in different organs of meat (chicken, goat and cow) samples, commercially available in local markets of Lahore, (Pakistan) exceed to the permissible limits set by international health organizations. The high concentrations of non-essential metals have identified the high risk vulnerability of the local population on consumption of the contaminated meat products. Therefore, this study suggests a critical need to formulate and implement national food safety standards in Pakistan to ensure the availability of safe meat products in Pakistan. Further studies are needed to investigate the risk associated with bioaccumulation of these trace metals in locals due to consumption of the contaminated meat products.

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Distribution and Abundance of Marine Debris Along the Coast of Karachi (Arabian Sea), Pakistan

Rashida Qari* and Moniba Shaffat
Institute of Marine Science, University of Karachi, Karachi 75270, Pakistan

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Abstract. This study reports the first assessment of distribution and abundance of marine debris along the coast of Karachi (Arabian Sea), Pakistan. The quadrate method was used for estimating the debris material. Total 40 quadrates were made for collecting the debris on 4 beaches: Sandspit, Buleji, Paradise Point and Korangi Creek in the year of 2012. Nine different types of debris comprising of plastics, glasses, thermopore, clothing, rubber, paper, pot pieces and cigarette filters were collected. The study revealed that, plastic was found in high quantity at all four beaches of Karachi. Other most common items were as follow: plastic at Paradise Point and Sandspit; pot pieces at Korangi Creek and rubber at Buleji. A total weight of 12277.45 g debris was recorded during the whole study period. It was also noted that Paradise Point is the dirtiest beach (5612.6 g) when compared with other studied beaches.

Keyword: marine pollution, beaches, debris, plastic, Pakistan

Introduction

Marine debris or marine litter is one of the global marine pollutions produced by human and released accidentally or deliberately in the ocean. Marine debris not only affects the marine organisms (animals and plants) and environment qualitatively but also hampers the commercial economy activities related to marine foods (e.g., fish). Many types of debris materials are released in the ocean. Cigarette filters, beverage bottles and cans, food wrappers, fishing line, nets and gear are some of the most common debris type that enter the ocean environment from any source (Coe and Rogers, 1997). Much of the debris reaches the ocean after people engaged in beach-going activities have discarded it. The debris is often blown into the water and other debris comes from activities in the water, including vessels, offshore drilling rigs and platforms and fishing piers.

Many forms of marine debris especially derelict fishing gear pose serious threats to wildlife. According to the U.S Marine Mammal Commission, 136 marine species have been reported in entanglement incidents, including six species of sea turtles, 51 species of seabird, and 32 species of marine mammals (Clark, 2008). Derelict fishing or ghost fishing gear also causes damage when abandoned fishing gear and nets continue to catch and kill ocean life. Discarded gear may cause significant losses of some commercially valuable fish and crab species (Laist, 1997).

Major category of solid waste is plastic which is practically indestructible. According to Clark (2007) drift nets, especially monofilament gillnets do not catch the fish alone, but a large number of birds and sea mammals are also trapped by them and drown. Sea birds trapped by gillnets include the Laysan albatross (*Diomedea immortabilis*) Fulmarus (*Fulmarus glacialis*) Shear water (*Pufinus griseus*) and Tufted Puffins (*Launda cirrhata*).

Most of the studies regarding debris along the shoreline are focused on large, visible material found on beaches, with only a few studies describing abundance of material in the water column (Lattin *et al.,* 2004). Mistaken ingestion of micro plastic occurs in seabirds, fish and various planktivores (Laist, 1997; Carpenter *et al.,* 1972). Injection of plastic can have harmful effects, such as diminished food consump-tion, loss of nutrition, internal injury, intestinal blockage, starvation and death (Derraik, 2002; Redford *et al.,* 1997). Plastic is a threat to humanity because plastic does not degrade readily but is only broken into small particles called microplastics, may accumulate in plankton consuming animals, and could be passed up in the food chain (Gago *et al.,* 2014; Derraik, 2002). In addition, plastic pellets and fragments can transfer chemical pollutants to organisms (Derraik, 2002). Recently European Marine Strategy Framework Directive (MSFD) working group develop the monitoring guidance for marine litter

*Author for correspondence; E-mail: rqari2002@yahoo.com

in European Sea (Galgani *et al.,* 2013b). It is an estimate that each year millions of tonnes of plastic enter the ocean (Edyvane *et al.,* 2004). The present study is the first comprehensive attempt that deals with the density, distribution and composition with complete picture of marine debris on the coast of Karachi, Pakistan with basic aim to reduce pollution in the coastal areas and protection of marine environment.

Materials and Methods

A debris study was conducted in the month of August 2012 at low tide on 4 beaches (Sandspit, Buleji, Paradise Point and Korangi Creek), along the coast of Karachi during 2012 (Fig. 1). The sampling method of Chapman (1964) was used for estimating the debris presence and abundance. A quadrate frame made of wood (one-meter square) was employed. Ten replicates (ten feet apart) parallel to the coast line were randomly sampled in the intertidal belt horizontally at each beach. Debris falling inside each quadrate were taken and placed individually in prelabeled plastic bags and returned to the laboratory.

In the laboratory, each sample of debris was placed on a sheet of white paper and all samples of debris were sorted by category (plastic, glass, clothing, paper, thermopore, rubber, fishing nets, pot pieces and cigarette filters) placed in separate containers, and labeled with location and type. After sorting, each sample was weighed with a fine degree of accuracy (0.001 g). Subsequently, the total weight and mean of each debris type was calculated in ten quadrates at each site.

Beach characteristics. The site of Sandspit is situated south west of Karachi. It is rocky and white sandy area where many sandy pits have been found. The sea is very calm and quiet from October to March and very rough during the south west monsoon. Sandspit is quite

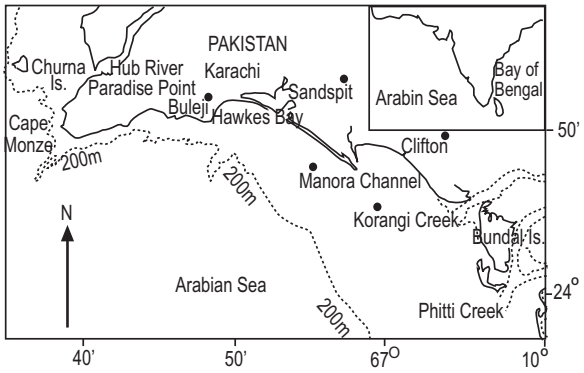

Fig. 1. Map of the Karachi Coast showing locations of the four beaches.

popular picnic and recreational spot in Karachi. During south west monsoon, high tides often cross over and enter into the backwater mangrove area, and refurbish the sediments with coastal sand.

Buleji is the triangular rocky plate Island. It lies at 24°54N and 66°48E of Karachi, between Hawks Bay and Paradise Point covering a distance of about 800 meters. The shore with small and large pools of water exposed at low tides. Boulders of various sizes are visible but mostly near the higher watermark standing on a rocky base or on sand, gravel, cobbles and pebbles etc. The middle and lower area of the ledge are made-up of rather flat continuous rocks and comparatively small boulders.

Paradise Point is also recreational beach. The coast is open to sea front and the wave action is intense all along the coast. The rocky ledge of Paradise Point is mostly wave swept shore (Qari and Siddiqui, 2010; 2005). The beach has attraction for families and tourists.

Korangi Creeks area is dominated by mangroves (Shahzad *et al.,* 2009) and worst pollution affected, where the effluents from Korangi, Landhi, Karachi Export Processing Zone, Bin Qasim Industrial Area, and Pakistan Steel Mill are directly discharged into the sea. Untreated waste water from the industries is discharged into the fourth studied beach (Korangi Creek) through a poorly maintained drainage network (Abbas, 2006). In addition to industrial effluents, discharges from Bhains (buffalo) Colony cattle farms and domestic sewage from residential areas also end up in the Korangi Creek system.

