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Research Article

Statistical Modeling of Groundwater Quality for Source and Ionic Relationships: A Case Study for Drinking Water Quality

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Abstract: A study was conducted to check the ground water quality of the University of Engineering and Technology (UET) campus, Lahore during October 13, 2014 to November 10, 2014. For this purpose grab samples were collected from four tube wells and eight end users for five weeks. These samples were analyzed for chloride (Cl^-), total dissolve solids (TDS), fluoride (F^-), pH, electrical conductivity (EC) and heavy metals (Cr, Pb, Ni, Fe) using prescribed methods and Atomic Absorption Spectrophotometer, respectively. Statistical tools were used for the source and correlation of ground water quality. Symmetrical (Cl^-, pH, EC, F^-, TDS, Ni, Fe) and non-symmetrical (Cr, Pb) distribution was observed. The Spearman and Pearson correlation matrix showed a correlation among heavy metals and physical parameters. Analysis of Variance (ANOVA) results also supported this correlation. The Principal Component Analysis (PCA) and the Cluster Analysis (CA) data identified four sources of chemical species in ground water, i.e. landfill leachates, emissions from vehicles, seepage of industrial emissions and tanneries wastewater, which enhanced the levels of heavy metals contamination in groundwater. Enrichment factor (EF) also indicated anthropogenic activities for the elevated levels of heavy metals in the ground water. The mean concentration of Cr (0.52 mg L^{-1}), Pb (0.08 mg L^{-1}) and Ni (0.08 mg L^{-1}) were higher than the permissible values while that of Fe was within permissible limit for drinking purposes.

Keywords: Heavy metals, physical parameters, Statistical Analysis, ground water, drinking water quality, ionic relationships, spatial and temporal contaminant variation

1. INTRODUCTION

The contamination of ground water is a critical issue throughout the world and several studies have been conducted to assess the severity of the problem [1-5]. Ground Water could be polluted by physical (pH, temperature, turbidity etc.) or chemical parameters (heavy metals, Fl, etc.) [3, 5-7]. The presence of heavy metals has been attributed to groundwater aquifer or through anthropogenic activities [1, 5, 8, 9]. The heavy metals in water pose serious issues due to their toxic and carcinogenic characteristics not only for drinking but for other life sustaining activities [9].

Earlier studies on groundwater of Lahore have exposed some critical facts that exacerbates the situation revealing serious levels of contaminants [1, 2, 10], like high levels of TDS and EC. Similarly, the industrial areas are more prone to contamination of groundwater [5] with high levels of Cd, Cr, Fe, As, Pb and Zn [2]. In order to have a clear idea of quality of groundwater, statistical modeling of groundwater was done for various physical and chemical parameters. The data were used for modeling to identify the source of emission and extent of anthropogenic activities. For this purpose various statistical tools like the Descriptive Statistics, the Box and Whisker plots, the Pearson and Spearman Correlations, Analysis of Variance (ANOVA), Principal Component Analysis (PCA) and Cluster Analysis (CA) were performed using SPSS IBM software. Enrichment

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Factor (EF) was calculated to ascertain the effects of anthropogenic activities for heavy metals.

2. MATERIALS AND METHODS

2.1 Study Area

Lahore is the second largest city of Pakistan with a population of 7.566 Millions in 2011 [11]. The water supply for domestic, industrial and commercial uses mainly comes from the groundwater which is estimated at 3.79, 0.92 and 0.77 MCM/day, respectively. The ground water quality of Lahore has been deteriorated due to excessive water use, untreated wastewater discharge into rivers and open dumping of un-segregated solid waste [12, 13]. To check the quality of ground water in terms of pollution levels, for source identification and correlation, an educational institute the University of Engineering and Technology (UET) Lahore was selected. Distribution system and sewerage system of UET is still the same from time of establishment, i.e. the year 1921. There is a tannery only 2-3 km away, a waste water and solid waste disposal point ≈ 1-2 km away from the UET campus which could be assumed major sources of ground water pollution (Fig. 1). Sampling sites (four tube wells) were selected in whole university. These were taken as source and eight different locations (Tap water supplied from these tube wells) were considered as end users (Table 1).

Table 1. Description of water sampling locations.

Location	Location Symbol	No. of Samples
Annaxie tube well (direct, 690 ft depth)	TW-1	5
Annaxie juice center (tap)	L-2	5
Annaxie (before entering)	L-3	5
Tube well behind shopping center (direct, 625 ft depth)	TW-4	5
Sultan Mehmood Ghaznavi hostel (tap)	L-5	5
Inside shopping center (tap)	L-6	5
Staff colony tube well (direct, 653 ft depth)	TW-7	5
40-B Staff colony, Operator's house	L-8	5
R-94 Staff colony (tap)	L-9	5
Tube well Khadija hall (direct, 715 ft depth)	TW-10	5
Khadija Hall hostel (tap)	L-11	5
GSSC UET (tap)	L-12	5

2.2 Sampling

Grab samples were taken from tube wells which were pumped sufficiently (10 min) in order to ensure that they represented the ground water. Samples from distribution system were taken after

Fig. 1. Google map showing locations of water samples.

flushing the lines sufficiently (30 min) ensure that samples were representative of supply system [6, 14]. Sampling was done from October 13, 2014 to November 10, 2014 in which a total of 60 samples were collected; 5 samples from each location with an interval of one week. This interval of one week was selected to have a good idea of quality of water. There can be changes in aquifer due to some weather conditions and geological conditions proving longer time the best for the sake of analytical study. Samples were collected with care to avoid any contamination that might cause any uncertainty in results.

2.3 Analytical Procedures

After sampling, the collected samples were analyzed in the laboratory for different parameters using standard methods [15].

3. RESULTS AND DISCUSSION

Different statistical tools like Descriptive Analysis, Box & Whisker Diagrams, Spearman's and Pearson's Correlation Coefficient, ANOVA, PCA and CA were applied to check the time based variation on water quality and finding the ultimate sources of contamination.

3.1 Descriptive Analysis

The Descriptive Statistics in the forms of mean, variance (V), standard deviation (SD), standard error (SE), median, range of variation, and percentile at 95 %, 75 % and 25 % (P95 %, P75 %, P25 %) has been computed [1] which is shown in Table 2. The mean value of Cl (186.89 mg L^{-1}), pH (7.55), Fl (0.332 mg L^{-1}) and TDS (519.46 mg L^{-1}) were within the permissible values of WHO (Table 2). The mean values of Cr, Pb, Ni and Fe were 0.518, 0.083, 0.079 and 0.091 mg L^{-1}, respectively. The values of Cr, Pb, and Ni exceeded the permissible limits by WHO while that of Fe was within limits set for taste detection (Table 2) [6]. The smaller values of standard error and standard deviation for the measured parameters reflected that the samples were more representative of the overall study area. The values of skewness (close to zero but not exactly zero) reflect symmetrical and non-symmetrical distribution of parameters among locations.

3.2 Distribution Patterns

In the Box Whisker plots data remain symmetrical if the data are evenly split at the median, and data will be asymmetrical or skewed if median is either right or left side of plots [7]. The results of Box Whisker graphs (Fig. 2) show that data is not normally distributed and there is a lot of un-symmetry in data. Median ranges for each parameter are different in every week. The Cl, pH and F showed negative skewness. The EC showed

Table 2. Descriptive statistical data for all the parameters for the sampling period (n=60).

Parameter	Cl (mg L^{-1})	pH	EC (µS cm^{-1})	F (mg L^{-1})	TDS (mg L^{-1})	Cr (mg L^{-1})	Pb (mg L^{-1})	Ni (mg L^{-1})	Fe (mg L^{-1})
Mean	186.89	7.55	791.08	0.33	519.47	**0.52**	**0.08**	**0.08**	0.09
Standard Error	11.86	0.04	35.88	0.01	32.03	0.13	0.03	0.01	0.01
Median	166.77	7.46	756	0.3	469	0.01	0	0.09	0.03
Standard Deviation	91.86	0.34	277.92	0.07	248.09	1.01	0.21	0.06	0.11
Skewness	0.16	0.68	0.32	0.54	0.42	1.54	2.7	-0.15	0.86
Minimum	40.02	6.96	339	0.2	142	0	0	0	0
Maximum	366.88	8.52	1396	0.5	1020	2.66	0.96	0.17	0.34
WHO Guidelines [6]	250	6.5-8.5	---	1.5	1000	0.05	0.01	0.02	---

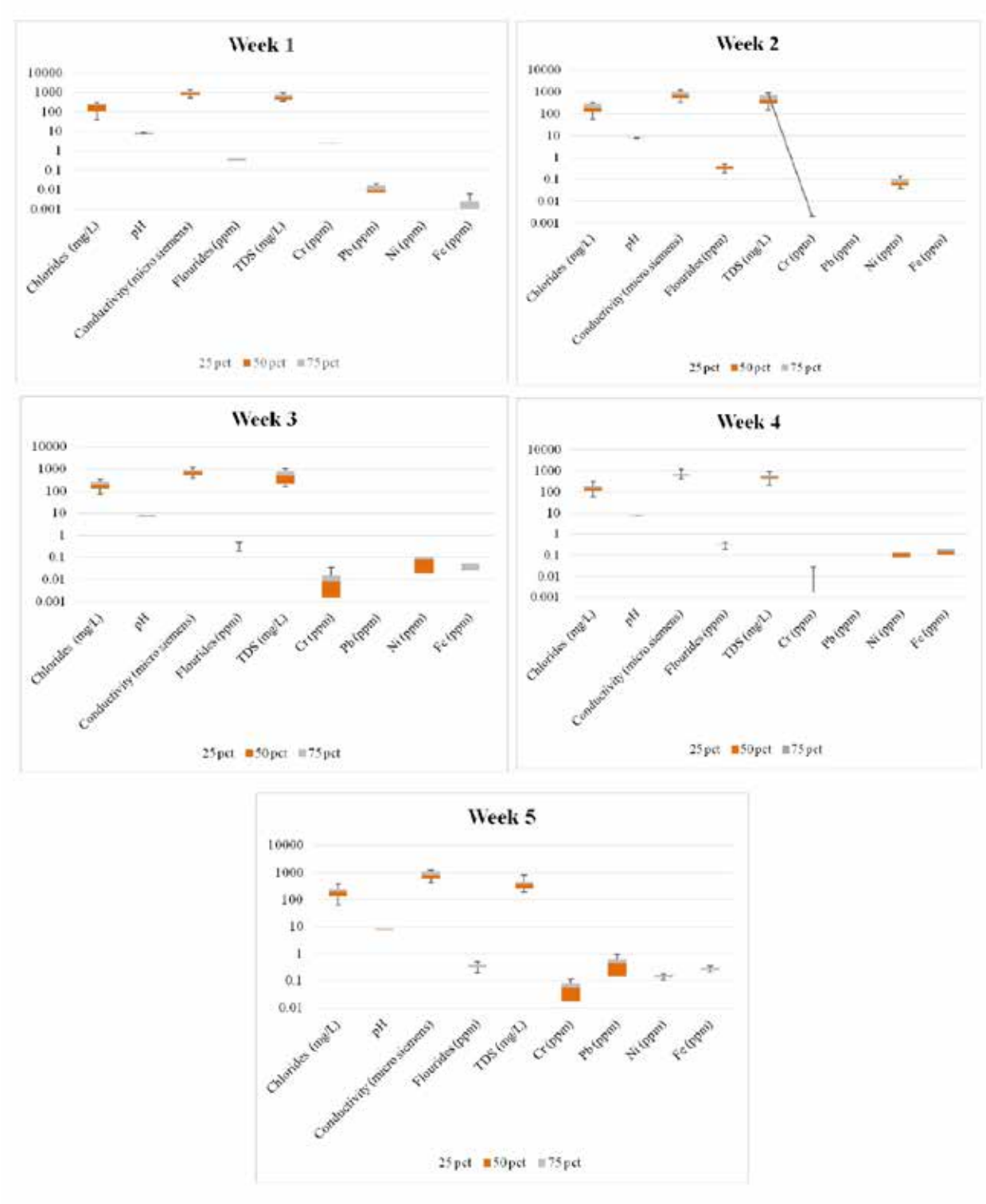

Fig. 2. Box and Whisker plots for five weeks of water sampling.

Table 3. Spearman correlation coefficient matrix (r-values) among all the parameters for five weeks of sampling.

	Cl^-	pH	EC	F	TDS	Cr	Pb	Ni	Fe
					Week-1				
Cl^-	1								
pH	-0.639*	1							
EC	0.884**	-0.554	1						
F^-	-0.231	0.54	-0.358	1					
TDS	0.663*	-0.428	0.804**	-0.307	1				
Cr	-0.701*	0.476	-0.501	-0.128	-0.116	1			
Pb	-0.637*	0.432	-0.466	-0.103	-0.018	0.958**	1		
Ni	0	0	0	0	0	0	0	1	
Fe	-0.07	-0.251	0.029	0.214	-0.149	-0.124	-0.12	0	1
					Week-2				
Cl^-	1								
pH	-0.743**	1							
EC	0.921**	-0.594*	1						
F^-	-0.853**	0.651*	-0.846**	1					
TDS	0.942**	-0.622*	0.965**	-0.796**	1				
Cr	0.131	0.131	0.044	0.19	0.218	1			
Pb	0	0	0	0	0	0	1		
Ni	-0.088	-0.049	0.06	-0.019	0.144	-0.044	0	1	
Fe	0	0	0	0	0	0	0	0	1
					Week-3				
Cl^-	1								
pH	-0.825**	1							
EC	0.930**	-0.822**	1						
F^-	-0.831**	0.752**	-0.840**	1					
TDS	0.837**	-0.746**	0.844**	-0.755**	1				
Cr	-0.538	0.676*	-0.646*	0.472	-0.678*	1			
Pb	0.039	-0.067	0.09	0.201	0.147	0.011	1		
Ni	0	0	0	0	0	0	0	1	
Fe	-0.569	0.194	-0.476	0.289	-0.356	0.39	0.096	0	1
					Week-4				
Cl^-	1								
pH	-0.771**	1							
EC	0.752**	-0.760**	1						
F^-	-0.279	0.185	-0.277	1					
TDS	0.661*	-0.238	0.531	-0.416	1				
Cr	0.378	-0.175	0.514	-0.199	0.239	1			
Pb	0	0	0	0	0	0	1		
Ni	-0.204	0.023	0.046	-0.301	0.14	-0.57	0	1	
Fe	-0.837**	0.613*	-0.664*	0.517	-0.552	-0.395	0	0.042	1
					Week-5				
Cl	1								
pH	-0.659*	1							
EC	0.648*	-0.309	1						
F	-0.732**	0.588*	-0.388	1					
TDS	0.746**	-0.372	0.51	-0.583*	1				
Cr	-0.757**	0.214	-0.545	0.54	-0.727**	1			
Pb	-0.702*	0.214	-0.515	0.596*	-0.658*	0.963**	1		
Ni	-0.074	0.207	-0.378	0.184	0.007	0.238	0.273	1	
Fe	0.081	-0.509	0.133	-0.219	0.091	0.378	0.441	-0.147	1

* Significant at 0.05 level (2-tailed).
** Significant at 0.01 level (2-tailed).

Table 4. Pearson correlation coefficient matrix (r-values) for all the parameters for five weeks of sampling (n=60).

	Cl^-	pH	EC	F	TDS	Cr	Pb	Ni	Fe
Week 1									
Cl	1								
pH	-0.805**	1							
EC	0.957**	-0.779**	1						
F	-0.226	0.359	-0.322	1					
TDS	0.664*	-0.567	0.811**	-0.384	1				
Cr	-0.757**	0.634*	-0.572	-0.176	-0.103	1			
Pb	-0.699*	0.574	-0.548	-0.059	-0.016	0.912**	1		
Ni	0	0	0	0	0	0	0	1	
Fe	0.198	-0.231	0.181	0.054	-0.044	-0.17	-0.237	0	1
Week 2									
Cl	1								
pH	-0.765**	1							
EC	0.959**	-0.795**	1						
F	-0.760**	0.855**	-0.831**	1					
TDS	0.949**	-0.688*	0.930**	-0.701*	1				
Cr	-0.608*	0.739**	-0.618*	0.492	-0.620*	1			
Pb	0	0	0	0	0	0	1		
Ni	0.211	0.009	0.157	0.02	0.317	0.169	0	1	
Fe	-0.408	0.096	-0.288	0.042	-0.325	0.352	0	0.033	1
Week 3									
Cl	1								
pH	-0.702*	1							
EC	0.958**	-0.638*	1						
F	-0.834**	0.732**	-0.822**	1					
TDS	0.957**	-0.623*	0.984**	-0.822**	1				
Cr	0.195	0.148	0.042	0.165	0.148	1			
Pb	0	0	0	0	0	0	1		
Ni	-0.171	-0.07	-0.071	-0.049	-0.017	-0.1	0	1	
Fe	0	0	0	0	0	0	0	0	1
Week 4									
Cl	1								
pH	-0.742**	1							
EC	0.922**	-0.604*	1						
F	-0.532	0.192	-0.521	1					
TDS	0.757**	-0.326	0.767**	-0.447	1				
Cr	0.348	-0.146	0.333	-0.137	0.155	1			
Pb	0	0	0	0	0	0	1		
Ni	-0.066	0.23	0.086	-0.332	0.162	-0.589*	0	1	
Fe	-0.864**	0.736**	-0.744**	0.532	-0.48	-0.368	0	0.028	1
Week 5									
Cl	1								
pH	-0.708*	1							
EC	0.673*	-0.404	1						
F	-0.741**	0.545	-0.303	1					
TDS	0.795**	-0.475	0.607*	-0.623*	1				
Cr	-0.741**	0.368	-0.615*	0.502	-0.712**	1			
Pb	-0.682*	0.309	-0.52	0.512	-0.607*	0.966**	1		
Ni	0.039	0.083	-0.35	-0.038	0.103	0.107	0.107	1	
Fe	0.253	-0.453	0.163	-0.336	0.36	0.232	0.368	0.09	1

* Significant at P= 0.05 level (2-tailed).
** Significant at P= 0.01 level (2-tailed).

positive skewness in week-4 and negative during remaining weeks. The TDS reflected negative skewness in week-2 and 5 and positive during the remaining weeks. The Cr and Pb showed positive skewness in almost all weeks. The Ni and Fe gave positive skewness in first 3 weeks and negative skewness in weeks-4 and 5. Overall, there is little symmetry in data (Fig. 2).

3.3 Pearson Correlation Coefficients

The results of Spearman's correlation coefficients were performed by SPSS using two-tailed correlation and revealed that the Cl have strong negative correlation with pH, F and Pb while strong positive correlation with EC, TDS and Cr. Similarly, pH showed moderate negative correlation with EC. It also showed strong positive correlation with First two weeks and moderate positive in last two week. This variation indicated some anthropogenic activities affecting the ground water quality [12]. The pH showed moderate negative correlation with TDS while moderate positive correlation with Cr and Pb. The EC showed moderate negative correlation with F while, it showed strong positive correlation with TDS. The EC showed moderate negative correlation with Cr and moderate negative correlation with Pb. Fluoride showed moderate negative correlation with TDS while it showed moderate positive correlation with Cr. Similarly, TDS showed moderate negative correlation with Cr in one week and strong negative correlation in one week. TDS also showed moderate negative correlation with Fe while Cr showed strong positive correlation with Pb. The variations among correlation indicate that these physicochemical parameters, affecting the quality of ground water, are not originating from the same source; otherwise it showed strong correlations which are supportive of the effect of anthropogenic activities [4, 7, 12].

3.4 Spearman Correlation Coefficients

Chloride showed strong negative correlation with pH, strong positive correlation with EC while Chloride showed strong negative with F. It showed strong positive correlation with TDS while strong negative correlation with Cr. Chloride indicated moderate negative correlation with Pb in one week while strong negative in other week. Chloride indicated moderate negative correlation with Fe in one week and strong negative correlation in one week. Similarly, pH showed moderate negative correlation with EC and it showed moderate positive correlation with F. pH showed moderate negative correlation with TDS, moderate positive correlation with Cr, moderate positive correlation with Fe in one week but moderate negative correlation in the other week. The EC showed moderate negative correlation with F in two weeks and strong negative correlation in the other two weeks. The EC indicated strong positive correlation with TDS while moderate negative correlation with Cr, moderate negative correlation with Pb and Ni. Fluoride showed moderate negative correlation with TDS but moderate positive correlation with Cr. The TDS showed moderate negative correlation with Cr in one week and strong negative correlation in the other one week and moderate negative correlation with Fe. Similarly, Cr exhibited strong positive correlation with Pb and moderate positive correlation with Fe.

3.5 Analysis of Variance (ANOVA)

The ANOVA was used to evaluate the relationships between metals and other parameters. For this two null hypothesis (H_o) was formulated, i.e. heavy metals don't have any correlation with physiochemical parameters, i.e., Cl, EC, F, pH or TDS at $\alpha < 0.05$ and there is no correlation among physicochemical parameters. The results of ANOVA, physicochemical parameters are shown in (Table 5). It revealed that Cl has some correlation with pH and F. Pearson and Spearman correlation also supported these findings. The ANOVA for heavy metals and physicochemical parameters (Table 6) revealed that metals have correlation with EC and TDS due to their high ionic solubility, but have no correlation with other parameters.

Table 5. Analysis of variance for correlation of chlorides with physical parameters.

ANOVA		
Physical Parameters	**Chloride Effect**	
	F value	**Significance**
pH	1.203	1.581
F	0.325	0.125

Table 6. Analysis of variance for correlation of physical parameters with heavy metals.

Metal	**Cl**		**EC**		**F**		**pH**		**TDS**	
	F	**Sign.***	**F**	**Sign.***	**F**	**Sign.***	**F**	**Sign.***	**F**	**Sign.***
Cr	5.043	0	0.459	0.899	0.854	0.471	0.661	0.858	0.309	0.923
Pb	2.195	0.025	0.542	0.851	1.865	0.146	0.944	0.438	0.325	0.916
Ni	2.859	0.005	0.442	0.909	0.438	0.727	0.786	0.74	0.273	0.939
Fe	1.523	0.145	1.041	0.582	0.218	0.884	0.538	1.102	0.947	0.256

*Significant at P = 0.05

3.6 Principal Component Analysis (PCA)

The PCA is based on an imaginary Eigen values. In the present study, all the Eigen values < 1 were ignored. The components having Eigen value > 1 are grouped based on same source. The PCA using rotation method of Varimax and Kaiser Normalization was performed and the results have been shown below in Table 7. The tool was applied on all the parameters for source identification. The PCA gave three components named as PC 1, PC 2 and PC 3 which explained a total of 78.329 % of variance. The PC 1 explained the highest share (39.43 %) of the total variance followed by PC 2 (27.01 %) and PC 3 (11.87 %). The PC 1 expressed the highest loading for Cl, EC, F and TDS which reflected seepage to groundwater aquifer from sewage effluent discharges, urban runoff, industrial waste discharges and contamination form refuse leachate to the ultimate problem. Moreover, dissolution of salt deposits in the aquifer can increase chloride levels and waters in the areas of Palaezoic and Mesozoic sedimentary rocks have higher TDS levels, ranging from as little as 195 to 1100 mg L^{-1} [16]. The PC 2 showed the highest loading for Pb and Fe which indicated dissolution of rocks and minerals in the aquifer or anthropogenic activities like seepage of wastewater from pigments, ammunition, caulking, cable sheathing, iron related industries, acid mine drainage and landfill leachates as sources. The PC 3 showed maximum loading for Cr which reflected contamination from the seepage of industrial emissions and tanneries wastewater [6].

Table 7. Principal Component loadings for water quality parameters using Varimax with Kaiser Normalization**.**

Rotated Component Matrix[a]			
Parameter	**Component**		
	1	**2**	**3**
Cl	0.909	-0.019	0.047
pH	-0.599	0.026	0.307
EC	0.866	0.101	0.371
F	-0.781	0.165	0.19
TDS	0.865	-0.168	0.213
Cr	0.051	-0.103	0.899
Pb	-0.161	0.93	-0.001
Ni	0.011	0.534	-0.733
Fe	-0.008	0.81	-0.429

Table 8. Enrichment factor for heavy metals for five weeks taking crustal average of Fe as reference.

Metals	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
Fe	1	1	1	1	1
Cr	115.5	137.6	164.0	97.6	184.2
Pb	333.0	326.0	332.5	442.3	333.9
Ni	351.6	347.7	393.6	361.9	308.5

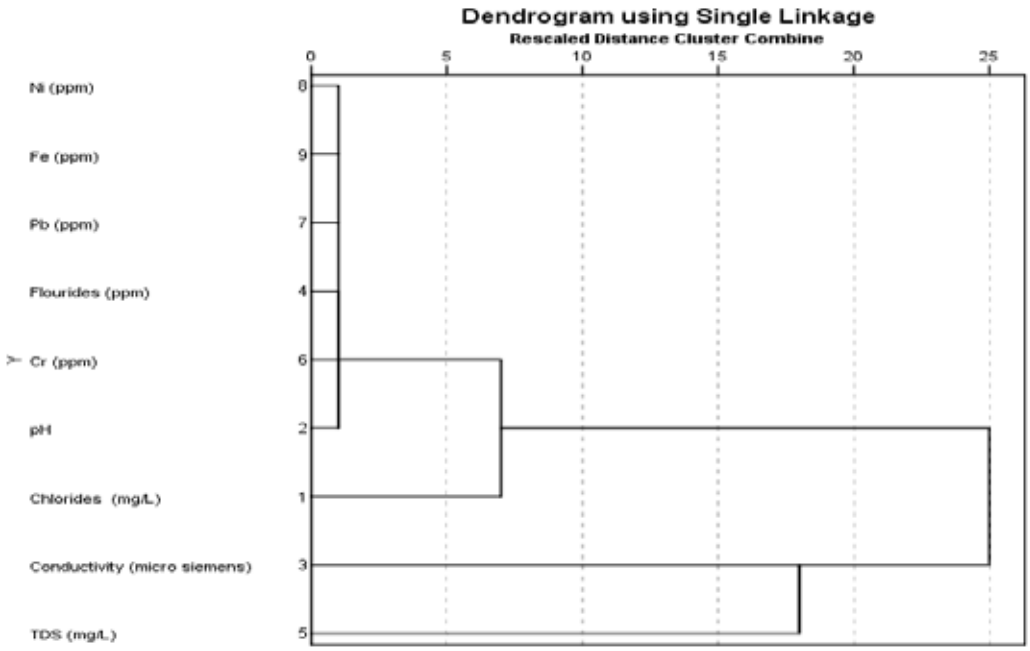

Fig. 3. Dendrogram of all parameters using Cluster Analysis showing various grouping on the basis of correlations.

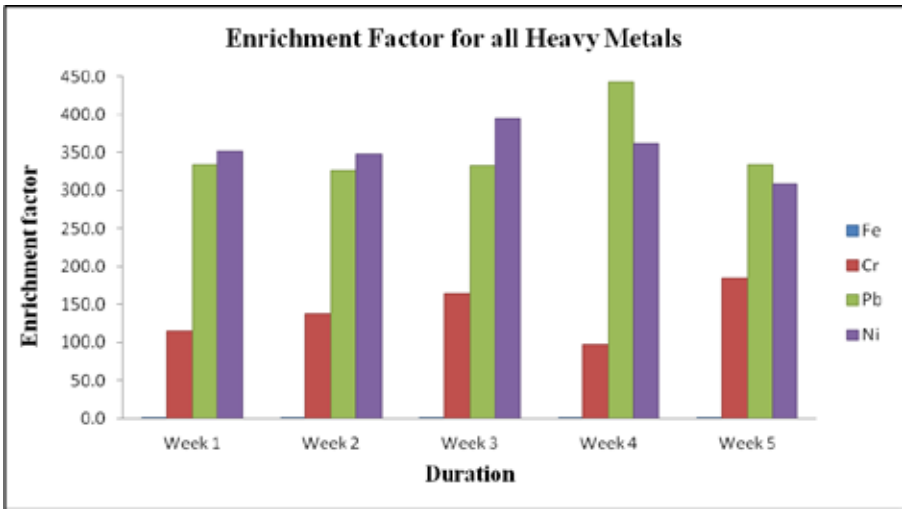

Fig. 4. Enrichment factor for all heavy metals using Fe as reference metal. It showed that the anthropogenic activities were responsible for contamination of groundwater.

3.7 Cluster Analysis (CA)

A tree diagram shows the agglomerative hierarchical clustering algorithms available in the data and is called Dendrogram [7]. The extent of correlation among parameters using Cluster analysis Dendrogram (single linkage) has been performed on average values of each parameter on all the 12 locations (Fig. 3). These groups were formed on the basis of CA, i.e., G_1, G_2 and G_3. The Ni, Fe, Pb, F, Cr and pH formed a single group (G_1). Euclidean distance was less so it showed strong relation as is evident in the Fig. 3 and in the results of PCA. The EC and TDS formed another group (G_2), Euclidean distance was more, so relation can be supposed as weak. Third group was formed by G_1 and Cl (G_3) relation was not too strong. The G_2 and G_3 groups are distinct from each other (Fig. 3) having large euclidean distance

which reflect that both have different sources. The Dendrogram relationship indicated that investigated heavy metals have strong correlation among themselves and poor correlation with other physicochemical parameters.