Results and Discussion

The studied beaches: Sandspit, Buleji, Paradise Point and Korangi Creek of Karachi are highly polluted by man-made debris. There is much variation observed in numbers and weight of debris items throughout the coastal belt of studied beaches of Karachi coast. Nine different types of debris items (plastic, glass, clothing, paper, thermopore, rubber, fishing nets, pot pieces and cigarette filters) were identified throughout the study period (Fig. 2). The weight, composition and use of each debris item are described in Table 1. A great variation was found in debris items and their weight that were collected from 10 quadrates at 4 different coastal areas of Karachi coast: Sandspit, Buleji, Paradise Point and Korangi Creek (Fig. 3A-D). The total weight of debris collected at all beaches was 12278.05 g. The highest quantity of debris was found at Paradise Point (5612.6 g) as compared to Sandspit (140.65 g), Buleji (3944.8 g) and Korangi Creek (2580 g) (Fig. 4). Paradise

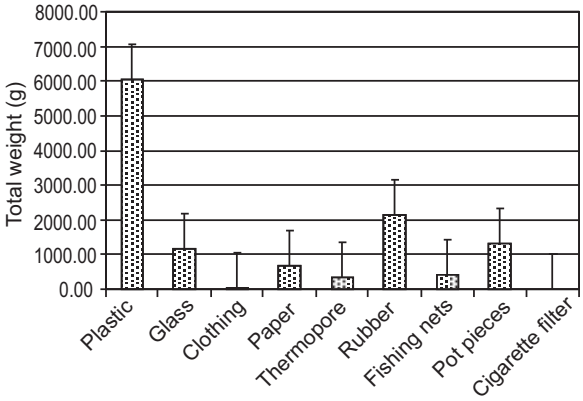

Fig. 2. Total weight of different debris material found at Karachi coast (Bars represent standard error of the mean).

Point was found to be the most polluted coast (5612.6 g) and the less polluted coast with 140.65 g debris was Sandspit (Fig. 3AC and Fig. 4).

The present study revealed that plastic is the dominant debris (49.43%) as compared to other debris items (Fig. 2). The plastic debris was abundantly found at all four studied beaches; Sandspit (7.57 g/m^2), Buleji (104.5 g/m^2), Paradise Point (507.76 g/m^2) and Korangi creek (11.54 g/m^2) of Karachi coast (Fig. 3). The total plastic debris weight found at all 4 studied beaches of Karachi coast was 6070.17 g in the whole study period (Table 1). Plastic debris contained small and large bags, juice boxes, container bottles and packing materials. It was also observed that at Paradise Point the highest amount of plastic was found (90.46 %). Paradise Point showed the greatest number of small plastic bags. There was above 60 pieces of different food products counted in each quadrate at the beach of Paradise Point.

Rubber debris (17.67 %) consisted of only shoes. It was at Buleji 402.8 g/m^2 and at Paradise Point 155.6 g/m^2 (Fig. 3 A and D). It was not found at Korangi Creek and Sandspit. The pot pieces (10.74 %) were found only at Sandspit (26.5 g/m^2) and Korangi Creek (184.57 g/m^2), respectively (Fig. 3 A and D). Glass (9.72 %) was found only at two beaches, Sandspit (7.76 g/ m^2) and Korangi Creek (148.3 g/m^2) as shown in Fig 3 A and D. Glass was the second highest at Korangi Creek (Fig. 3 D). Paper debris was 5.54 % of total debris, composed of juice boxes, newspapers and wrappers of burgers and other food items. Paper debris was found only at Buleji (81.25 g/m^2) and Paradise Point (71.0 g/m^2) beach (Fig. 3 B and C). Total weight of paper collected from these two beaches was 680.80 g (Table 1 and Fig. 2).

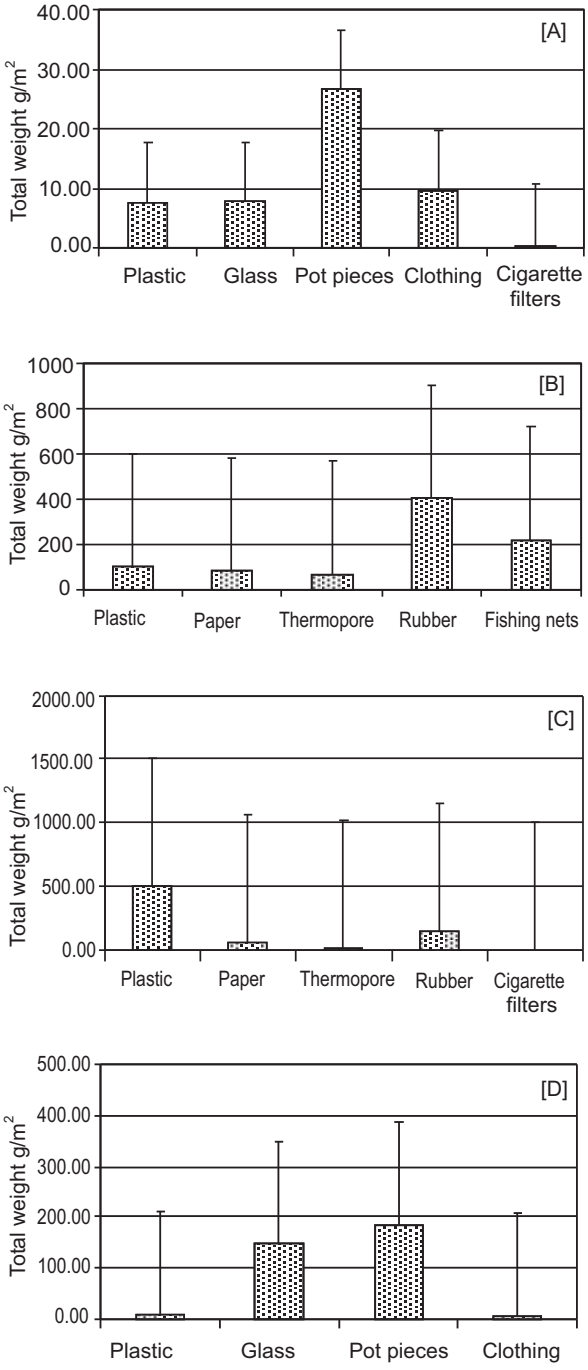

Fig. 3. Total debris material collected at the Karachi coast: (A) Sandspit, (B) Buleji, (C) Paradise Point, (D) Korangi Creek (Bars represent standard error of the mean).

Fishing nets (3.56 %) were found only at Buleji (218.8 g/m^2) because Buleji is a coast used for fishing (Fig. 3 B). Thermopore or packing material was 2.86 % of total debris and it was found only at Buleji (66.2 g/m^2) and Paradise

Table 1. Total weight of debris items collected from different beaches of Karachi coast

S.No	Type of debris	Sampling beach	Total weight (g)	Composition	Use
1	Plastic	Sandspit	75.77	Organic polymers	Bags for food items
		Buleji	836.00	(Polystyrene, Polyvinyl	and packing
		Paradise Point	5077.6	Chloride)	
		Korangi Creek	80.80		
		Total	6070.17		
2	Glass	Sandspit	7.76	Silica, potassium,	In building windows
		Korangi Creek	1186.4	alumina, sodium,	and making
		Total	1194.16	magnesium, calcium	cutlery
3	Pot pieces	Sandspit	26.50	Clay	Decoration purposes
		Korangi Creek	1292.8		
		Total	1319.30		
4	Clothing	Sandspit	28.82	Cotton	Cloth
		Korangi Creek	20.00		
		Total	48.82		
5	Paper	Buleji	325.60	Cellulose, hemi	Writing, roofing,
		Paradise point	355.20	cellulose, lignin	flooring
		Total	680.8		
6	Thermopore	Buleji	331.60	Polystyrene	For insulation and
		Paradise point	20.40		packing
		Total	352		
7	Rubber	Buleji	2014	Polybutadiene,	Slippers, rubber band,
		Paradise point	155.60	Polystyrene and	holding and tighting
		Total	1319.30	Natural rubber	purposes
8	Fishing nets	Buleji	437.60	(Polyisoprene) thread	for catching fish
		Total	437.60		
9	Cigarette filters	Sandspit	1.8	Cellulose acetate	Smoking
		Paradise point	3.8		
		Total	5.6		