3.8 Enrichment Factor for Trace Metals

To calculate the extent of anthropogenic activities for relative increase in trace metals concentration; Enrichment Factor (EF) was measured. Enrichment factor of metals relative to earth crust composition was calculated. It demonstrated that the metals concentration increased due to human activities. The EF was computed by knowing the mean concentration of metals in earth crust and in samples of study area [15]. The Fe levels were taken as reference for enrichment factor calculation in this study and EF were calculated by using following relationship:

$$EF = \frac{\left(\frac{C_{Me}}{C_{Fe}}\right)Sample}{\left(\frac{C_{Me}}{C_{Fe}}\right)Crust} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where C_{Me} represents the concentration of metal, which is to be compared with concentration of Fe.

Trace metals having EF values ≤ 5 are not considered as enriched because some degree of uncertainty is present in the composition of the Earth crust [17]. Elements having EF values 10-100 are moderately enriched and have sources other than the Earth crust while metals having EF values ≥ 100 are highly enriched and have different sources of emission, i.e. anthropogenic activities [17]. The EF values of Fe, Cr, Pb and Ni are shown in Table 8. All the values are > 100 during the study period (five weeks) strongly supporting anthropogenic activities to be the sources of heavy metals (Fe, Cr, Pb, and Ni) in the groundwater. The order of EF is in the increasing order of Pb > Ni > Cr > Fe.

4. CONCLUSIONS

The analysis of groundwater quality has been done for important parameters and statistical analysis was performed for source identification and correlation. The following conclusions could be drawn on the basis of study:

- The concentration of physical parameters and Fe among heavy metals was found within WHO guidelines but the concentration of Cr, Pb, and Ni remained higher than their respective permissible limits.
- The concentration of metals was in the decreasing order of Cr > Pb > Ni. Concentrations of Cl, pH, EC, F, TDS, Ni and Fe showed almost symmetrical distribution while a little non-symmetrical trend was recorded for Cr and Pb.
- Spearman and Pearson correlation showed that Ni, Cr, Pb and Fe have low correlation with physical parameters. The ANOVA results reflected that physical parameters were associated with heavy metals except Cl which effected metals; and showed relation only with Fe amongst all the heavy metals present.
- The PCA and CA data identified various anthropogenic and natural sources for the enhanced levels of heavy metals in water samples from UET campus. The assumed sources identified included vehicular emissions, acid mine drainage, landfill leachates, seepage of industrial emissions and tanneries wastewater in the aquifer as the sources of contamination (Fig.1).
- Enrichment Factor showed moderate and higher contribution to heavy metals by anthropogenic activities and trend observed was Pb > Ni > Cr > Fe.
- Environmental rules and legislations should be strictly implemented and industries should be forced to dispose wastewater after treatment in compliance with the available standards of the country. Community should be given awareness on importance of wastewater treatment and safe disposal of the resulting effluent. Septic tank can be best option in such localities the effluent of which can be disposed into some nearby drain.

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Research Article

Analysis of Raw Meat for Heavy Metals and Bacterial Contamination and Comparison of Antibiotic Susceptibility of Isolated Bacteria

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Abstract: The focus of the study was to analyze the commercially available meat for its heavy metal contents and bacterial contamination. The meat samples were collected from four commercial markets of Lahore, i.e., as Wafaqi Colony (Site I), Township (Site II), G-1 Market (Site III) and Zenith (Site IV), and analyzed for heavy metal [i.e., manganese (Mn), nickel (Ni), chromium (Cr), cadmium (Cd) and copper (Cu)] contents and bacterial contaminants (*E. coli, Pseudomonas sp., Bacillus sp.* and *Salmonella sp.)* Atomic absorption spectrophotometery was employed for the detection of the heavy metals and plate count method was used for the detection of bacterial contaminants. The Ni concentration in the Site II sample only and Cd concentration in all meat samples were found above the standard value and the concentration of other metals (Cu, Cr, and Mn) was less than the standard concentrations. Bacterial (*E. coli, Pseudomonas sp., Bacillus sp., Salmonella sp.* and *Staphylococcus sp.*) contamination was found in all meat samples; however, the number was a little lower in the Site IV samples. Statistical analysis was done, by one-way ANOVA using SPSS, to compare heavy metal contamination in the meat samples. The results showed distribution of heavy metals in all meat samples; there was significant difference of Ni concentration in the meat samples. The measure of antibiotic susceptibility showed that isolated species of bacteria were resistant to lincomycin, streptomycin, tertracyclin, ampicillin, amoxicillin and doxycyclin, but did not survive in the medium containing ofloxacin.

Keywords: Heavy metal, Bacterial contamination, *E. coli, Pseudomonas sp., Bacillus sp., Salmonella sp., Staphylococcus sp.*

1. INTRODUCTION

Heavy metals are called trace metals when these are present in low concentration. Heavy metals are of two types, essential and non-essential heavy metals. When trace metals (Fe, Mn, Zn, and Cu) are found in body in small concentration, they are essential for the existence and survival of the living organisms. The trace metals can be found naturally in the rocks, water and soil bodies. Presence of heavy metals can cause vey toxic and harmful effects on the exposed plants, animals and human beings [1]. The combustion of fossil fuels, the utilization of antiseptics and disinfectants, the exhausted batteries, poor agricultural practices and disposing of industrial waste are the big sources for the entrance of heavy metals in the ecosystem. Trace heavy metals affect the quality of water, production of agriculture and health of human beings [2]. Industrial evolution has played an important role in causing the heavy metal pollution [3].

Most of the countries of world are facing the heavy metal pollution. Usually, heavy metals such as cadmium and chromium are accumulated in liver and kidney. Lead is a toxic heavy metal and can have the capacity to make strong bonding with enzymes, having sulfhydryl groups and disturbs the normal function of enzymes. Lead affects the

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blood, nervous, genital, urinary and gastric system and in experimental animals, it can cause carcinogenesis, mutagenesis and teratogenesis. Lead toxicity can cause the headache, learning disabilities, brain damages and hearing problems as well. Cadmium is another toxic heavy metal which causes the high blood pressure, mutations and prostate cancer. Other metals like iron, copper, zinc and manganese are important in physiological functions and act as co-factor of enzymes but the presence of these metals in excessive amount can also have the toxic effects.

Meat is represented as the ecosystem for the growth of many pathogenic organisms including *Brochothrix thermosphacta, Pseudomonas sp., Carnobacterium sp., Enterobacteriaceae, Lactobacillus sp. and Leuconostoc sp.* These organisms are responsible for the spoilage of refrigerated meat and meat products [4, 5, 6, 7, 8]. Meat is a good medium for the growth of these species [9, 10, 11]. *Listeria monocytogenes, E. coli O157:H7, Campylobacter jejuni and Yersinia enterocolitica* are recognized as foodborne pathogens spreading the foodborne diseases [12, 13, 14, 15]. Many food industries use *E.coli* and Total Coliform bacteria as indicators for the presence of pathogenic microbes [16].

More than three lac and seventy thousand animals are slaughtered annually in Pakistan [17]. Mostly organisms are transferred to the consumers through improper sanitary handling and lack of awareness regarding food safety. So the proper management of animal husbandry and meat processing is required for the safety of health and for maintaining the quality of meat and meat products for long time period.

The objectives of this study were to analyze the commercially available meat for its heavy metal contents and bacterial contamination. The comparison was also made between the selected sites to find the most contaminated and least contaminated sampling site. Antibiotics resistance of isolated bacteria was measured by growing bacteria in a medium exposed to various antibiotics.

2. MATERIALS AND METHODS

This study has been conducted to detect the presence of heavy metals and bacterial species in commercial meat collected from different markets of Lahore.

2.1. Site Selection

The samples were collected from four different commercial markets of Lahore. These four sites were Wafaqi Colony (Site I), Township (Site II), G-1 Market (Site III) and Zenith (Site IV). The samples were collected for the detection of heavy metals and bacteria in them.

2.2. Pretreatment of Samples for Heavy Metal Detection

The meat samples collected from different markets of Lahore were washed with distilled water to remove the dust particles on samples and to get rid of excessive blood. These meat samples were kept in oven for drying purpose at 80^0C for 48 hours.

2.3. Heavy Metals Analysis

The wet digestion method was performed by adding conc. HNO_3 in to the meat samples. The 0.5g of meat samples and 5mL of conc.HNO_3 were taken in to the digestion flask. Hot plates were used for digestion at 80-90^{o}C and raised to 150 ^{o}C. More acid was added up to 3-5mL until clear solution was obtained. The samples were cooled at room temperature and filtered through filter assembly and the volume was raised up to 25 mL with the help of distilled water. The blank sample was also prepared. Atomic Absorption Spectrophotometer was used for the detection of heavy metals presence in the meat samples (FAAS, Shimadzu AA-7000F).

2.4 Statistical Analysis

A one way ANOVA was performed to measure the difference between the concentrations of individual heavy metals in the meat samples collected from different markets of Lahore.

2.5. Preparation of Meat Sample Homogenate

Twelve grams of meat was weighed from each meat samples collected from different sites and

dissolved them in 100 mL of BPW. The orbital shaker was used to make the homogenate of meat samples for 15-20 minutes. Desired dilutions were prepared. The Petri dishes were sterilized in autoclave and the nutrient agar was poured in to the Petri dishes. The meat inoculums were taken from each dilution and poured in the nutrient agar. The sample was spread over the medium in uniform manner and allowed to solidify properly. The Petri dishes were kept in an incubator at 32^0C for 48 hours.

2.6. Detection and Identification of Salmonella

The meat homogenate was transferred in to the 500mL sterilized bottle and placed it in incubator for 15-20 hours at 37^0C. Ten milliliters of sample from sterilized bottle was taken and transferred in to tetra-thionate broth and other 10 mL was transferred to the selective medium and placed in incubator for 2 days at 42-43^{o}C. The Petri plate were taken and sterilized. The Salmonella/Shigella agar was poured and streaked carefully and plates were kept in incubator for 24 hours and examined the presence of *Salmonella*.

2.7. Detection of Bacillus sp.

For the identification of the *Bacillus sp.*, MacConkey agar was prepared by adding 5g agar in 100mL distilled water. The Petri plates were sterilized and inoculated with meat sample through streaking method and placed in incubator for 24 hours. The colonies appeared and confirmed the presence of *Bacillus sp*.

2.8. Detection of *E. coli*

To identify the presence of *E.coli,* Eosine Methylene Blue (EMB) agar was prepared by adding 5g of agar in 100mL of distilled water. The Petri plates were sterilized in autoclave for 15 minutes at 121^{o}C and meat sample was inoculated by streaking on the Petri dishes. The Petri dishes were placed in incubator for 24 hours. The growth of colonies on the Petri plates indicated the presence of *E. coli.*

2.9. Detection of Pseudomonas sp.

Pseudomonas agar was prepared by adding 5 g agar in 100 mL distilled water for *Pseudomonas sp.* identification. After washing the meat sample was transferred to the Petri dishes and streaked. The *Pseudomonas sp.* grew on the agar and confirmed its presence in meat samples.

2.10. Detection of Total Coliform Bacteria by Dilution Formation

The test tubes were taken and filled with 5mL of EC broth. The test tubes were covered with cotton and aluminum foil and kept in autoclave for 15 minutes at 121^0C. The test tubes were cooled at room temperature. Five dilutions of 0.1mL sample, 5 dilutions of 1mL sample and 5 dilutions of 10mL sample were made by pouring samples in the sterilized test tubes containing media except 1 test tube serving as blank. The test tubes were kept in incubator for 2 days. After the incubation period these were compared with blank to count the total coliform MPN/kg [18].

2.11. Detection of Fecal Coliform Bacteria

Samples were analyzed for faecal coliform bacteria following the procedure mentioned in Standard Methods of American Public Health Association [18] and reported as MPN/kg.

2.12. Analysis of Antibiotics Susceptibility

For analysis of antibiotic resistance, filter paper disks impregnated with selected antibiotics were placed on the surface of growth medium containing bacterial isolates. Growth of bacteria around the disks impregnated with specific antibiotic was observed to check its susceptibility for that antibiotic.

3. RESULTS AND DISCUSSION

3.1. Heavy Metal Contamination in Meat

The samples were analyzed for individual heavy metals by Atomic Absorption Spectrophotometer and are shown in Fig. 1.

The concentration of manganese was not same for all samples. The highest concentration (0.0124 ppm) was found in the samples collected from Site II and in other three sites Mn was BDL (Below Detection Limit). Many previous studies have

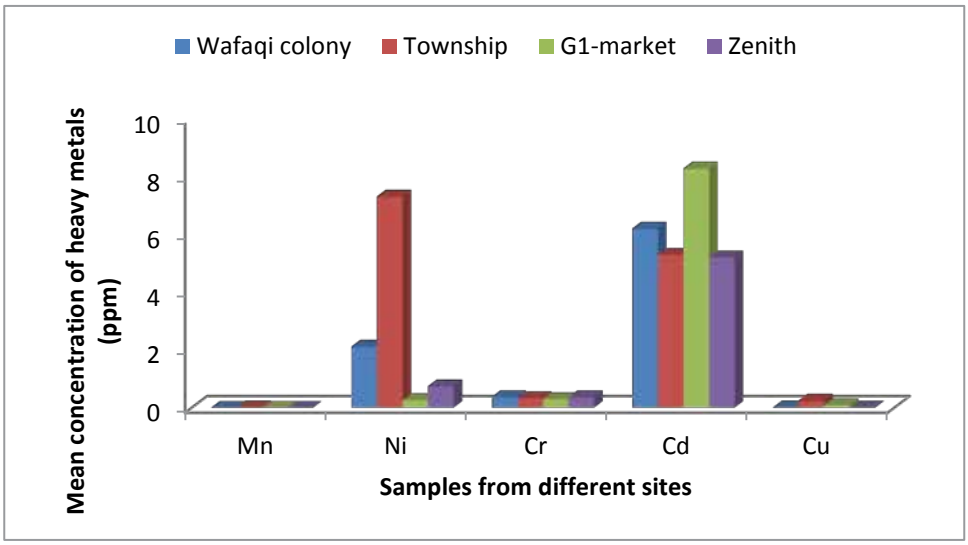

Fig. 1. Heavy metal concentrations in meat samples collected from different markets of Lahore.

been conducted to detect the presence of Mn in meat products. Cabrera et al. [19] analyzed the meat samples and found the Mn contents in meat (0.05-0.17mg/kg). Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for Mn is 2-5mg/kg [19]. The comparison of current study with standard values indicates the safe level of Mn in all samples of meat of selected sites. The concentration of nickel varies in all samples in order of Site II>Site I>Site IV> Site III. The highest concentration of Ni was found in Site II (7.32ppm), which was quite higher than standard value while other samples have showed the less concentration of nickel in comparison with standard value. According to Food and Agriculture Organization [20], the standard of nickel for food items is 5 mg/kg. Demirezen et al. [21] conducted the study to analyze the meat and meat products and find the contents of trace metals in meat. Nickel contents were present in a range of 8.2-24µg/g in the study conducted by Demirezen et al. [21]. The concentration of chromium also varied among all samples in order of Site I>Site IV>Site II>Site III The standard value of chromium is 2.3 mg/kg according to FAO [20]. The result indicates that all samples have less chromium concentration than the standard value. Bratakos et al. [22] found chromium contents in lamb meat (0.08–0.16µg/g) and chicken meat (0.11–0.21µg/g). In case of cadmium concentration, the highest concentration was found in Site III (8.29ppm). FAO has given the standard value of cadmium in food items which is 0.2 mg/kg [22]. Our results showed that the concentration of cadmium in all meat samples is quite high and the utilization of that meat was harmful for health. Farmer et al. [23] analyzed the horse meat and found the mean concentration of Cd to be at 128 mg/kg. The copper concentration in meat samples collected from different sites of Lahore. The copper concentration was highest in the Site II sample and results showed its concentration greater than 0.25 mg/kg in Site II sample and 0.1 mg/kg in Site III. The standard value of copper concentration in food items is 40 mg/kg and all samples have less copper contamination. Demirezen et al. [21] found copper concentration in meat samples (7.18–10.01µg/g). The copper contents in current study are lower than study conducted by Demirezen et al. [21], as well as recommended value of FAO [20].

3.2. Bacterial Contamination in Meat

Samples in triplicate were collected from all four sites for the determination of bacterial contents by streaking and inoculation on selected media. In the samples of Site I the *E.coli* and *Salmonella* were

seen by observing pink color colonies and reddish green colonies of *Bacillus sp.* were observed (Table 1). Previous studies were also conducted to isolate the bacterial species from meat and meat products. *E.coli* and *Salmonella sp.* were isolated by Nychas et al. [7]. For samples of Site II, the EMB agar and Salmonella/shigella agar gave positive results by confirming the presence of pink colonies. *E.coli, Bacillus*, *Pseudomona s*and *Salmonella species*were observed (Table 1). Mrema et al. [24] during their study isolated the different species of *Salmonella* and Barrera et al. [25] isolated the *E.coli* from meat samples. The comparison indicates that results are similar to the previous research results. The samples collected from Site IV, only EMB agar showed the pink colonies of *E.coli*, the reddish green colonies of *Bacillus species,* other selected media showed negative results and confirmed the presence of Staphylococcus *sp.* (Table 1). Ali et al. [26] isolated the *E.coli, Salmonella, Sheigella and Staphylococcus aureus* during their studies. Marty et al. [27] also isolated the *Staphylococcus species* during their study. The comparison of this study with above mentioned studies indicated the accurate results to some extent. In sample of Site III, there were pink colonies of *E.coli* and *Pseudomonas species*. There were *Bacillus sp.* and *Salmonella sp.* also observed (Table 1). Audenaert et al. [28] isolated *lactobacillus species* along with Lactic Acid Bacteria. Results of Audenaert et al. [28] also indicated the presence of *E.coli, Salmonella and Staphylococcus* in their results but the *Pseudomonas sp.* and *Bacillus sp.* were not isolated which indicates that these two bacterial species are found less frequently in meat and meat products.

3.3. Comparison of Total Coliform and Fecal Coliform in Meat Samples of all Sites

Samples in triplicate collected from all sites were analyzed to detect the presence of Coliform Bacteria and fecal Coliform. The number of bacteria in all samples was measured by MPN (Most Probable Number) method. The mean value of Site I samples (3.1×10^4), Site II and Site III samples (9.4×10^4) and Site IV samples (2.0×10^4) were recorded (Table 1). Odumeru et al. [16] also measured the Total Coliform Bacteria in ground beef during their studies. The mean values for Site I (0.1×10^4), for Site II (0.02×10^4), for Site IV (0.01×10^4) and for Site III (0.03×10^4) were measured (Table 2). The results showed the highest Fecal Coliform in Site I meat sample and lowest in Site IV meat samples. The number of Fecal Coliform varied from site to site in order of Site I>Site III >Site II>Site IV. Bhandare et al. [29] also found the Fecal Coliform in meat samples by using MPN method.

Table 1. Isolation of various bacteria by growing them at selected media in meat samples of all sites.

Source	EMB agar	Pseudomonas agar	MacConkey agar	Salmonella agar	Results
Site I	+	–	+	+	*E. coli* *Salmonella sp.* *Bacillus sp.*
Site II	+	+	+	+	*E.coli* *Bacillus sp.* *Salmonella sp.* *Pseudomonas sp.*
Site III	+	–	+	+	*E.coli* *Bacillus sp.* *Salmonella sp.*
Site IV	+	–	+	+	*E.coli* *Bacillus sp.* *Salmonella sp.*

Table 2. Determination of total Coliform and Fecal Coliform in meat samples collected from different sites.

Samples	Site I (MPN/kg)	Site II (MPN/kg)	Site III (MPN/kg)	Site IV (MPN/kg)
Total Coliform	3.1×10^4	9.4×10^4	9.4×10^4	2.0×10^4
Fecal Coliform	0.1×10^4	0.02×10^4	0.03×10^4	0.01×10^4

3.4. Statistical Analysis for the Heavy Metals in Meat Samples

For the comparison of heavy metals from different locations ANOVA was performed. The significant value is 0.05 by default. The concentration of heavy metals (Cu=0.496, Cr= 0.688, Cd=0.913 and Mn=0.441) showed the value greater than significant value (0.05) which means concentration of metals were same and could not differ among different sites. Whereas only Ni metal showed the significant value (0.023) which means that only the concentration of this metal varies significantly between the groups or selected sites.

3.5. Antibiotics Susceptibility Profile

Antibiotic resistance in bacteria isolated from samples of selected sites is illustrated in Fig. 2. All the Bacterial strains were found highly resistant to Lincomycin, streptomycin, tertracyclin and ampicillin but were less resistant to amoxicillin and doxycyclin. Bacteria were not survived in medium containing ofloxacin therefore none of the bacteria were found resistant to Ofloxacin. However Pseudomonas was found more resistant to all antibiotics except ofloxacin, in comparison to other bacteria.

4. CONCLUSIONS

Lahore is a mega and overpopulated city which attracts the people due to many facilities found here. People of Lahore demand food products containing meat. The current study showed that quality of meat of different commercial markets of Lahore is not so good. Meat samples were collected from different markets (Site I, Site II,

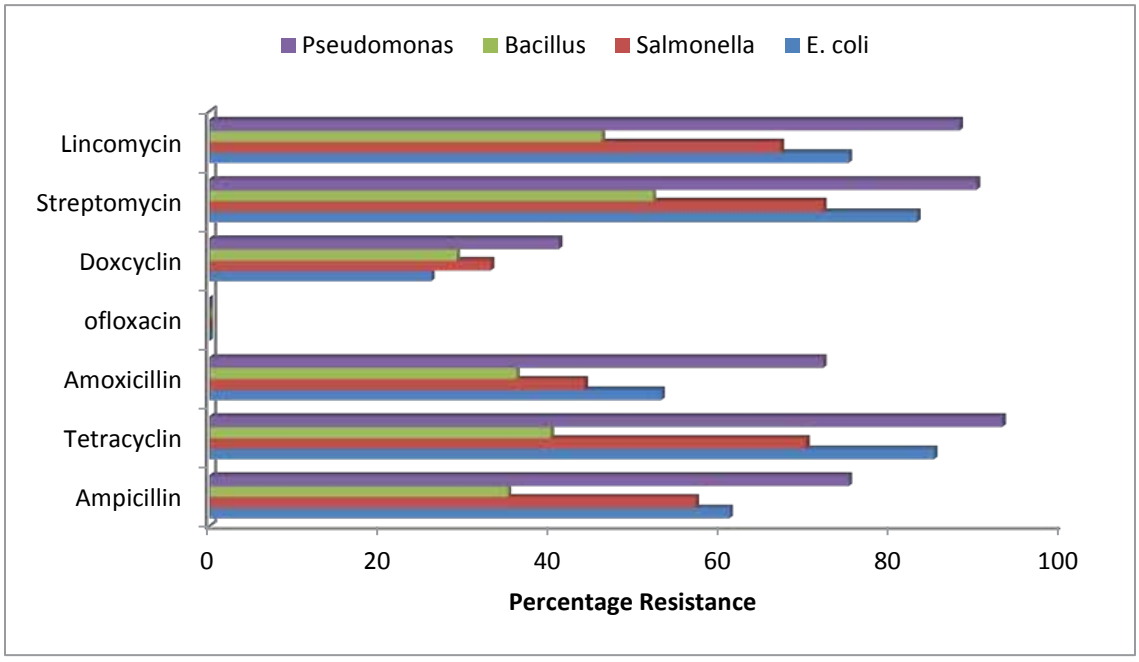

Fig. 2. Antibiotic resistance percentage isolated bacteria from meat samples.

Site IV and Site III) of Lahore to check the quality of meat. The meat samples were analyzed for heavy metals and bacterial contamination. Results showed the presence of Cu, Ni, Cr, Cd and Mn metals in all the samples but concentration was higher in Site II samples and less in Site IV samples. Concentration of Ni was found significantly higher in all sites in comparison to other heavy metals. The bacteria (*E.coli, Salmonella sp., Staphylococcus sp., Pseudomonas sp.* and *Bacillus sp*.) were also isolated from all the samples. Bacteria were found resistant to all antibiotics except ofloxacin. Results indicated that meat if not properly cooked can be harmful for human health and its long term use can cause heavy metals accumulation and toxicity in the body.

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Research Article

Logistic Regression and Multiple Classification Analyses to Explore Risk Factors of Under-5 Mortality in Bangladesh

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Abstract: Logistic regression (LR) analysis is the most common statistical methodology to find out the determinants of childhood mortality. However, the significant predictors cannot be ranked according to their influence on the response variable. Multiple classification (MC) analysis can be applied to identify the significant predictors with a priority index which helps to rank the predictors. The main objective of the study is to find the socio-demographic determinants of childhood mortality at neonatal, post-neonatal, and post-infant period by fitting LR model as well as to rank those through MC analysis. The study is conducted using the data of Bangladesh Demographic and Health Survey 2007 where birth and death information of children were collected from their mothers. Three dichotomous response variables are constructed from children age at death to fit the LR and MC models. Socio-economic and demographic variables significantly associated with the response variables separately are considered in LR and MC analyses. Both the LR and MC models identified the same significant predictors for specific childhood mortality. For both the neonatal and child mortality, biological factors of children, regional settings, and parents' socio-economic status are found as 1st, 2nd, and 3rd significant groups of predictors respectively. Mother's education and household's environment are detected as major significant predictors of post-neonatal mortality. This study shows that MC analysis with or without LR analysis can be applied to detect determinants with rank which help the policy makers taking initiatives on a priority basis.

Keywords: Neonatal, post-neonatal, & child mortality; logistic regression analysis; multiple classification analysis; priority index

1. BACKGROUND

Childhood mortality known as under-5 mortality is a worldwide problem particularly in the developing countries. Though much improvement has been achieved to meet the fourth Millennium Development Goal (MDG) regarding worldwide child mortality (reduce two-thirds during 1990 to 2015), still now a large number of children (about 6.9 million in 2011) are failing to survive until their fifth birthday [1]. The scenario is very worse in the low-income countries (50 per 1000 children) specifically in Sub-Saharan Africa (92 per 1000 children) and Southern Asia (67 per 1000 children). In Bangladesh under-5 child mortality rate also declined to about 53 in 2007/11 from 146 in 1990/91 through successful programs for immunization, control of diarrhoeal diseases and vitamin A supplementation [2]. This achievement indicates partial fulfillment of the MDG (48 per 1000 by 2015). Though the overall rate has been declined, major vulnerable groups among the population are still needed to address. The researches indicate that the disparities between poor and rich households have significant relationship with the rate of child vulnerability in developing countries [3]. The children from the poorest households have about three times more risk to die than those from the richest households. Thus identifying the risk factors behind the early childhood death is still an important area of research.

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A number of studies have been done to find the associated factors of early childhood mortality in many countries as well as in Bangladesh [4-9]. Socio-economic, demographic, environmental, and maternal & child health care variables are found to have significant impact on early childhood mortality. To find out the influencing predictors on childhood mortality, generally exploratory and LR analyses have been used in most of the studies [9-11]. Exploratory analysis does not permit for quantification or testing the strength of inter-relationship between two variables. In contrast, LR analysis is employed to examine the association of all the selected explanatory variables with the dependent variable simultaneously. However, it is not possible to identify which predictor is the most effective one among the significant predictors. In another way, it can be said that the significant variables cannot be ranked according to their influence on the dependent variable in LR analysis. The significant variables can be ranked according to their contribution in the variation of the study variable applying multiple classifications (MC) analysis [12]. Moreover, the MC analysis can be done for both nominal and interval scale dependent variables while LR analysis for nominal variables.

The MC analysis provides how much variation of the target variable is explained by a predictor included in the regression model and hence provides an assessment of the importance of the predictors. In another way, the degree of relationship between a predictor variable and the dependent variable as well as predictive power of the predictor variable can be assessed by MC analysis. It shows both bivariate (without presence of other predictors) and multivariate relationships (with presence of other predictors) between the predictors and the dependent variable. While in LR analysis separate analyses are required to obtain such relationships. Thus MC analysis is an additive model with wider range of application than the linear and the generalized linear regression model. The aim of the study is not only to find out the significant predictors but also to observe their predictive power to read the variation in the childhood mortality in Bangladesh through both LR and MC analyses. The article is organized as: LR and MC analyses are discussed in Section 2; description of the data obtained from Bangladesh Demographic and Health Survey (BDHS) 2007 is given in Section 3; results of the LR and MC models are described in Section 4 and the conclusion is made in Section 5.

2. LOGISTIC REGRESSION AND MULTIPLE CLASSIFICATION ANALYSES

In this study binary logistic regression (LR) model has been developed to find the significant predictors of childhood mortality, while MC model helps to rank those significant predictors according to their priority on explaining the variation of the response variable.