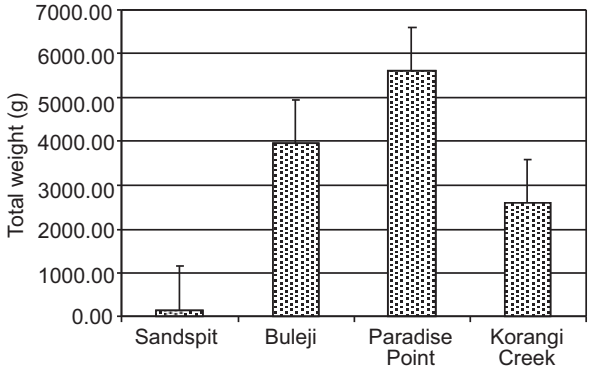

Fig. 4. Total debris material at different sites of Karachi coast (Bars represent standard error of the mean).

point (10.02 g/m^2) (Fig. 3 B and C). The debris item clothing (0.39 %) was found only at Sandspit (9.6 g/m^2) and Korangi Creek (6.67 g/m^2) (Fig. 3 A and D). Cigarette filters (0.04 %) were found at two locations Sandspit (0.45 g/m^2) and Paradise Point (1.27 g/m^2) (Fig. 3 A and C), both of these beaches are picnic point.

The data of debris items at different coasts were analysed by looking at the relationship between different coasts and debris items. There was no particular correlation found in between type of debris studied at different coasts except Sandspit and Paradise Point debris showed positive significant correlation (r^2 =0.930). Most of the types of debris showed positive significant correlation: plastic and cigarette filters (r^2 = 0.838), plastic and paper (r^2 = 0.735), glass and pot pieces (r^2 = 1.000), paper and thermopore (r^2 = 0.575), paper and rubber (r^2 = 0.588), paper and fishing nets (r^2 = 0.526), thermopore and rubber (r^2 = 1.000), thermopore and fishing nets (r^2 = 0.998) and fishing nets and rubber (r^2 = 0.997), clothing and rubber (r^2 =0.735), pot pieces and fishing net (r^2 =0.762) thermopore and clothing (r^2 = 741).

From the observation of debris items it has been noted that, sources of most of the debris are human activities. The plastic items (49.43 %) were the most abundant marine debris type in the whole study period (Fig. 5), followed by rubber (17.67%), pot pieces (10.74%) and glass (9.72%). The present results are similar with the results of study conducted at the beaches of northern New South Wales, Australia (Taffs and Cullen, 2005). Similar results were also observed by Lazar and Gracan (2011) when they studied the occurrence and impacts of marine debris ingestion by logger head sea turtles, *Carette caretta* in the foraging habitats of the eastern Adriatic Sea, recorded 35.2% turtles eaten marine litter with plastic dominant. In the present study, majority of plastic debris consists of bags in addition to other items of plastic that were abundantly found at all studied beaches. The possible reason could be the use of plastic in our modern life. Plastic is an environmental hazard as it does not break down easily in the environment and its presence in various forms causes harm to marine life. Harmful effects from the ingestion of plastics include blockage of gastric enzyme secretion, diminished feeding stimulus, lowered steroid hormone levels, delayed ovulation and reproduction failure (Derraik, 2002). Gago *et al.* (2014) also reported that, most common element found in beach litter was made of plastic with average percent of 63, 38 and 83 when a seasonal series of sampling was conducted on three beaches of Galician coast, Spain for the assessment of the situation of beach litter. The ingestion of plastic debris by small fish and seabirds for instance, can reduce food uptake, cause internal injury and death following blockage of intestinal tract (Derraik, 2002; Ryan, 1987). The issue of plastic connects to several European water policies for the good environmental status of marine waters (Galgani *et al.,* 2013a)

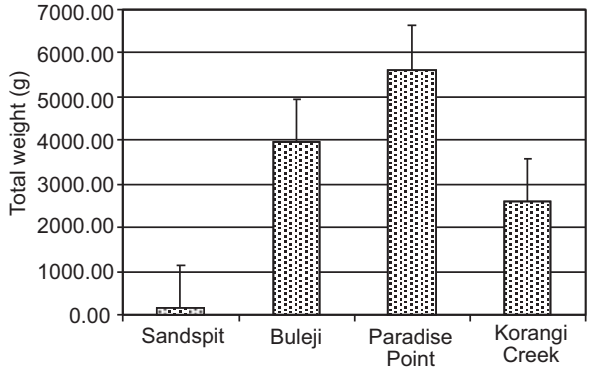

Fig. 5. Total plastic debris material at different sites of Karachi coast (Bars represent standard error of the mean).

Conclusion

It is concluded that the present study provide baseline data on density, distribution and composition of manmade debris at different beaches of Karachi. It is also concluded that the management of beaches needs to be focused on reducing the debris or litter especially plastic pollution entering the marine environment from different sources. It is also assumed that plastic is major threat to marine mammals, turtles and birds through entrapment and digestion. It is very necessary to display restriction on all the beaches of Karachi for all kinds of debris especially plastic. The trash containers should be fixed for all debris like pieces of fishing line, net or other litter along the coast. The debris material should be reduced, reused and recycled. It is also necessary to place all trash on ship or boat for proper disposal on land and at the beach. People should take trash home with them when they return from picnics. Public awareness programmes should be under taken to keep the beaches clean.

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Concentration of Heavy Metals in Available Fish Species (Bain, *Mastacembelus armatus*; Taki, *Channa punctatus* and Bele, *Glossogobius giuris*) in the Turag River, Bangladesh

Rezuana Afrin[a], Md. Younus Mia[a]*, Md. Aminul Ahsan[b] and Ahedul Akbor[b]

[a]Department of Environmental Science and Resource Management, Mawlana Bhashani Science and Technology University (MBSTU), Tangail, Bangladesh

[b]Institute of National Analytical Research Service (INARS), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh

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Abstract. This study was conducted to assess the concentration level of heavy metals in three available fish species (Bain, *Mastacembelus armatus*; Taki, *Channa punctatus* and Bele, *Glossogobius giuris*) of the Turag river, Bangladesh during the months from January to March, 2014. In case of bio-concentration of heavy metals in fish, the levels of Pb, Cd, Cr, Cu and Fe ranged from 0.01-0.13 mg/kg, 0.001-0.02 mg/kg, 0.17-0.48 mg/kg, 0.30-0.74 mg/kg and 4.05-46.86 mg/kg, respectively while Hg was below detection level. These values indicate that the heavy metals were concentrated in fish flesh at a higher level than water. The highest values of Pb, Cd and Cr were found in Bain fish, Fe was found in Taki fish and Cu was found in Bele fish. Most of the heavy metals have crossed the permissible limits in fish, especially for the values of Cr, Cu and Fe. From the results of the present investigation, it can be concluded that the available fish species are harmful for their consumers.

Keywords: heavy metals, river fish, bio concentration, ecosystem

Introduction

Environmental problems related to heavy metals have a long history worldwide (Khan, 2008). Heavy metals can cause harm to human, animals and other organisms. As fish are often at the top of aquatic food webs and may concentrate large amounts of metals from the water and sediments, heavy metals can enter into human body very easily (Mansour and Sidky, 2002). But people commonly have no awareness about this problem and most of them have no knowledge about heavy metal exposure and its effects on health, especially in the developing countries.