2.1 Logistic Regression Analysis

Logistic regression analysis is conducted to find a relationship between a categorical response variable and one or more explanatory variables. The analysis helps to determine the predictor variables significantly associated with the dependent variable with exact significance level. Here the conditional probability $Y_i = 1$ given $X_i = x_i$ is expressed as $P_i = \Pr(Y_i = 1 \mid X_i = x_i) = \exp(x_i^T \beta) / (1 + \exp(x_i^T \beta))$ and that of $Y_i = 0$ as $1 - P_i = \Pr(Y_i = 0 \mid X_i = x_i) = 1/(1 + \exp(x_i^T \beta))$ where Y_i is the dichotomous variable having values 1 for success (say a child dies before his/her 1st birth day) and 0 for failure (the child is still alive), x_i is a vector of independent variables and β is a vector of unknown parameters. Then the odds and the log of odds being success are respectively

$$\frac{pi}{1-pi} = e(x_i^T \beta) \text{ and } \ln\left(\frac{pi}{1-pi}\right) = x_i^T \beta \,. \qquad (1)$$

This equation for the log of odds is known as LR model. For a binary explanatory variable, odds ratio (OR) can be easily calculated by taking the exponential of the corresponding regression coefficient (e^{β}) which helps to interpret the probability of being success for a target group compared to a reference group. It is noted that an OR greater than 1.0 suggests more possibility to

occur, while an OR less than 1.00 indicates a decreased likelihood compared to the reference category. Maximum likelihood method with a suitable iterative process such as Newton's method is used to estimate the regression coefficients [13]. The Hosmer-Lemeshow (H-L) test is done for assessing the goodness of fit of the fitted LR model [14]. To assess the significance of an individual predictor, several tests such as likelihood ratio, Wald, and score tests are recognized in LR theory [13]. These tests are asymptotically equivalent [14]. The binary LR model is extended to multinomial and ordinal LR models when the dependent variables are measured in nominal scale (more than two categories) and ordinal scale [15].

2.2 Multiple Classification Analysis

The MC analysis is a multivariate technique for assessing the interrelationship of some explanatory variables with a response variable through an additive model. MC analysis determines the effect of each predictor before and after adjustments for its inter-correlations with other predictors included in the additive regression model. The MC analysis technique can be considered as a multiple regression analysis where the explanatory variables are dummy variables [16-17]. Unlike simple multivariate methods, MC analysis can handle explanatory variables measured on nominal scale and any form of interrelationships between the explanatory variables and the response variable. However, dependent variable is required to be measured on an interval scale without extreme skewness or to be a dichotomous variable without extremely unequal occurrences. The predictor variables can be measured on nominal scale (categories), ordinal scale (ranking), and interval scale (classes of numerical variable).

In MC analysis approach, each subgroup of an independent variable is transmitted into a dummy variable depending on whether the case falls or not in that particular subgroup. The MC model assigns a coefficient to each category of each predictor and the response value of an individual is expressed as the sum of the coefficients assigned to the categories indicating the individual's characteristics, the average of the response values for all cases, and a random error term. Thus the additive model in MC analysis can be expressed as

$$(2) \quad y_{ijk\ldots n} = \mu + a_i + b_j + \ldots\ldots\ldots + e_{ijk\ldots n}$$

where, $y_{ijk\ldots n}$ is the response value for a particular respondent who falls into i^{th} category of predictor A, j^{th} category of predictor B and so on; μ is the grand mean of the dependent variable Y, a_i is the added effect of i^{th} category of predictor A (difference between grand mean μ and the mean of the i^{th} category of predictor A), b_j is the added effect of j^{th} category of predictor B (difference between grand mean μ and the mean of the j^{th} category of predictor B); $e_{ijk\ldots n}$ is the random error term.

The coefficients are estimated in such a way that the sum of squared errors is minimized. Thus like least squares method, some normal equations or least squares equations are needed to solve for estimating the MC analysis coefficients. These equations can be solved by following a suitable iterative procedure. The diagnostic of the fitted model can be done by checking whether all the predictors can explain a significant proportion of the variation in the values of the dependent variable. In the similar way, the significance of each predictor can be answered by one-way analysis of variance. For details please see Andrews et al. [12] and Nagpaul [18].

For assessing the importance of a predictor (the degree of relationship between a predictor and the dependent variable), two correlation ratios called Eta (η) and Beta (β) statistics are calculated from the model before and after adjustment the other predictors respectively [19]. The value of the adjusted statistic β indicates the importance of a predictor on the response variable. The ranks of β statistics indicate the relative importance of the various predictors to explain the variance of the dependent variable [20]. Eta (η) statistic is used for assessing the bivariate relationship between a predictor and the dependent variable. Both statistics range from 0 to 1. The statistics for a predictor I are estimated by [12]:

$$\eta_I = \sqrt{U_I / T}\ ,\ U_I = \sum_j \left(\sum_k W_{Ijk} \right) \left(\bar{Y}_{Ij} - \bar{Y} \right)^2 ,$$

$$T = \sum_k w_k y_k^2 - \left(\sum_k w_k y_k \right)^2 \Big/ \left(\sum_k w_k \right),$$

$$\beta_I = \sqrt{D_I / T} \text{ , and } D_I = \sum_j \left(\sum_k W_{Ijk} \right) a_{Ij}^2 .$$

Here w_k is the weight of k^{th} individual, y_k is the value of Y for k^{th} individual, $\overline{Y}_{Ij}$ is the mean value of Y for the j^{th} category of independent variable I, W_{Ijk} is the weight of k^{th} individual fall in j^{th} category of I independent variable, and a_{Ij} is the adjusted deviance of j^{th} category of independent variable I on final iteration. Thus U_I and D_I are the sum of squares based on unadjusted and adjusted deviations respectively for predictor I and T is the total sum of squares. Squares of η_I and β_I indicate the proportions of variation in Y explained by the predictor I before and after adjustments of other predictors respectively. The specific formulas are illustrated in [12, 18, 20].

Though MC analysis method is like an ANOVA, it has no problem of accounting correlated predictors in the additive model. In comparison to multiple regression analysis, MC analysis has no problem of considering non-interval scale predictors in the model. The gain of MC analysis over multiple regression analysis is estimating the effect of each predictor on the dependent variable both before and after considering the effects of all other predictors without any constraints, while some restriction are needed to consider in multiple regression analysis [18].

3. STUDY MATERIALS

In BDHS 2007, a nationally representative sample of 10,819 households has been selected following a two-stage stratified sampling method. A total of 10,996 ever-married women age 15-49 years (eligible respondents) were asked to inform regarding their socio-economic characteristics, reproductive history, knowledge and use of family planning methods, vaccinations, and childhood illnesses etc. [21]. All ever married women were also requested to provide a complete history of their live births including their survival status and age at the survey date or at death. Since the units of this analysis in this study are under five dead children, the study can be considered as a retrospective study where information regarding the death children is based on mothers' recall. NIPORT et al. [21] conducted several tests (sampling and non-sampling errors, underreporting and misreporting of infant deaths, etc.) for checking the validity of the mortality data and confirmed the accuracy of the collected data.

Before analysis, it was expected that only the live births during the last 5 years preceding the survey were enough to conduct the study. However, the number of live births died during this period was not sufficient to find significant association of the response variable (whether a live birth is still alive or died) with the considered explanatory variables. The problem can be solved by considering the live births that were born in last 10 or 15 years. In this case, there is some suspicion about the perfect comparison between the case group (who died before their fifth birthday) and the control group (who are still alive). If the live births of last 10 years are considered, a large number of children who still survive after their first five years of life will be in the control group, while in case group the members are under-5 death children ignoring their date of birth. To overcome such problem, all the children who died before their fifth birth day are considered so that both the case and control groups have at least one common characteristic that they are died before 5 years of age. So in total 3426 children who died before five years of age were extracted from the total live births. However, these children were born during 36 years preceding the survey date. The problem of considering all these death children are incompleteness of death reports and inaccuracy in mothers' recall for the children whose mothers are very older now. For reducing inaccuracy, we consider only those children (2132) who were born during the last 20 years and failed to survive up to their fifth birth day. The consideration for 20 years is to increase the sample size so that significant association between the dependent and independent variables can be obtained.

The variable of interest is the age of child death measured in months that is used to define

three binary variables for explaining the early childhood mortality indicators: neonatal death (1132) referred to deaths during first four weeks of birth, postnatal death (555) referred to deaths during 1 to11 months after birth and child death (445) referred to deaths during 12 to 59 months after birth [2]. Bivariate relationship between the dependent variables and the independent variables are observed before LR and MC analysis. Type of explanatory variables associated with the binary response variable varies with the type of mortality indicators. For this reason, different explanatory variables are used to develop three independent models for three mortality indicators involving all the death children. Since the LR model was developed for binary response variable, same binary variable was used to develop the corresponding MC model. Both LR and MC analyses are done in SPSS using LOGIT and ANOVA with MCA function, respectively. The sampling weight given in BDHS data was considered to perform the analysis.

4. RESULTS

To meet the target of United Nation's MDG and to set strategies, the policy makers and donors require not only the rate of child mortality but also its risk factors. It will be beneficial for the policy makers if they can make an order of initiatives based on the ranks of the significant predictors. An attempt has been made in this section which is split into three sub-sections as pattern of neonatal, postnatal and child mortality in Bangladesh since 2000, significantly associated predictors determined in the LR analysis, and the ranking of the significant predictors based on MC analysis.

4.1 Childhood Mortality Pattern in Bangladesh

Bangladesh has gain a great achievement in the reduction of all stages of child mortality. In 1970s, infant mortality and child mortalities were respectively 147 and 86 per 1000 live births while in 2000s, the rates have come down to 51 and 17 per 1000 live births respectively [22]. According to recent BDHS 2011, 1 in each 23 children fails to survive after one year of age, while 1 in each 19 children dies before 5 years of age [2]. Though the overall childhood mortality has been reduced over the last few decades, the rate of decline is not same at different stages of childhood mortality. According to DHS surveys in Bangladesh since 1993/94 to 2011, infant mortality has declined by 51 percent (87 to 43 per 1000 live births), while child mortality and under-5 mortality have declined by 78 percent (50 to 11 per 1000 live births) and 60 percent (113 to 53 per 1000 live births) respectively.Among the infant mortalities (neonatal and post-neonatal), the proportion of neonatal death out of infant deaths is still high over the periods (60% in 1993/94 and 74.42% in 2011). Thus initiatives still require to decline the infant death specially the neonatal death to reduce the variation of child mortality at different stages of childhood.

To examine the situation of child death during the last decade, the three consecutive BDH surveys (BDHS-2007, BDHS-2004 and BDHS-2000) are used to draw the Figure 1 where the proportions of child deaths at different stages out of total under-5 deaths are shown [21, 23-24]. The children who died before their fifth birthday are considered here without seeing their date of birth. It is explicitly clear that proportion of child death during neonatal period (<1 month) is highest over the last decade. About 50 percent of child deaths occurred during the neonatal period and the rate doesn't change. While the proportions of postnatal death and young child death are near about 25 percent. It is also noticeable that percentages of child death at different stages are almost same but the rate of childhood mortality decreased superficially during the last eight years. The reason might be the increasing sizes of live births considered in the corresponding surveys.

The trend analysis shows that the rate of under-5 child mortality has been decreased significantly and is in the right track to meet the MDG-4 by 2015. It also identifies the significant variation in mortality rate at different stages of childhood. The most vulnerable stage is the 1st month after birth and followed by the next 11 months. These variations also suggest that the risk factors of child mortality might vary with

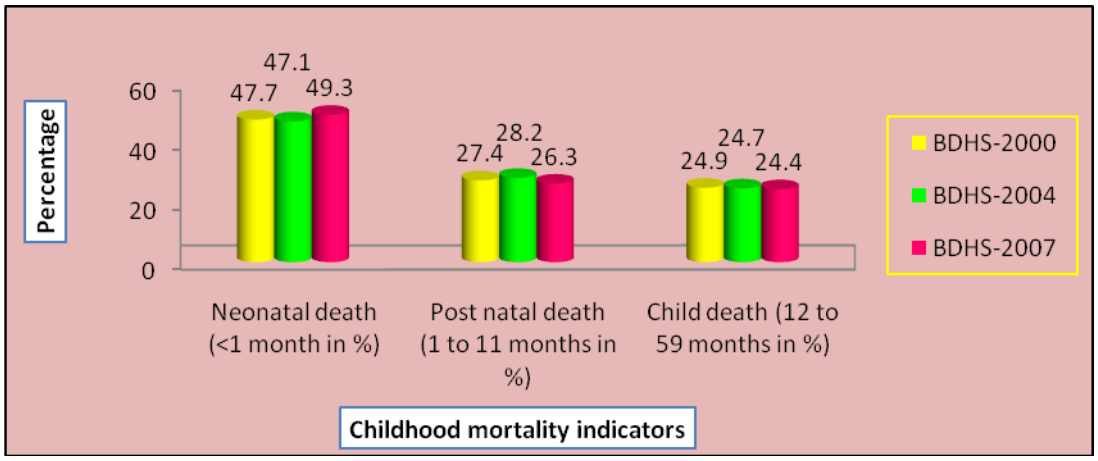

Fig. 1. Comparison of child death under age five in three consecutive BDH surveys (Source: BDHS-2000 [23], BDHS-2004 [24], BDHS-2007 [21].

childhood stages. This is the basic reason of separate LR and MC analysis for neonatal, postnatal and child mortality.

4.2 Risk Factors of Early Childhood Mortality

Before fitting a binary LR model, the independent variables are chosen through cross-tabulation analysis between binary outcome variable (whether a child died in a given period or not) and categorical explanatory variables. Eleven categorical explanatory variables are found to have significant association (through chi-squared test not shown in the paper) with childhood mortality indicator variable. These associations vary with the considered dependent variables which indicate death of children at different stage of life. To free from multicollinearity problem, all influential covariates are not included to build up the regression model. Three binary LR models as well as three MC additive models have been developed to find out the significant factors of neonatal, post-neonatal and young child death. The results of both LR and MC models shown in Table 1 to Table 3 indicate that a considerable number of predictors have significant effect on less than five deaths and also the predictors vary with the models. The H-L tests indicate that all the fitted LR models are perfect for the given data set (p-value>0.05). The results are discussed below by chronological order of neonatal, post-neonatal, and child mortality.

Sex differential has a significant relationship with childhood mortality. In Bangladesh ratio of male-to-female infant and under-5 mortality is more than 100 since 1970s while in case of child mortality this ratio is always less than 100 [22]. In our study it is observed that sex of child has significant association with child death at the very beginning (neonatal) stage of life. Table 1 shows that male children (OR: 1.0 and adjusted predicted mean, APM: 0.51) were more likely to die at the neonatal stage than those female children (OR: 0.71 and APM: 0.44). The reason may be due to the fact that boys are biologically weaker and more susceptible to diseases and premature death compared to girls [25].

Hong [26] observed a strong negative association of infant mortality with multiple births in Bangladesh from the analysis of 2004 Bangladesh Demographic and Health Survey data. In this study the fitted LR model also indicates that number of foetus has highly significant effect on neonatal death. It is expected that women who gave multiple births are more likely (OR=2.9, APM=0.68) to face neonatal child death than those who have single birth (APM=0.45). The reason may be multiple births create more complicacy during delivery than the single birth and babies become physically week, as a result the risk of neonatal mortality is found higher for the multiple births.

Table 1. Distribution of neonatal mortality and its risk factors in Bangladesh determined by logistic regression (LR) and multiple classification (MC) models, BDHS 2007.