The river Turag running by the side of the Dhaka City, the capital of Bangladesh, is one of the most polluted rivers in Bangladesh (DoE, 2003) and has been steadily experiencing complicated problems like pollution and encroachment that have almost suffocated the valuable lifelines of the city (Hossain, 2011). Main pollution sources of the Turag river water are various consumer goods industries and most of the industries discharge their effluents directly or indirectly into the Turag river without any treatment causing pollution of the surface water (Rahman *et al.,* 2012).

*Author for correspondence; E-mail: mdmia1998@gmail.com

The river water is already polluted by various heavy metals discharging from industrial wastewaters and contamination of freshwater fish with heavy metals (HMs) is a recognised environmental problem (Staniskiene, 2006). Fish resources play an important role in the economy of Bangladesh, accounting for about 5% of GDP and it is an important source of protein (MFL, 1998). Fisheries in Bangladesh contribute its role in mitigating animal protein shortage as well as providing jobs to millions of people. Fish provides 63% of the total animal protein supply and the per capita annual fish intake is about 15.04 kg (Sarder, 2007). As fish is an important natural resource and good food source, it is very much needed to know the concentration levels of harmful heavy metals in fish living in polluted water. The present study was conducted by considering this reason for leading a safer and better life. As Turag river water is very much polluted, only the fish species that can survive in polluted water and in low DO level of water are available here. In the present study, 3 available fish species i.e., *M. armatus* (Bain), *C. punctatus* (Taki), and *G. giuris* (Bele) were collected from Turag river and analysed for some heavy metals to know their concentra-tions in the muscles of those fish species.

Fig. 1. Map showing the Turag River and the study area.

Materials and Methods

Sample collection. Samples of three fish from each of the 3 available species *(Mastacembelus armatus, Channa punctatus,* and *Glossogobius giuris)* were collected from Ashulia bridge area (Table 1) and kept them in an ice-box.

Fish samples were collected for 3 times in January, February and March, 2014. After collection, the samples were preserved and labeled properly and kept at -20 °C. Then the fish samples were analysed for heavy metals (Pb, Cd, Cu, Cr, Hg and Fe) at Institute of National Analytical Research and Service (INARS), BCSIR Laboratories, Dhaka, Bangladesh.

Table 1. Fish specimens (3 specimens for each species) recollected from Turag river in Bangladesh

S. No.	Local name	Scientific name
Species 1	Bain	*Mastacembelus armatus*
Species 2	Taki	*Channa punctatus*
Species 3	Bele	*Glossogobius giuris*

Sample analysis. ***Sample preparation.*** For heavy metal analysis, ashing process was followed for fish samples except Hg. At first the fish were gutted and flesh was separated. The flesh was put in a watch glass for each sample. Then they were weighted (above 10 g for each sample) properly and taken in dry beakers. The beakers were put in furnace at 100 °C for 1 h, then at 200 °C for 1 h, then at 300 °C for 1 h and then at 450 °C for 4-5 h until the samples became totally dry or ash. After drying in furnace, concentrated HNO_3 and distilled water (1:1) were added in the beakers to make them wet. Then the beakers were put in hot-plate and evaporated until they were dry again. After that the beakers were kept in the furnace at 550 °C for 5-6 h. After taking out from the furnace, 20 mL HNO_3 was put into each beaker. Then they were put on the hot-plate by keeping watch glass on each beaker and heated until boiling.

After boiling the beakers were taken out from the hot-plate. Then the samples were taken in 50 mL volumetric flasks and filled with distilled water up to the mark. At last they were filtered and preserved in labeled containers for each sample.

For Hg analysis in fish, acid digestion process was followed. At first the weight (above 10g for each sample) of the fish samples were taken properly and then 20 mL HNO_3 and 10 mL $HClO_4$ were added to each sample in beakers. The beakers were boiled on the hot-plate until the samples became totally colourless. Then they were taken in 50 mL volumetric flasks and filled with distilled water up to the mark. After that the samples were filtered properly and kept in separate containers (APHA, 1998).

Instrumental analysis. The Pb, Cd and Cr concentrations of fish samples were analysed using Zeeman Atomic Absorption Spectrometer (Model: Varian, AA 240Z and Method: APHA 3113.B). The prepared samples were taken in vials and put in specific positions of Atomic Absorption Spectrometer (AAS) (AA 240 Z). Hg concentration of water samples was analysed using Cold Vapor Hydride Generation Atomic Absorption Spectrometer (Model: Varian, AA 220FS and Method: APHA 3112.C). Cu and Fe concentrations of water samples were analysed using Flame Atomic Absorption Spectrometer (Model: Varian, AA 240FS and Method: APHA 3111.B) (APHA, 1998). The recovery ranges for each parameter were 100% ± 20%. The detection limit for Fe, Cu, Cr, Cd, Hg and Pb were 0.027, 0.01, 0.0035, 0.00012, 0.00019 and 0.003 ppm, respectively. For lower concentration of heavy metals, the samples were pre-concentrated and for higher concentration of heavy metals, the samples were diluted. The standards used for Pb, Cd, Cr, Cu, Hg and Fe were 30 ppb, 2 ppb, 10 ppb, 10 ppb, 5 ppm and 1 ppm, respectively.

Results and Discussion

Heavy metals in fish. *Lead (Pb).* The highest value (0.13 mg/kg) of Pb was observed in species 3 in January and the lowest (0.01 mg/kg) was observed in species 2 in January (Table 2). The order of Pb accumulation in fish is kidney> gill> liver> muscle (Abdel-Baki, 2011). Afrin *et al.* (2014) found the highest value of Pb in Ashulia bridge area of the Turag river water as 0.005 mg/L in March, 2014.

Lead (Pb) can affect every organ and system in the body (CHSR, 2009). The symptoms of acute lead poisoning are headache, irritability, abdominal pain and various symptoms related to the nervous system. Long-term lead exposure may also give rise to kidney damage and long-term low-level lead exposure in children may lead to diminished intellectual capacity. Experiment suggests a weighted mean decrease in IQ of 2 points for a 0.48 µmol/L (10 µg/dL) increase in blood lead level (Jarup, 2003).

Ahmad *et al.* (2010) found the highest level of Pb in chapila (*Gonialosa manmina*), (13.52 mg/kg) during monsoon and the lowest in tatkeni, *Cirrhinus reba* (8.03 mg/kg) during pre-monsoon in the Buriganga river. Ahmed *et al.* (2009b) studied the heavy metal concentration in fish from the Dhaleswari river, Bangladesh and found the seasonal variation of Pb from 7.03 to 12.18 mg/kg. Ahmed *et al.* (2009a) investigated the heavy metal concentration in fish and oyster from the Shitalakhya river, Bangladesh and found seasonal variation of Pb ranging from 9.16 to 13.09 mg/kg. These values differ from the present study because the values of Pb were observed in the whole body in the previous results. But it was observed only in the muscle or flesh of fish in the present study, where the bio-accumulation level is very low.

According to Indrajith *et al.* (2008), concentration of Pb ranged from 0.01-0.08 mg/kg in *E. suratensis* and 0.004-0.06 mg/kg in *A. commersoni* in Negombo estuary, Srilanka. Nwani *et al.* (2010) studied the mean concentration of Pb in the muscle of the six fish species which

Table 2. Concentrations of heavy metals (mg/kg) in fish (flesh) samples

Heavy metals	Species 1			Species 2			Species 3		
	Jan 2014	Feb 2014	Mar 2014	Jan 2014	Feb 2014	Mar 2014	Jan 2014	Feb 2014	Mar 2014
Pb	0.03	0.1	0.03	0.01	0.03	0.06	0.13	0.02	0.03
Cd	0.003	0.02	0.005	0.001	0.01	0.007	0.003	0.002	0.003
Cr	0.48	0.36	0.27	0.27	0.17	0.30	0.42	0.30	0.43
Cu	0.61	0.74	0.72	0.43	0.30	0.60	0.76	0.48	0.63
Hg	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
Fe	7.08	5.83	8.41	4.85	8.46	46.86	8.03	4.05	5.70

*N. D. = Not Detectable

varied from minimum of 0.10±0.01 mg/kg to a maximum value of 0.31 ± 0.01 mg/kg in lotic freshwater ecosystem at Afikpo, Nigeria. Daka *et al.* (2008) obtained 0.01-0.06 mg/kg for Pb in fish species from Azuabie Creek in the Bonny Estuary, Nigeria. Oguzie (2003) reported Pb concentration of 0.007-0.03 mg/kg in fishes from Ikpoba River Nigeria. Burgera and Gochfeld (2005) found Pb ranged from 0.04 to 0.12 mg/kg in some marine fish of New Jersey, USA. The previous values are mostly similar to the present study.

Cadmium (Cd). Here, the highest value (0.02 mg/kg) of Cd was observed in species 1 in February and the lowest (0.001 mg/kg) was observed in species 2 in January (Table 2). The order of Cd accumulation in fish is liver > gill > kidney > muscle (Abdel-Baki, 2011). Afrin *et al.* (2014) found the highest value of Cd in Ashulia bridge area of the Turag river water as 0.00003 mg/L in January, 2014.

Cadmium and their compounds are known human carcinogens. Ingesting very high levels severely irritates the stomach, leading to vomiting and diarrhoea. Long-term exposure to lower levels leads to a buildup in the kidneys and possible kidney disease, lung damage, and fragile bones (CHSR, 2009).

According to Ahmad *et al.* (2010), Cd concentration was the highest in batashi, *Neotropius atherinoides* (1.25 mg/kg) during monsoon and the lowest in tatkeni, *Cirrhinus reba* (0.73 mg/kg) during post-monsoon in Buriganga river. Ahmed *et al.* (2009b) studied the heavy metal concentration in fish from the Dhaleswari river, Bangladesh and found the seasonal variation of Cd (0.52-0.8 mg/kg). Sharif *et al.* (1993) studied the heavy metal concentration in *T. vagina* and found the concentration of Cd as 0.11 ± 0.00 mg/kg (dry weight basis). All these values differ from the present study due to different accumulation levels of Cd in different organs of fish and also for abundance of Cd enriched pollutants in water.

According to Indrajith *et al.* (2008), concentration of Cd ranged from 0.002 to 0.048 mg/kg in *E. suratensis* and 0.001-0.030 mg/kg in *A. commersoni* in Negombo estuary, Srilanka. Burgera and Gochfeld (2005) found Cd ranged from 0.0001 to 0.01 mg/kg in some marine fish of New Jersey, USA. The values of the previous study are mostly similar to the present study.

Chromium (Cr). The highest value (0.48 mg/kg) of Cr was observed in species 1 in January and the lowest (0.17 mg/kg) was observed in species 2 in February (Table 2). The order of Cr accumulation in fish is kidney > gill > liver > muscle (Abdel-Baki, 2011). Afrin *et al.* (2014) found the highest value of Cr in Ashulia bridge area of the Turag river water as 0.024 mg/L in March, 2014.

Chromium (VI) compounds are toxins and known human carcinogens, whereas breathing high levels of chromium (III) can cause irritation to the lining of the nose, nose ulcers, runny nose, and breathing problems; such as asthma, cough, shortness of breath, or wheezing. Skin contact can cause skin ulcers. Allergic reactions consisting of severe redness and swelling of the skin have been noted. Long term exposure can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation (CHSR, 2009).

According to Ahmad *et al.* (2010), Cr concentration was the highest in chapila, *Gonialosa manmina* (7.38 mg/kg) during monsoon and the lowest in tengra, *Mystus tengara* (5.27 mg/kg) during monsoon in the Buriganga river. Ahmed *et al.* (2009b) studied the heavy metal concentration in fish from the Dhaleswari river, Bangladesh and found the seasonal variation of Cr (9.38-19.65 mg/kg). Ahmed *et al.* (2009a) investigated the heavy metal concentration in fish and oyster from the Shitalakhya river, Bangladesh and found seasonal variation of Cr ranged from 8.12 to 9.07 mg/kg. All these values differ from the present study due to different accumulation levels of Cr in different organs of fish and also for abundance of Cr enriched pollutants from tannery industries in river water.

According to Indrajith *et al.* (2008), concentration of Cr ranged from 0.02-0.28 mg/kg in *E. suratensis* and 0.01-0.24 mg/kg in *A. commersoni* in Negombo estuary, Srilanka. Nwani *et al.* (2010) studied the mean concentration of Cr in the muscles of fish species which varied from minimum of 0.28 ± 0.04 mg/kg in *M. tapirus* and *C. anguillaris* to a maximum of 0.66 ± 0.04 mg/kg in *C. nigrodigitatus* and *T. zillii* in lotic freshwater ecosystem at Afikpo, Nigeria. These values are in line to the present study.

Copper (Cu). Here, the highest value (0.74 mg/kg) of Cu was observed in species 1 in February and the lowest (0.30 mg/kg) was observed in species 2 in February (Table 2). The order of Cu accumulation in fish is liver > kidney > gill > muscle (Abdel-Baki, 2011). Afrin *et al.* (2014) found the highest value of Cu in Ashulia bridge area of the Turag river water as 0.09 mg/L in March, 2014.

Cu is one of the essential elements for humans and the adult daily requirement is about 2.0 mg (De, 2005). But long term exposure to Cu has deleterious effects on human health. In case reports of humans intentionally or accidentally ingesting high concentrations of copper salts (doses usually not known but reported to be 20-70 g copper), a progression of symptoms was observed including abdominal pain, headache, nausea, dizziness, vomiting and diarrhoea, tachycardia, respiratory difficulty, hemolytic anemia, massive gastrointestinal bleeding, liver and kidney failure, and death (Stern *et al.,* 2007).

Ahmed *et al.* (2009b) studied the heavy metal concentration in fish from the Dhaleswari river, Bangladesh and found the seasonal variation of Cu (7.55-11.50 mg/kg). Ahmad *et al.* (2010) studied that Cu level was the highest (6.34 mg/kg) in chapila, *Gonialosa manmina* during post-monsoon and the lowest in tatkeni, *Cirrhinus reba* (3.36 mg/kg) during the same time in the Buriganga river. Ahmed *et al.* (2009a) investigated the heavy metal concentration in fish and oyster from the Shitalakhya river, Bangladesh and found seasonal variation of Cu ranged from 5.47-8.19 mg/kg. All these values differ from the present study due to different accumulation levels of Cu in different organs of fish and also for abundance of Cu enriched pollutants in water.

According to Indrajith *et al.* (2008), concentration of Cu ranged from 0.02 to 0.37 mg/kg in *E. suratensis* and 0.01-0.25 mg/kg in *A. commersoni* in Negombo estuary, Srilanka. Nwani *et al.* (2010) studied the mean concentration of Cu in the muscles of fish species which varied from minimum of 0.56 ± 0.03 mg/kg in *C. anguillaris* to a maximum of 1.33 ± 0.06 mg/kg in *T. zillii* in lotic freshwater ecosystem at Afikpo, Nigeria. These values are mostly similar to the present study.

Mercury (Hg). Here, the level of concentration of Hg was not detectable for all fish species (Table 2) because there was no or a very little source of Hg containing pollutants in the Turag water. Normally the order of Hg accumulation in fish is kidney > liver > muscle > gill (Abdel-Baki, 2011). Afrin *et al.* (2014) found Hg as not detectable in Ashulia bridge area of the Turag river water.