Background Characteristics	Percentage of Mortality	Number of Children	**Model I: Neonatal Mortality**[†] **Results from LR Model**		**Results from MC Model**[‡]	
			β	OR	APA	UPA
Sex of child						
Male [R]	56.8	1125	-	-	0.5089	0.5135
Female	49.0	1008	-0.342	0.711***	0.4367	0.4320
No. of foetus						
Singleton [R]	51.4	1968	-	-	0.4531	0.4511
Multiple	73.2	164	1.057	2.877***	0.6794	0.6995
Previous birth interval						
<24 [R]	50.1	513	-	-	0.5008	0.5057
24+	45.7	869	-0.217	0.805**	0.4567	0.4539
Mother's age at first birth						
<19	52.8	1582	0.214	1.238*	0.4886	0.4858
19+ [R]	53.9	551	-	-	0.4264	0.4349
Mother's access to mass media						
No media	52.8	1892	0.335	1.398**	0.4784	0.4807
One or more medias [R]	55.4	242	-	-	0.4225	0.4007
Region						
Barisal [R]	43.1	130	-	-	0.3754	0.3814
Chittagong	44.8	426	0.012	1.012	0.3912	0.3844
Dhaka	52.9	698	0.393	1.481*	0.4794	0.4742
Khulna	54.3	188	0.379	1.460	0.4653	0.4708
Rajshahi	63.5	460	0.855	2.350***	0.5765	0.5862
Sylhet	53.0	232	0.438	1.549*	0.4833	0.4852
Constant	-	-	-0.746	0.474***	-	-
Goodness of fit (p-value)			**H-L test:** $\chi^2_{(8)}$=14.804 (0.063)		***F*-test:** $F_{10,1370}$=6.971 (0.000)	
R^2 value			**Cox & Snell**: 0.048		0.048	

*** P<0.05; ** P<0.10; * <0.01
[R] Refers to reference category
[†] Total children for Model-I: 2132 (weighted)
[‡]APA & UPA refer to adjusted and unadjusted predicted average, respectively

The previous studies regarding child mortality in Bangladesh indicate that the children born within 24 months of preceding and successive birth had higher risk of early childhood death [27-29]. It is also observed in the study that mothers with previous birth interval of more than 24 months is 0.81 times (APM=0.46) less likely to have neonatal mortality compared to mother with less than 24 months birth interval (APM=0.50). It is because of the fact that during the short birth interval period mothers did not get sufficient time to revert their reproductive health and so they have increased risk of getting premature birth as well as neonatal death. Mother's age at first birth is also found mildly significant (p-value<0.15) with the child neonatal death after adjusting the other covariates. It is observed that mothers who provided birth before 19 years of age had higher risk (OR: 1.24, APM=0.49) of facing neo-natal death compared to their counterparts (APM=0.43). In a case study of Rajshahi district, Mondal et al. [30] found mother's age at first birth as significant predictor of both infant and child mortality.

Table 2. Distribution of post-neonatal mortality and its risk factors in Bangladesh determined by logistic regression (LR) and multiple classification (MC) models, BDHS 2007.

Background Characteristics	Percentage of Mortality	Number of Children	**Model II: Post-neonatal Mortality**[†]			
			Results from LR Model		**Results from MC Model**[‡]	
			β	OR	APA	UPA
Antenatal visits during pregnancy						
No visit [R]	36.5	74	-	-	0.3608	0.3762
Visits	24.3	70	-0.801	0.449**	0.2460	0.2297
Mother's Education						
Illiterate [R]	25.7	1131	-	-	0.4001	0.4120
Primary	29.1	671	-0.607	0.545	0.2865	0.2902
Secondary or higher	20.8	331	-1.231	0.292***	0.2054	0.1879
Mother's Occupation						
Unemployed [R]	27.3	1313	-	-	0.2910	0.3102
Manual	22.5	694	-0.100	0.904	0.3532	0.2969
Non-manual	33.3	120	-0.188	0.829	0.2195	0.2772
Household's drinking water source						
Tube-wells [R]	25.7	1889	-	-	0.2780	0.2694
Tap (Pipped)	41.0	78	2.376	10.763***	0.6235	0.7541
Others	22.3	166	0.893	2.442	0.4139	0.4425
Region						
Barisal [R]	29.2	130	-	-	0.4123	0.4581
Chittagong	24.9	426	-1.133	0.322	0.2473	0.2465
Dhaka	28.7	698	-0.976	0.377	0.2747	0.2687
Khulna	29.3	188	0.690	1.993	0.4735	0.5909
Rajshahi	20.0	460	-0.958	0.384	0.2709	0.2660
Sylhet	28.3	233	-0.965	0.381	0.3528	0.2747
Constant	-	-	0.672	1.959	-	-
Goodness of fit (p-value)			**H-L test:** $\chi^2_{(8)}$=3.791 (0.875)		***F*-test**: $F_{12,131}$=1.497 (0.136)	
R^2 value			**Cox & Snell**: 0.118			0.120

*** P<0.05; ** P<0.10; * <0.15
[R] refers to reference category
[†] Total children for Model-II: 2132 (weighted)
[‡]APA & UPA refer to adjusted and unadjusted predicted average, respectively

Now-a-days maternal and child health care depend on mothers activities such as access to updated information through mass media like newspaper/magazine, radio and television programs, participating in different programs related to child and maternal health care, morbidity and mortality. In this study mother's access to mass media (TV & radio) are found significantly associated with neonatal mortality. Mothers who had not the habit of watching TV or listening radio are 1.4 times (APM=0.48) more likely to have neonatal mortality than the mothers who had such habit (APM=0.42). Existing mass media of Bangladesh are now broadcasting sufficient awareness program regarding child health care after birth, as a result mothers who maintain media access are more conscious about neonatal health and have more knowledge about the causes of neonatal death compared to the mother who is not maintaining any media.

The one and only variable which is found significantly associated with all types of childhood

Table 3. Distribution of child mortality and its risk factors in Bangladesh determined by logistic regression (LR) and multiple classification (MC) models, BDHS 2007.

Background Characteristics	Percentage of Mortality	Number of Children	**Model III: Child Mortality†**			
			Results from LR Model		**Results from MC Model‡**	
			β	OR	APA	UPA
		Sex of child				
Male[R]	17.2	1125	-	-	0.1730	0.1718
Female	24.9	1007	0.504	1.656***	0.2498	0.2511
		No. of foetus				
Singleton[R]	22.3	1969	-	-	0.2235	0.2237
Multiple	3.7	163	-2.009	0.134***	0.0396	0.0366
		Parents' education				
Illiterate (Both)	23.1	1258	0.580	1.786***	0.2314	0.2351
Primary / Secondary (at least one)	18.6	671	0.227	1.254	0.1863	0.1777
Secondary or higher (both)[R]	14.8	203	-	-	0.1484	0.1540
		Mother's occupation				
Unemployed [R]	19.6	1313	-	-	0.1964	0.1943
Manual	24.4	694	0.306	1.358***	0.2437	0.2447
Non-manual	15.0	120	-0.195	0.823	0.1516	0.1685
		Region				
Barisal [R]	27.7	130			0.2770	0.2896
Chittagong	30.5	426	0.137	1.146	0.3041	0.3158
Dhaka	18.5	697	-0.660	0.517***	0.1857	0.1794
Khulna	16.6	187	-0.691	0.501***	0.1681	0.1763
Rajshahi	16.6	459	-0.820	0.440***	0.1653	0.1570
Sylhet	18.9	233	-0.603	0.547***	0.1881	0.1883
Constant	-	-	-1.558	0.210***	-	-
Goodness of fit (p-value)			**H-L test**: $\chi^2_{(8)}$=6.465 (0.595)		***F*-test**: $F_{11,2116}$=10.727 (0.000)	
R^2 value			**Cox & Snell**: 0.057			0.053

*** P<0.05; ** P<0.10; * <0.15
[R] refers to reference category
† Total children for Model-III: 2132 (weighted)
‡APA & UPA refer to adjusted and unadjusted predicted average, respectively

mortality in bivariate analysis is the location (division) where the children are living. It is observed that the proportion of child death under five years of age significantly varies with the regional settings. However, in LR analysis region has significant effect on neonatal and young child deaths. The children belonging to Rajshahi division had higher risk (2.35 times) of being died within the neonatal period compared to the children of Barisal division. Also the risk of neonatal mortality was found significantly higher among the children of Sylhet (1.55 times) and Dhaka (1.48 times) divisions compared to Barisal division. The corresponding unadjusted and adjusted predicted means are also found significantly higher than that of Barisal division (Table 1).

A different set of significant predictors are found for post-neonatal and child mortality in both LR and MC analyses. Receiving antenatal care during pregnancy which decreases the risk of maternal mortality as well as early childhood mortality is found significant in both LR and MC models (Table 2). It is observed that mothers who pursued regular antenatal visits during pregnancy are 0.45 times less likely to have post-neonatal mortality than their counterparts. The OR of post-neonatal death is found significantly varied

with Mother's education. Secondary or higher educated mothers are 0.292 times less likely to have post-neonatal child death compared to the illiterate mothers. The reason of such result is expected since educated mothers are better able to access medical services, follow advice and engage in child care practices that reduce post-neonatal death. Sources of drinking water, categorized according to Das et al. [31], as a household environmental variable were found significantly associated with post-neonatal mortality. The risk of post-neonatal mortality is about 10.76 and 2.44 times more likely for the children, whose family used tap and other sources water than tube-well water. Adjusted and unadjusted predicted means in MC model also suggest the similar. It is necessary to mention that there is no variation by regional settings which clearly indicates the post-neonatal deaths are almost similar over the whole country. These results might be due to small portion of childhood deaths during the post-neonatal period in the data set.

In the similar manner of neonatal mortality, sex differential has a significant relationship with child mortality (Table 3). Though male children were found more likely to die at the neonatal stage than that female children (OR: 0.71), the opposite result is observed in case of child mortality. Female children are 1.66 times more likely to die in the period of 12-59 months than the male children. This significant variation might be due to the sex inequalities in health care and feeding practices which are still common in Bangladesh. The male children get more preference for providing health and nutrition care, and so they make progress in reducing the risk of death. Recently Brinda, Rajkumar, and Enemark [32] have also shown a significant positive association between child mortality and Gender Inequality Index developed by United Nations Development Programme (UNDP) from a cross-national study of 138 countries. In case of foetus status, also an opposite scenario is observed in child mortality case. The risk of child mortality is 0.13 times lower for the multiple births than the single birth (Table 3), while the risk of neonatal death was more than double (Table 1). This might be due to the more care of family members for the multiple births at young child period (12-59 months) after facing the infant complicacy.

The parents' education is significantly associated with childhood mortality [33]. Though the education was not found significant in the earlier stage childhood death, LR model indicates that risk of child mortality (death during 12-59 months) consequently decreasing with the increase of mother's and father's educational level (Table 3). Children with illiterate parents were 1.8 times more likely to experience with child mortality in comparison to children with highly educated. This may be due to the fact that higher educated parents had more knowledge about child health compared to illiterate parents.

Results obtained from the fitted LR and MC models indicate that mother's occupation has also significant effect on child death during the 12-59 months of child age. Mother who is related with manual work (such as farmer, agricultural worker, fisherman, unskilled labourer, domestic labourer, non-agricultural land owner and labourer) is 1.35 times more likely to have child mortality compared to unemployed mother, however the risk is 0.82 times lower for the mother who is engaged with non-manual work. The reason may be mothers are generally educated who is engaged in non-manual work compared to unemployed and manual working mothers.

At last the regional influence is also observed in case of child mortality as neonatal child death but with different scenario. Though the children of Rajshahi division were found more experienced with neonatal death (OR: 2.35), they are least experienced (OR: 0.44) with child death in comparison to children of Barisal division. Except in Chittagong, the risk of child mortality is also found lower in other divisions (please see OR and APM in Table 3). These results indicate that the risk of child mortality is higher in Barisal and Chittagong divisions; however risk of neonatal death was lower there. Thus the regional settings have a great influence in overall childhood mortality situation which is not captured in the overall improvement of national childhood mortality rate.

Table 4. Multiple Classification (MC) analysis for neonatal, post-neonatal, and child mortality in Bangladesh, BDHS-2007.

Variables	Model I: Neonatal Mortality			Model II: Post-neonatal Mortality			Model III: Child Mortality		
	η_I	β_I	p-value	η_I	β_I	p-value	η_I	β_I	p-value
Sex of child	0.072	0.082	0.002				0.094	0.097	0.000
No. of foetus	0.129	0.141	0.000				0.120	0.123	0.000
Previous birth interval (months)	0.043	0.050	0.059	-	-	-	-	-	-
Mother's education	-	-	-	0.181	0.207	0.070	-	-	-
Parent's education	-	-	-	-	-	-	0.070	0.078	0.002
Mother's occupation	-	-	-	0.074	0.019	0.977	0.064	0.062	0.016
Mother's Access to Mass media	0.033	0.047	0.076	-	-	-	-	-	-
Antenatal visits during pregnancy	-	-	-	0.125	0.159	0.078	-	-	-
Household's drinking water source	-	-	-	0.154	0.209	0.054	-	-	-
Mother age at first birth	0.054	0.044	0.101	-	-	-	-	-	-
Region	0.131	0.140	0.000	0.150	0.220	0.259	0.132	0.149	0.000

4.3 Importance of Early Childhood Mortality Predictors

The LR analysis helps to find out the variables significantly associated with the child mortality but can't rank the variables according to the magnitude of their influence on the variable of interest. The MC analysis provides a way to rank the significant variables according to their relative importance on dependent variable. Similar to LR analysis, three separate MC models are developed for different stages of childhood mortality. The model fit statistics indicate that the MC models for neonatal and child mortality is significantly fitted with lower R-squared values (Table 4). However, the MC model for post-neonatal mortality is found insignificant at 5% but significant at 15% level of significance. The reason is the fewer number of cases at this stage. The lower R-squared values in all the MC models are due to the binary response variable which is clear also from the R-squared values of LR models. The MC model for neonatal mortality (Table 4) shows that the number of foetus is explaining highest contribution (β=0.141), followed by regional settings (β=0.140). Though regional settings is found to have significant higher contribution to neonatal mortality, on an average the biological factor (sex, foetus, & birth interval) is explaining the highest variation (β=0.273=0.082+0.141+0.050). Thus now it is very easy to rank the significant predictors as: biological factors as first, regional settings as second and mother's characteristics as third.