Mercuric chloride and methyl mercury are possible human carcinogens. The nervous system is very sensitive to all forms of mercury. Exposure to high levels can permanently damage the brain, kidneys, and developing fetuses (CHSR, 2009). A high dietary intake of mercury from consumption of fish has been hypothesised to increase the risk of coronary heart disease (Jarup, 2003). According to Indrajith *et al.* (2008), concentration of Hg ranged from 0.03 to 0.33 mg/kg in *E. suratensis* and 0.04-0.26 mg/kg in *A. commersoni* in Negombo estuary, Srilanka. All these values differ from the present study due to different accumulation levels of Hg in different organs of fish and also for abundance of Hg enriched pollutants in water.

Iron (Fe). The highest value (46.86 mg/kg) of Fe was observed in species 2 in March and the lowest (4.05 mg/kg) was observed in species 3 in February (Table 2). Afrin *et al.* (2014) found the highest value of Fe in Ashulia bridge area of the Turag river water as 6.33 mg/L in March, 2014.

Iron is an essential element in human nutrition. Estimates of the minimum daily requirement for iron depend on age, sex, physiological status, and iron bioavailability and range from about 10 to 50 mg/day. The average lethal dose of iron is 200-250 mg/kg of body weight, but death has occurred following the ingestion of doses as low as 40 mg/kg of body weight. Adults have often taken iron supplements for extended periods without deleterious effects and an intake of 0.4-1 mg/kg of body weight per day is unlikely to cause adverse effects in healthy persons (WHO, 2003).

Nwani *et al.* (2010) studied the concentration of Fe in the muscles (mg/kg) of the fish species which varied from minimum of 186.00 ± 0.07 mg/kg in *M. tapirus* and *C. anguillaris* to maximum of 443.20 ± 0.08 mg/kg in *C. nigrodigitatus* and *T. zillii,* respectively. These values differ from the present study due to different accumulation levels of Fe in different organs of fish and also for abundance of Fe enriched pollutants in water.

Conclusion

According to this study, heavy metals can be concentrated in fish species. The study observed a great amount of heavy metals especially Cr (0.17-0.48 mg/kg), Cu (0.30-0.74 mg/kg) and Fe (4.05-46.86 mg/kg) in fish flesh or muscle that can be lethal to fish, humans and other organisms. Concentrations of Pb (0.0-0.13 mg/kg) and Cd (0.001-0.02 mg/kg) were very low in fish and Hg was below the detection level. According to the previous studies, fish flesh or muscle has the lowest level of bio-concentration. So, the concentration levels of heavy metals are comparatively low in the present study. The concentration levels of Cd, Cr and Cu were highest in bain, *Mastacembelus armatus* (species 1).

Concentration of Pb was highest in bele, *Glossogobius giuris* (species 3) and Fe in taki, *Channa punctatus* (species 2). Highest bio-concentration levels of Pb and Cr were observed in January, Cd and Cu were observed in February and Fe in March.

In the present investigation, some heavy metals concentrations (Cr, Cu, Fe) are higher than the safe recommended values, which suggest that the Turag river is partly a heavy metal polluted river and the water and fish are not fully safe for human health and ecosystem. Again lower concentration of heavy metals (Pb, Cd) can be harmful to human health and organism in case of long term exposure.

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Short Communication

High Heritability in a Resistant Barley Genetic Source to Spot Blotch *(Cochliobolus sativus)*

Mohammad Imad Eddin Arabi*, Antonious Al-Daoude, Amina Shoaib and Mohammad Jawhar

Department of Molecular Biology and Biotechnology, AECS, P. O. Box 6091, Damascus, Syria

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Abstract. The objective of the present research was to assess and understand the heritability of the resistant barley genetic source cv. Banteng to spot blotch SB disease caused by *Cochliobolus sativus*. A cross was made between this resistant cultivar and the universally susceptible cv. WI 2291. Analysis of variance for the studied trait indicated highly significant differences among cultivars. High broad sense heritability was found ($H \approx 88$ %). However, in all cases, the results obtained for the F_2 plants demonstrated that the observed segregation pattern fitted 1:15 ratios.

Keywords: *Hordeum vulgare*, *Cochliobolus sativus*, fungus resistance, inheritance

Spot blotch (SB) caused by *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur [anamorph: *Bipolaris sorokiniana* (Sacc.) Shoem.] is economically one of the most important fungal diseases of barley (*Hordeum vulgare* L.) throughout the world (Mathre *et al.,* 2003).

The economic damage caused by SB and planting of resistant genotypes has been studied extensively by many researchers (Zhou and Steffenson, 2013; Ghazvini and Tekauz, 2008; Joshi *et al.,* 2007a; Bilgic *et al.,* 2006; 2005; Arabi, 2005a; Arabi and Jawhar, 2004; 2003; Steffenson *et al.,*1996).

The present study therefore, was initiated to investigate the inheritance pattern of SB resistance in the German barley Banteng cultivar to design suitable strategies to enhance resistance of barley cultivars.

Single plant selections of resistant (Banteng) and susceptible (WI2291) cultivars were multiplied and used in the cross. Resistant parent Banteng (a germplasm cultivar introduced from Germany) was crossed with the universal susceptible WI2291 (originated from the Waite Institute, Glen Osmond, Australia) which is otherwise higher yielding with good agronomic performance.

Parents and F_1 progenies (29 plants) were evaluated for resistance to SB under an induced epiphytotic created in the field at station, west of Damascus , Syria under rainfed conditions (500mm rainfall). Seeds were planted in a randomised complete block design, with three replicates. Plots of the F_1 generation consisted of two 2-m rows seeded 25 cm apart with 30 cm between plots. The susceptible barley cultivar WI 2291 was planted in the alleys and borders, two weeks before sowing the experiment to enhance the spread of disease. Soil fertilizers were drilled before sowing at a rate of 50 kg/ha urea (46% N) and 27 kg/ha superphosphate (33% P). The progenies of the cross were advanced to the F_2 generation (457 plants) following the method described by Joshi *et al.* (2004) where a random plant in each generation from each line was harvested for advancing the generation.

A mixture of equal ratio of pure aggressive isolates of *C. sativus* (Arabi and Jawhar, 2004) was used to inoculate the parents as well as plants of the F_1 and F_2 generation. A spore suspension (approximately 2×10^4 spores/mL) containing the surfactant Tween 20, was uniformly sprayed onto plants during the evening hours by using a hand-held atomizer, then plants were covered with polyethylene for 3 days to maintain humidity for infection and subsequent disease development (Joshi *et al.,* 2007a; 2007b).

Percentages were transformed into a 1-4 scale. Cultivars that scored less than 26% were considered resistant, between 26 and 50% as moderately susceptible; between 60 and 70% as susceptible, and those having higher than 70 as highly susceptible (Joshi *et al.,* 2007a; 2007b).

Statistical analysis. For every line, disease scores of all the plants including the most susceptible and most resistant ones were recorded. Broad-sense heritability

*Author for correspondence; E-mail: ascientific@aec.org.sy

(h^2b) was used to eliminate the influence of environment on the expression of disease severity (Mulitze and Baker, 1995) and computed as follows:

$h^2_b = \sigma^2_G / \sigma^2_P \times 100$, $\sigma^2_G = \sigma^2_P - \sigma^2_E$, $\sigma^2_E = \sigma^2_{P_1} + \sigma^2_{P_2} + \sigma^2_{F_1}/3$, $\sigma^2_P = \sigma^2_G - \sigma^2_E$

where:

σ^2_P = phenotypic; σ^2_E = environmental; σ^2_G = genetic; $\sigma^2_{P_1}$ = Banteng; $\sigma^2_{P_2}$ = WI 2291 variance.