In case of post-neonatal mortality, mother's education and household's drinking water source are explaining the most variation significantly at 10% significance level. In the absence of other predictors, mother's education had highest contribution (η=0.181) though in presence of other variables its contribution stands on 2nd position (β=0.207) after drinking water source (β=0.209). Among the other variables, only the contribution of ante-natal visits during pregnancy (β=0.159) is

found significant. On the other hand, almost similar pattern of neo-natal mortality is observed in case of child mortality. Here the regional setting is observed as the most effective determinant (η=0.132, β=0.149) to predict the number of child death during the period of 12-59 months before and after adjusting with other variables (Table 4). The close competitors are biological factors: no. of foetus (η=0.120, β=0.123) and sex of child (η=0.094, β=0.097) whose marginal effects and net effects are almost similar. The basic difference in child mortality is the contribution of parent's characteristics (education and occupation) in explaining the variation of child mortality. Thus the significant variables can be ranked here almost in the similar way of neonatal mortality. From the MC analysis results, it is also visible that, significant factors are same in MC as like LR analysis which clearly supports the additional facilities of MC analysis. However, LR analysis provides risk of success for a specific group compared to a reference group. The similar interpretation can be made from MC analysis but need to compare the adjusted predicted values of the compared group with reference group. It is easy to verify just comparing the predicted means with the odds ratio: the higher the odds ratio of a group, the higher the predicted mean.

5. CONCLUSIONS

Though much improvement has been done in the reduction of overall under-5 mortality in Bangladesh, still have significant differences in the rate of mortality at different stages of children life. Comparatively the rate of neonatal death is still considerably higher though the overall childhood mortality rate is now in control. Also the variations in child death by regional, socio-economic and demographic characteristics are still needed to ascertain to reach the goal of ultimate target. To identify the vulnerable groups, several studies have been done by following the classical approach of LR analysis as a multivariate technique. In this study it is also obvious that characteristics associated with the childhood death varies with respect to the age at death. Binary LR analysis determines child birth history (sex, number of foetus, birth interval), regional settings, and mother awareness through the access to mass media as significant predictors of neonatal death, while for post-neonatal mortality significant predictors are related to mother's characteristics (education and ante-natal care) and household environment (access to better drinking water source). Like neonatal mortality, children biological factors, regional settings, and parent's socio-economic status (parents' education, mother's occupation) are found as significant predictors of child mortality. The similar scenario is also revealed through MC analysis with an extra facility of ranking the significant predictors. The MC analysis result suggests to focus on child biological factors, regional settings and parents' socio-economic status with a priority basis for both neonatal and post-infant stage; while mothers education and household environment for post-neonatal stage. The main contribution of this study is to focus on the utility of the MC analysis with the LR analysis to make an order of the significant predictors so that the policy makers can take initiatives on a priority basis.

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Research Article

Spatio-temporal Analysis of Urban Expansion on Farmland and Its Impact on the Agricultural Land Use of Mardan City, Pakistan

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Abstract: In this study GIS and remote sensing techniques have been applied for assessing the increasing trend of farmland conversion of Mardan city, Pakistan into impervious surfaces. The study area was a part of the fertile and productive land of Peshawar vale. The analysis was undertaken using Landsat 30 m of 1990 and SPOT 2.5 m of 2010. The datasets were interpreted with Maximum Likelihood supervised classification approach in ArcGIS environment. In addition, ground verification and parcel data for the test years were obtained from the Land and Revenue Department, Mardan. The results were classified into major land use categories of built-up area, farmland and cultivable waste. The analysis revealed that rapid increase in population has resulted in haphazard urban expansion particularly after 1990. During the study period, the built-up area has been doubled from 30% of the total city area in 1990 to over 63% in 2010, thereby has doubled the impervious surfaces. Consequently, the prime agricultural land has shrunk from 1,339 ha (42%) to 1,109 ha (35%) during the two decades. Similarly, the share of cultivable waste was decreased from 827 ha (26%) in 1990 to 16 ha (0.5%) in 2010. It was found that the increasing demand for housing and other infrastructure was fulfilled by conversion of fertile agricultural and cultivable waste. The study further revealed that Mardan city is expanding haphazardly in all directions without strategic land use regulations and planning and thus is posing serious threats to the fragile environment.

Keywords: GIS, remote sensing, land use, cereal production, Mardan city

1. INTRODUCTION

Globally, the ongoing urbanization is considered to be one of the foremost threats for sustaining urban ecosystems [1]. Expansion of cities has been continuous for many decades [2]. This expansion particularly in the developing world is taking place at the expense of prime agricultural land [3, 4]. According to the United Nations [5] by the year 2050 the proportion of urban population will rise to 66% and 90% of the projected increase would occur in developing countries. It has also been predicted that most of urban growth will occur in small cities and towns.

Currently, a little over 1% of the earth surface is occupied by urban areas [6]. One of such emerging challenges is the conversion of agricultural land into built environment [7]. The farmland once lost has irreversible impacts on urban landscape and food security [8]. This in turn also influences human health, quality of life and natural environment [9, 10]. Parallel to this, the increasing built-up area at the cost of farmland is responsible for the high degree of impervious surfaces [11, 12]. Breuste [13] has identified numerous negative impacts of urban infrastructure, which include accelerating surface runoff, reducing recharge of underground aquifers and multiplying carbon footprints [14, 15].

Like other developing countries, in Pakistan the urban population has been increasing at a rapid pace and the World Bank has estimated that half of the country's total population will become urban by the year 2020 [16]. By way of increasing

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urban population, the prime agriculture land is being encroached by housing, infrastructure, industrial, educational and commercial uses [17, 18]. Change in urban land uses takes place either by reorganization of land uses or by outward expansion of urban areas [19]. In the reorganization processes, patches of urban agricultural land inside the city get converted into built up areas, whereas in the process of urban expansion, the loss of prime agricultural land in the suburban is irreversibly influenced and the food supply chain get affected adversely [20]. This situation is being aggravated by the rapid rate of farmland conversion into built-up areas [21].

This study focused on Mardan city, Pakistan. Geographically, Mardan city stretches from 34° 9' 4″ to 34°13' 21″ N latitude and 72° 3' 11″ to 72° 14' 48″ E longitude covering an area of 32 sq km (Fig. 1). It is located 64 km away from Peshawar, the provincial capital of Khyber Pakhtunkhwa (KP) and 148 km west of Islamabad, the federal capital of Pakistan. In the present study, GIS and remote sensing techniques were employed to detect changes in the land use pattern. Within Khyber Pakhtunkhwa province, Mardan is the 2nd largest and fastest growing city after capital city district Peshawar. During the past few decades, there has been significant urban expansion over the peri-urban agricultural land of this city. Likewise, the construction of District Head-quarters Hospital, Government Postgraduate College Mardan, Premier Sugar Mills Mardan and Tobacco industry were the other major developments that have attracted a large population to settle in the proximity to of old Mardan city. Since the 1990s, Mardan city has experienced a rapid growth both in terms of population and spatial extent [18]. The urban area has expanded on the fringes at the cost of prime agricultural and waste land. The rich agricultural products in neighborhood of the city have made it a thriving market and have enabled development of agro-based industries in the new vicinities. In addition, there are several bazaars, markets, shopping malls and plazas of commercial importance. A number of new markets and plazas are also under construction on the prime agricultural land. Besides serving Mardan district, the city is considered as one of the most important trade centers for the surrounding districts of KP province [18]. Mardan city is also characterized by complex urban and peri-urban landscapes, with a combination of diverse land uses at varying spatial scales. During the past two decades (i.e., 1990-2010), rapid urban expansion took place over the farmland. Such conversion of land use was reported more seriously at the city periphery as against rural section of district Mardan and so for no empirical research has been carried out on this aspect.

A massive growth in Mardan city had started after 1990 and the city authorities failed to exercise development control during this expansion. The process continued and it is imperative to monitor and quantify the increasing trend of farmland conversion into built-up area and devise strategies for mitigating the adverse impact. In 1901, urban population of Mardan City was mere 4000, which grew and rose to 77,932 in 1961. By the year 1998, the population further increased to 246,000, with the current growth rate the estimated population of the city was 402391 in 2015, and would reach to 512,778 in 2030. With the advancement in geoinformatics, the spatio-temporal satellite images are available to monitor and quantify the changes in land use with time. The remotely sensed data are being increasingly used as an important tool for detecting farmland conversion into built environment [19, 20, 21, 22, 23]. In this study an attempt has been made to apply multi-temporal images while analyzing the urban land use pattern in and around Mardan city, which is expanding rapidly with serious implications on for the peri-urban farmland.

2. MATERIALS AND METHODS

The data were collected both from primary and secondary sources. The primary data includes satellite imageries (Landsat 30 m for the year 1990 and Spot 2.5 m for 2010) of Mardan city were obtained from SUPARCO office, Peshawar (Fig.

2). In order to investigate the causes of urban expansion focused group discussions (FGDs) were conducted with the officials of urban authorities, District Revenue Office, politicians, landlords, elderly people and estate agents. In addition to this, secondary data were obtained from related Government departments. Similarly, maps of Mardan city were obtained from Mardan municipal committee, whereas population data were acquired from the Population Census Organization of Pakistan. Land use and crops statistics were obtained from the Bureau of Statistics and land records of the revenue office, Mardan.

The satellite images enabled us to quantify the changes in land use and soil sealing over the last two decades. Two images (Landsat 30 m for the year 1990 and Spot 2.5 m for 2010) of 20 years apart were chosen. Image classification technique helped to extract information about the land use and land cover type from the remotely sensed data. Both supervised and unsupervised classification approaches were used for processing the satellite images. Supervised classification is considered as an efficient method used to deal with the satellite images. Temporal images of Mardan city were processed using Supervised Maximum Likelihood Classified Algorithm technique in ArcGISenvironment. A total of 150 training samples (signatures) were generated for every land use class, i.e., built-up, agricultural land, cultivable waste and water bodies.With the help of GPS, ground verification was done for ground truthing and sample verification. This helped in processing the data for spatio-temporal analysis of urban expansion in the peri-urban environment. The areas under different land uses were then quantified.

The analyzed data were checked for quantitative and qualitative accuracy assessment for the final classified images of 1990 and 2010. The qualitative assessment is based on visual interpretation techniques and the same was tallied with the open source Google Earth, whereas, the

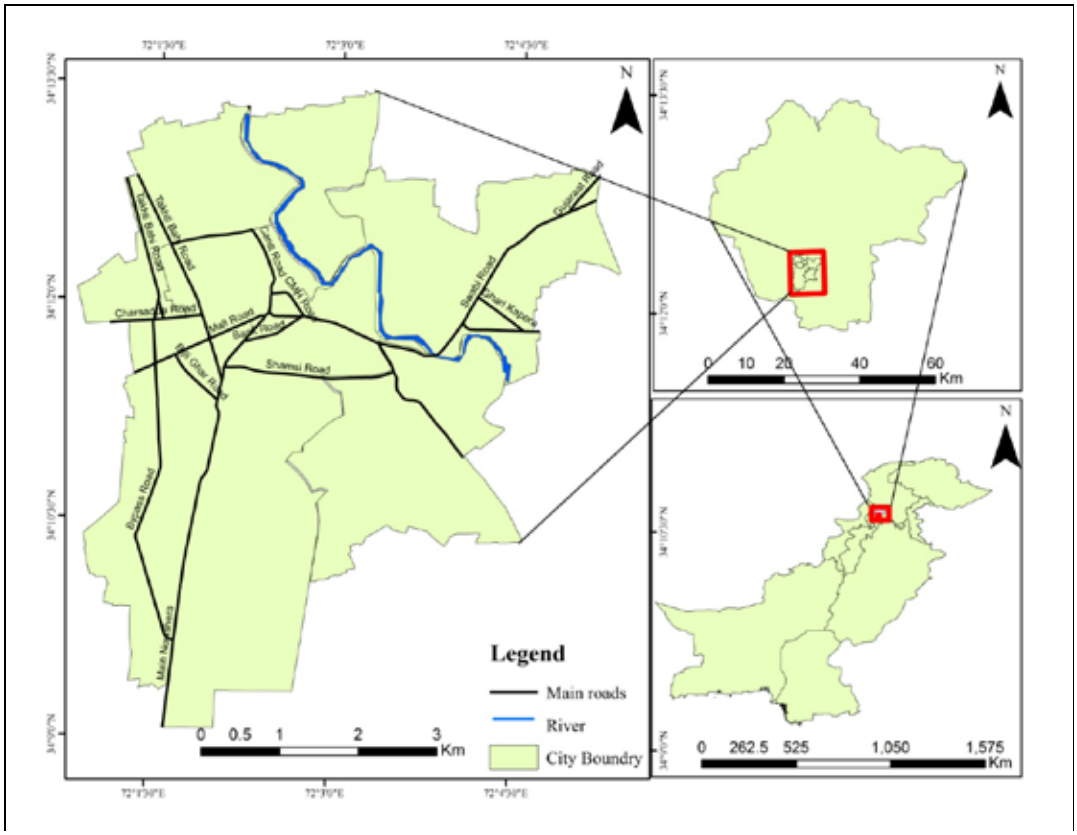

Fig. 1. Location of the study area, in Mardan city, KP, Pakistan

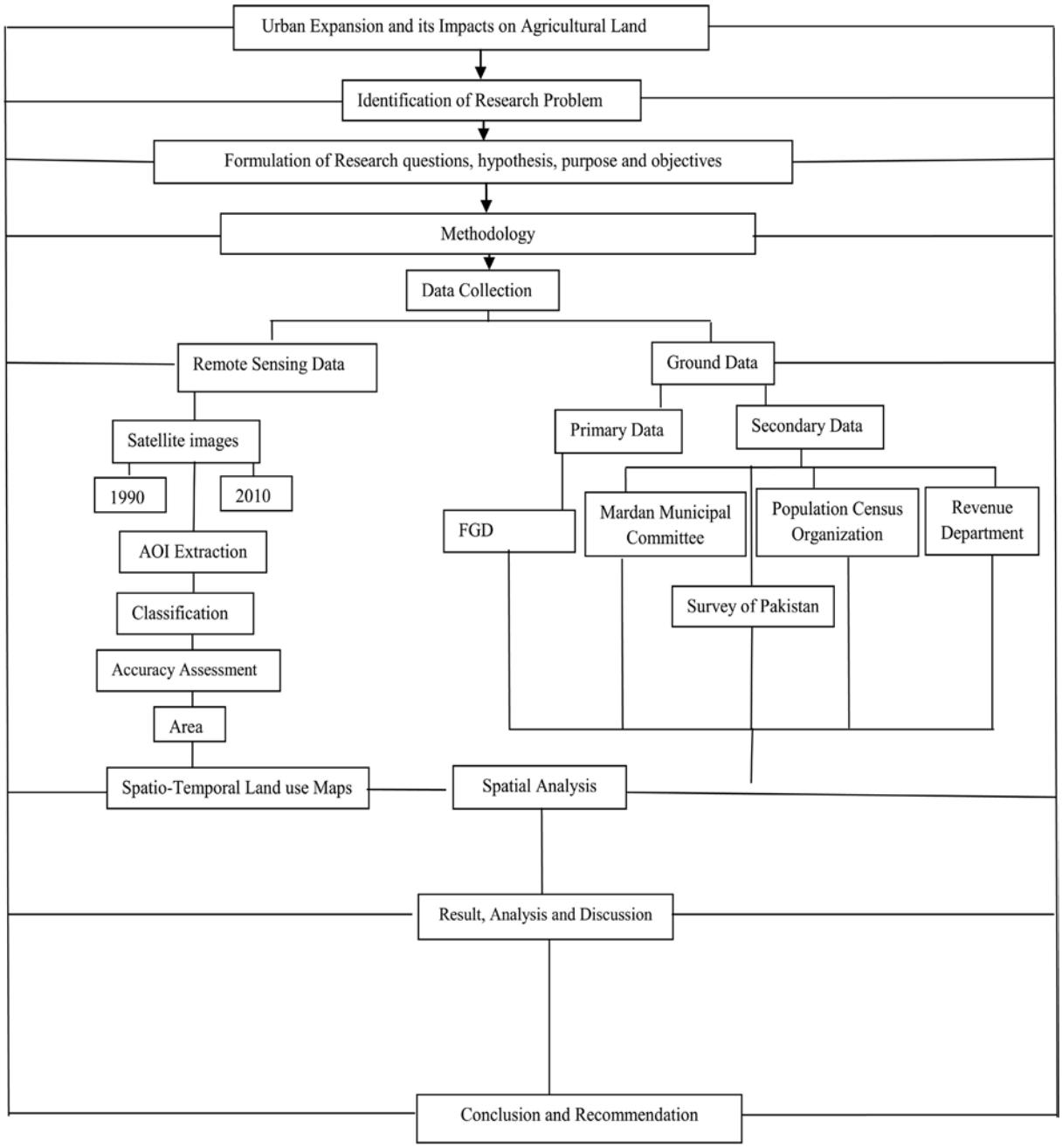

Fig. 2. Research process.