Significant differences (p = 0.05) in mean severity values were detected between the two barley cultivars. The cv. Banteng had a mean disease severity of 13.45 %, whereas the susceptible cv. WI 2291 had a mean disease severity 73.44% (Table 1). The general combining ability (GCA) mean square was significant at p = 0.05, which shows the variability of (GCA) of the parent. Estimates of GCA effects of each parental genotype are presented in Table 1. Compared to the parents, the SB severity of the 29 F_1 plants appeared to be intermediate (Fig. 1), indicating the absence of dominance for the genes governing resistance.

Moreover, F_2 progeny distributions in the cross (Fig. 1) indicated that resistance genes interacted in an additive manner. F_2 progeny exhibited a wide range of SB severities from 2 to 88 % (Fig. 1). As discrete classes were observed in the distribution of SB reaction in the F_2, the plants were classified within parental classes using phenotypic values observed for the parents grown in the same environment. This was only applicable to the class of the resistance of parent (≤ 20 %), as the range of variation of the standard resistance parent was similar to that of the F_1. However, in all cases, the results obtained for the F_2 plants demonstrated that the observed segregation pattern fitted 1:15 (X^2 =7.11; ∝ = 0.10) ratios.

Table 1. Range and mean spot blotch (SB) severity (%) of barley parents during two years of testing under different field conditions, and the general combining ability (GCA) estimates in the F_1 generation[y]

		SB reaction		GCA	
Genotype	Origin	Range	Mean	Seedling	Adult plants
Banteng	Germany	1.14-22	13.45	-0.90*	-0.92*
W1229	Australia	54-90	73.44	0.75*	0.72*
				0.18	0.15
F_1		35-50	42.00		
F_2		6-80	41.64		

*Significant at p = 0.05, [y] =Arabi, 2005a

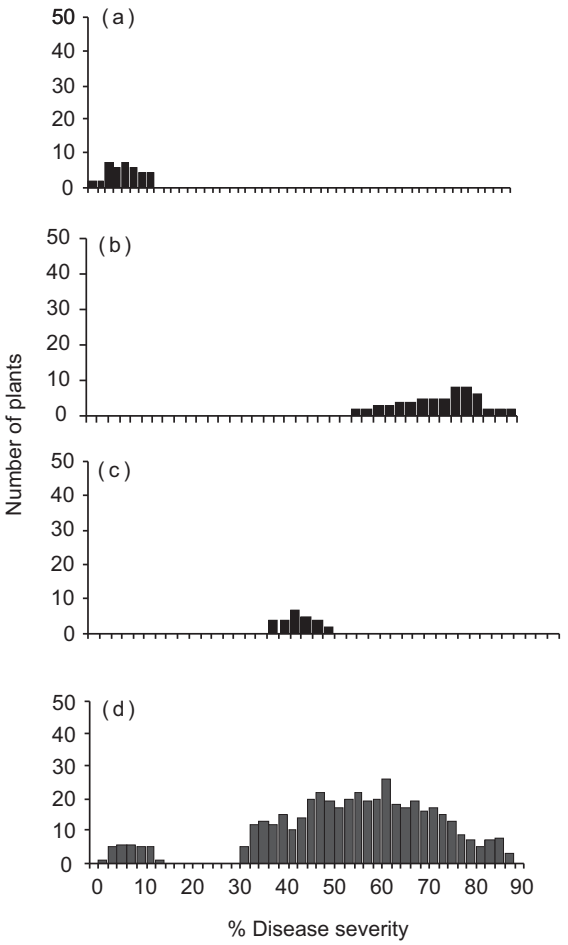

Fig. 1. Histogram of % disease severity in cv. Banteng (a), cv. WI 2291 (b), F_1 (c) and F_2 (d).

Chromosomes 1S and 5S have been identified in barley as harboring loci for SB resistance at the adult stage. Information regarding the genes controlling durable resistance is of a paramount importance to breeders. Some of the previous studies concerning the inheritance of SB resistance in barley also indicated the control by many genes (Kuldeep *et al.,* 2008), who reported that the heritabilities of SB resistance were moderately high and ranged from 0.77 to 0.83 across four environments.

The results of the present study indicate that the heritability of resistance in cv. Banteng was high at 88%. Thus, this cultivar should be considered as a possible donor in future breeding efforts. Since heritability was high, effective selection could be applied in early generations. Furthermore, the cv. Banteng was resistant to net blotch, barley stripe (Arabi, 2005b) and powdery mildew (Arabi and Jawhar, 2012), which could give it special interest in barley breeding programmes.

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Short Communication

Enhancing Shelf Life of Vegetable Oils Blend by Using *Moringa oleifera* Leaf Extract as Antioxidant

Muhammad Nadeem[a]*, Ansar Ullah[a], Muhammad Idnan[b] and Muhammad Ali[a]
[a]Department of Dairy Technology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[b]Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan

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Abstract. The antioxidant activity of ethanolic *Moringa oleifera* leaf extract for oxidative stabilisation of canola, sunflower and soybean oils was investigated at ambient temperature. The blend was prepared by mixing canola, sunflower and soybean oils in equal proportions. Ethanolic *M. oleifera* leaf extract was incorporated into vegetable oils blend at three different concentrations; 300, 600 and 900 ppm (T_1, T_2 and T_3), compared with a control and the sample added with 100 ppm tertiary butylated hydroxyl quinine (TBHQ) was used as a positive control. Filled in one litre transparent PET bottles, kept at room temperature (35-40 °C) for 3 months and sampled at 0 and 90 days for the assessment of oxidative stability. Peroxide value of three months stored blank, T_3 and TBHQ supplemented samples were 2.25, 0.84 and 0.78 (meqO_2/kg). Induction period of blank, T_3 and TBHQ supplemented vegetable oils blend was 3.46, 7.95 and 8.57 h. Peroxide value of blank, T_3 and TBHQ supplemented vegetable oils blend, after 5 days at 63 °C, was 7.55, 2.81 and 2.59 (meqO_2/kg).

Keywords: *Moringa oleifera*, leaf extract, vegetable oils blend, oxidative stability

Blended oils are naturally characterised with higher proportions of unsaturated fatty acids and susceptible to free radical mechanism. Thermal processing of edible oils almost completely eliminates the natural antioxidants (Fereidoon, 2005). To enhance the shelf life of vegetable oils, most of the edible oil producers are using synthetic antioxidants that cause harmful effects on human health. Tertiary butylated hydroxyl quinine (TBHQ) is regarded as the best antioxidant for the inhibition of oxidative breakdown in vegetable oils. Antioxidant potential of *M. oleifera* leaf extract for the stabilisation of olein based butter has been studied earlier (Nadeem *et al*., 2014). However, the antioxidant potential of *M. oleifera* leaf extract for the stabilisation of vegetable oils blend with high degree of unsaturation at ambient temperature has not been studied so far. Therefore, antioxidant activity of *M. oleifera* leaf extract was studied for the long term preservation of canola, sunflower and soybean oils blend on the basis of some chemical characteristics.

Refined, bleached and deodorised canola, sunflower and soybean oils without any additives were obtained from a reputed edible oil processing company. TBHQ was obtained from Rhodia Pakistan Ltd. *M. oleifera* leaves were collected from a village of district Muzzafar Garrh. The chemicals were HPLC grade and purchased from Sigma Aldrich, USA.

*Author for correspondence; E-mail: nadeem1220@gmail.com

Ethanolic *M. oleifera* leaf extract was prepared according to the method of Anwar *et al*. (2007). Canola, sunflower and soybean oils were blended in equal concentration (33.33%). *M. oleifera* leaf extract was incorporated into vegetable oils blend at three different concentrations; 300, 600 and 900 ppm (T_1, T_2 and T_3), compared with a control (blank; with no addition of extract) and the sample added with 100 ppm TBHQ was used as a positive control. Filled in one litre transparent PET bottles were kept at room temperature (35-40 °C) for 3 months. Sampling frequencies for the chemical analysis were 0 and 90 days of storage period.

Total phenolic content of *M. oleifera* leaf extract was determined in terms of gallic acid by following the method of Anwar *et al*. (2007). Schaal oven test (63 °C for 5 days), peroxide (Cd 8-53) and anisidine (Cd 18-90) values were determined according to the standard methods of AOCS (1995). Conjugated dienes and trienes were determined according to the standard methods of IUPAC (1987). Determination frequencies for peroxide value, anisidine value, conjugated dienes and conjugated trienes were 0 and 90 days. Induction period was determined by oxidising the 2.5 g samples in the reaction vessels by steady stream of oxygen at 120 °C by using Metrohm Rancimat Model-679 (Metrohm, 1993).

Statistical analysis. Each sample was analysed thrice and each treatment was replicated three times, one way

and two way analysis of variance techniques were used to find out the effect of storage and treatments (Steel *et al*., 1997). The significant variation ($P<0.05$) among the treatments was made by using Duncan's Multiple Range Test (DMR).

Total phenolic content of *M. oleifera* leaf extract was 6.9±0.14% gallic acid on dry basis; almost similar to the earlier findings (Nadeem *et al*., 2013a; Anwar *et al*., 2007). Peroxide value slowly and steadily increased during the storage period of 90 days. The rise in peroxide value was dependent upon the presence and concentration of *M. oleifera* leaf extract. The inhibition of lipid peroxidation at all the determination frequencies was in the order of $T_3 > T_2 > T_1 >$ blank (Table 1). The antioxidant activity of T_3 and 100 ppm TBHQ were at par with each other. The inhibition of peroxides and concentration of *M. oleifera* leaf extract were strongly correlated (Fig. 1, $R^2=0.9844$). The strong inhibition of autoxidation process can be attributed to the higher extents of wide range of phenolic compounds in leaves of *M. oleifera*. Supplementation of sunflower oil and butter oil with *M. oleifera* leaf extract strongly inhibited the autoxidation (Nadeem *et al*., 2013a; Anwar *et al*., 2007). Anisidine value indicates the secondary stages of autoxidation, which are characterised by the formation of aldehydes, ketonses, alcohols and odoriferous compounds (Table 1). Formation of secondary oxidation products were considerably inhibited by the addition of *M. olefiera* leaf extract, even then the storage temperature and concentration of unsaturated fatty acids were on higher side. The recommended temperature for the storage of butter fat is -18 °C, supplementation of butter with *M. oleifera* leaf extract enabled the storage of butter at refrigeration temperature (Nadeem *et al*., 2013b). Oxidation products in the form of conjugated dienes and trienes went increasing during 3 months storage period at varying rate, the yield of oxidation products in three months stored vegetable oils blend was in the order of $T_3 > T_2 > T_1 >$ blank. Supplementation of canola oil with wheat bran extract efficiently inhibited the generation of oxidation products (Chatha *et al*., 2011). Induction period and Schaal oven test were used to assess the antioxidant potential of antioxidants. Induction period of blank, T_3 and TBHQ supplemented vegetable oils blend was 3.46, 7.95 and 8.57 h. Peroxide value of blank, T_3 and TBHQ supplemented vegetable oils blend, after 5 days at 63 °C, was 7.55, 2.81 and 2.59 ($M_{eq}O_2$/kg) (Fig. 2-3). The strong antioxidant activity of sesame cake extract for the stabilisation of olein based butter has been reported in the literature (Nadeem *et al*., 2014; 2013a). *M. oleifera* leaf extract therefore, can be used for the long term storage of vegetable oils blend at ambient temperature.

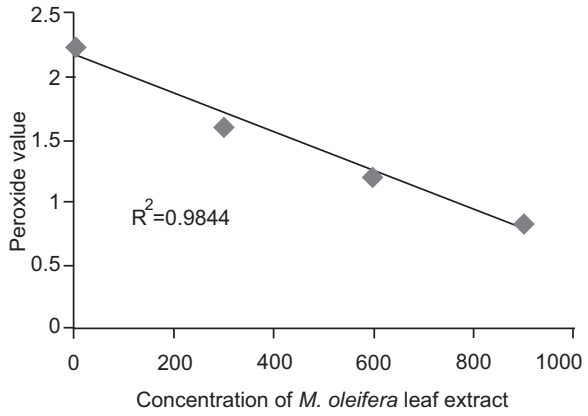

Fig. 1. Correlation between dose of extract and induction period.

Table 1. Effect of *Moringa oleifera* leaf extract on storage stability of vegetable oils blend

Parameters	Storage days	TBHQ	T_1	T_2	T_3	Blank
Peroxide value ($M_{eq}O_2$/kg)	0	0.25±0.02a	0.25±0.02a	0.25±0.02a	0.25±0.02a	0.25±0.02a
	90	0.78±0.04d	1.62±0.11b	1.24±0.05c	0.84±0.08d	2.25±0.06a
Anisidine value	0	4.59±0.19a	4.59±0.19a	4.59±0.19a	4.59±0.19a	4.59±0.19a
	90	8.89±0.25d	16.97±0.33b	11.79±0.18c	9.13±0.42d	24.37±0.91a
Conjugated dienes	0	0.16±0.01a	0.16±0.01a	0.16±0.01a	0.16±0.01a	0.16±0.01a
	90	0.74±0.12d	1.85±0.22b	1.14±0.05c	0.92±0.08d	2.58±0.11a
Conjugated trienes	0	0.05±0.01a	0.05±0.01a	0.05±0.01a	0.05±0.01a	0.05±0.01a
	90	0.35±0.04a	0.82±0.06b	0.59±0.04c	0.44±0.04a	1.29±0.14a

Values are mean ± SD, n = 3. Values followed by the same letter in rows are not significantly different ($p<0.05$).

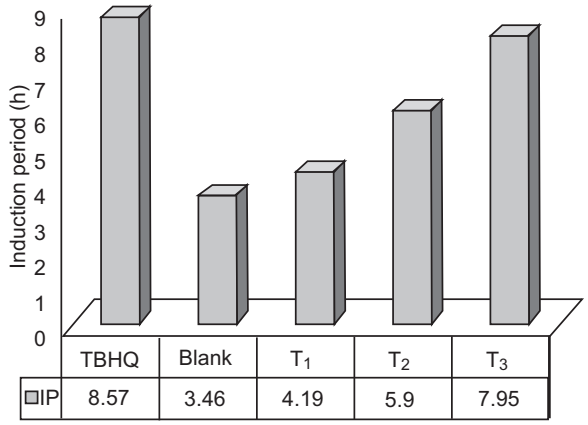

Fig. 2. Induction period.

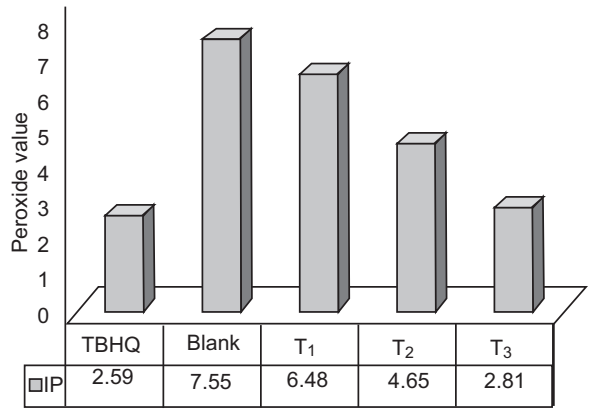

Fig. 3. Peroxide value in Schaal oven test ($M_{eq}O_2$/kg).

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