quantitative assessment was carried out through a standard technique of error matrix [24]. A stratified random sampling approach was applied to validate samples and the resultant thematic layer classes. Approximately 50 reference points were then generated for each land use class. Most of the sample reference points were checked during field surveys. Overall accuracy, user's accuracy, producer's accuracy and Kappa coefficient were calculated for 1990 and 2010 classified maps. The resultant maps met the overall minimum 85% accuracy as specified by the Anderson classification scheme [25]. As a result the quantified data for all the land use changes between 1990 and 2010 were compiled and tabulated. The land use maps of Mardan city for 1990 and 2010 were generated, which helped in comparing the changes in land use pattern and the growing pace of urban expansion over the farmlands.

3. RESULTS AND DISCUSSION

In order to achieve the objective, the present study focused on digging out the expansion of built-up area and its impacts on farmland and food production. The analysis revealed that in Mardan city population growth over the time period has caused great changes in the available land use. The demand for new houses increased with increasing population and growing income. The population increase has mainly been due to both natural increase and rural to urban migration. People from the surrounding areas have migrated to the city in search of better employment opportunities, education and health facilities.

The spatio-temporal dynamics in land use from 1990 to 2010 were extracted through the analysis of multi-spectral Landsat images. Rapid growth in population and subsequent urbanization is the main cause of enormous increase in land use changes in Mardan city. The overall land use changes that took place in 1990 and 2010 (Table 1; Fig. 3a, 3b). The urban built-up area that has increased substantially during the last two decades has been illustrated (Fig. 4). During the study period, Mardan city has undergone substantial expansion. Both visual and change detection maps revealed that most of the farmland in Mardan city has been consumed by residential, commercial and other uses indicating that arable soil has been converted to impervious surfaces. Likewise, in 1990 a big chunk of waste land was converted for such uses. The conversion of the food basket land and cultivable waste land into different land uses has been discerns (Fig. 5).

3.1 Status of Land Use/ Land Cover

In this study, a post-classification detection method was applied. The change metrics of Weng [26] has been used to quantify the extent of land utilization in 1990 and 2010 and the resultant gains and losses in each category were analyzed. The analysis revealed that in 1990 approximately 30% area of Mardan City (953 ha) was under built environment, 42% (1,339 ha) under agricultural land and the rest 26% (827 ha) was waste land (Table 1). In addition to this, 1.1% area (35 ha) was occupied by river Kalpani. During the year 2010, the built-up area was 63% (1994 ha), agricultural land 35% (1,109 ha), and cultivable waste 0.5% (16 ha), whereas area under water bodies remained unchanged during the study period (Table 1). This expansion of built-up area had engulfed the surrounding farmland, whereas the waste land has increased. With the rapid growth of population, the housing demands also increased and the nearby cropped land was converted into built-up environment. Along with land use conversion, the land value within the city limits has escalated several times.

Table 1 Mardan city: Land use change, 1990-2010.

Land use	1990		2010		Change 1990-2010	
	Area (ha)	Percent Share	Area (ha)	Percent Share	Change in Area (ha)	Percent change (%)
Built-up area			407.5	12.9		
Commercial	953	30	85	2.7	1041.5	33
Residential			1502	47		
Agricultural land	1339.1	42.4	1108.8	35.1	-230.3	-7.3
Cultivable waste	827.4	26.2	16.4	0.5	-811	-25
River	35	1.1	35	1.1	0	0
Total	3154.7	100	3154.7	100		

Source: LANDSAT 1990, SPOT 2010

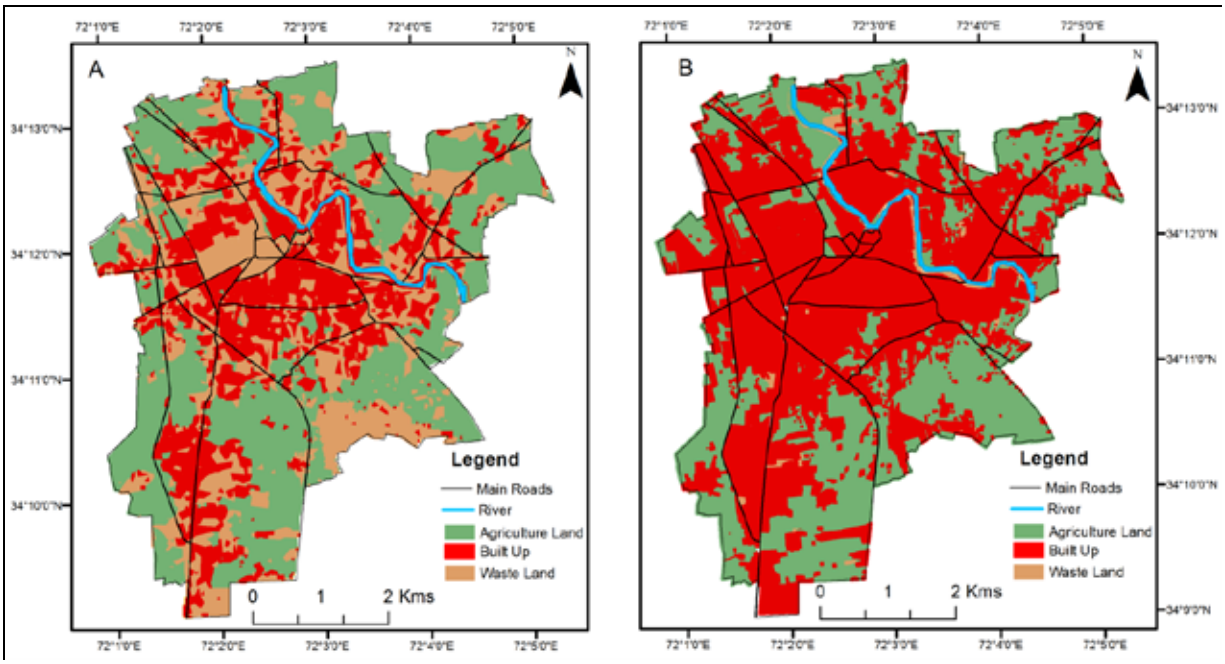

Fig. 3. Mardan city: Spatial distribution of land use, 1990-2010: (a) Land use in 1990; (b) Land use in 2010.

3.2 Spatio-temporal Analysis of Land Use/ Land Cover

The analysis revealed that, during the last two decades, both negative and positive changes have occurred in the land use and land cover pattern of Mardan City (Table 1; Fig. 3). The built environment has more than doubled as it has increased from 953 ha in 1990 to 1994 ha in 2010. The increase in built up area accounts for 52% of the total area. This increase in built-up environment was occurred as a result of rapid growth in urban population and creation of better transport facilities and other amenities within the city. As a result, the demand for housing has increased, which allowed the people to construct new housing units on the available farmland haphazardly without taking into consideration proper planning and management. Transport infrastructure has become another determining factor in expansion of Mardan city and accessibility from newly built-up areas. Anjum and Hameed [27] noted that those housing schemes were more successful which were located close to the existing built-up areas. Perhaps these provided a better sense of security in a new scheme to a potential dwelling owner. This appears to be one of the reasons for the rapid expansion which took place in the existing villages which were eventually engulfed by the city and the agricultural land was converted into residential areas.

The comparative analysis revealed that a gradual decrease has occurred in the farmland from 1,339 ha in 1990 to 1,109 ha in 2010, which is 17% of the total area. The main reason behind this decline in cropped land was the increase in land prices which encouraged the poor rural households to sell their land at higher prices. Having small parcels of land, they could not meet their family needs; therefore, with better price offers they sold their farmlands and opted out for alternative sources of income. Similarly, the cultivable waste land has decreased drastically, from 827 ha in 1990 to mere 16 ha in 2010. Most of this waste land has been transformed into built-up areas. However, the area under water bodies remained same during the two decades (1990-2010).

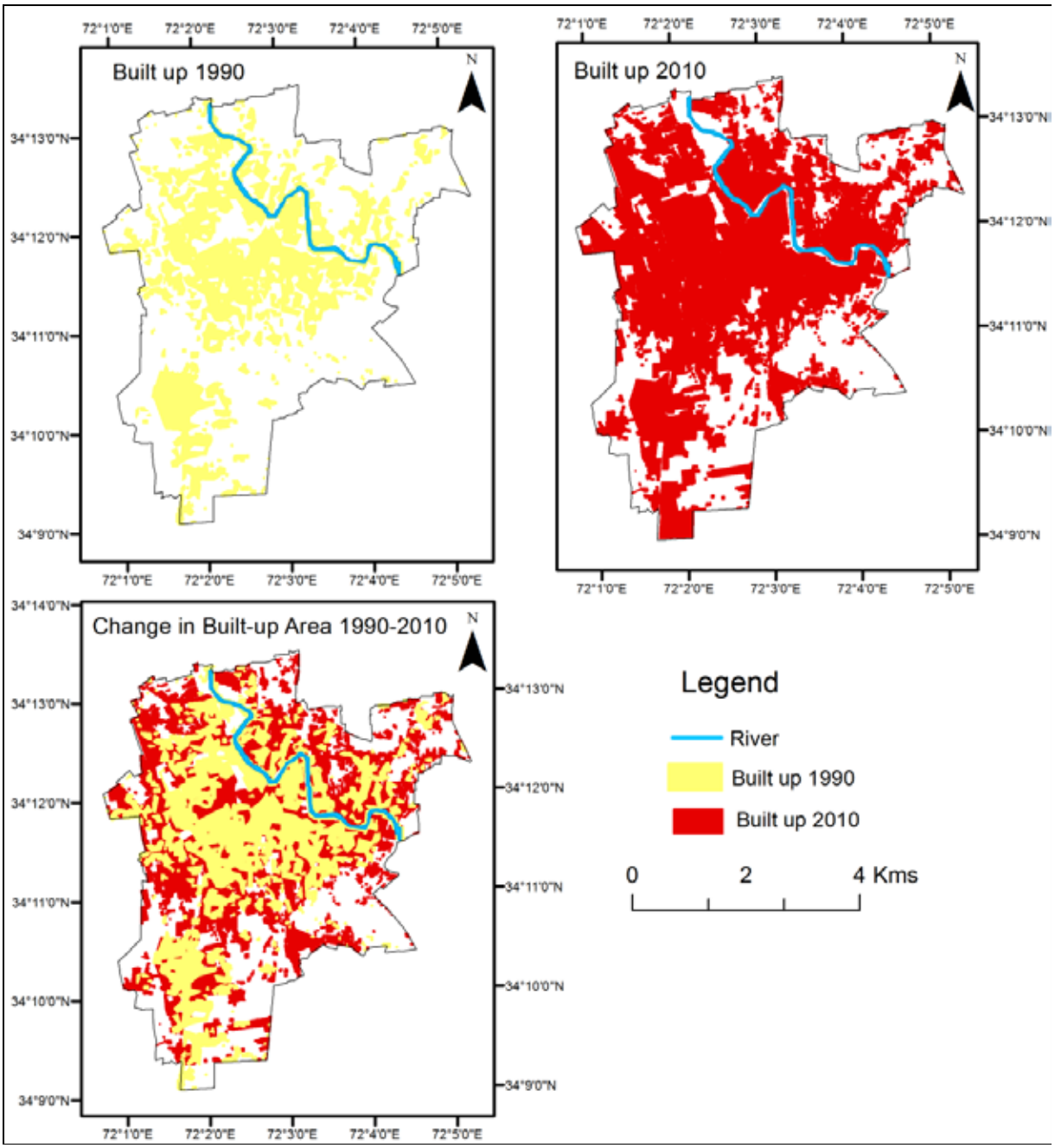

Fig. 4. Mardan city: Spatial growth of built-up area, 1990-2010.

To understand land encroachment in different land use categories, a change detection matrix (Table 2; Fig. 5) was prepared which revealed that during the two decades (1990-2010), the farmland within the city limits has been gradually transformed. The highlights of these transformations in Mardan City over 1990-2010 period are given in Table 2. The largest consumer of farmland was built-up environments. A total 613 hectare of waste land was lost to built-up uses over the study period of 1990-2010. This means that 52% of the newly built environment was developed over the waste land. However, almost 33% of farmland was also lost to built-up area during this period. Nevertheless, there was also gain in the agricultural land mainly by bringing around 141 hectares of cultivable waste land under plough. The spatial pattern of these changes has been shown in Fig. 5.

Table 2 Farmland conversion in Mardan city, 1990-2010.

Land use change 1990-2010	Area lost (hectares)	Percent of total loss
Waste Land to Built-up area	613	54.5
Farmland to Built-up area	371	33
Waste Land to Farmland	141	12.5
Total	**1125**	**100**

Source: LANDSAT 1990, SPOT 2010.

3.3 Trend of Cereal Production in Mardan City since 1990

The food production has seen marked growth during the last half-century, allowing a dramatic decline in the percentage of hungry people across the world, in spite of doubling of the total population [28]. However, more than one out of seven people today still do not have access to balanced food [29]. Currently, the world is facing a new set of intersecting challenges. The global population will continue to increase and it is estimated that approximately 9 billion people will be living on this tiny planet by 2050. With

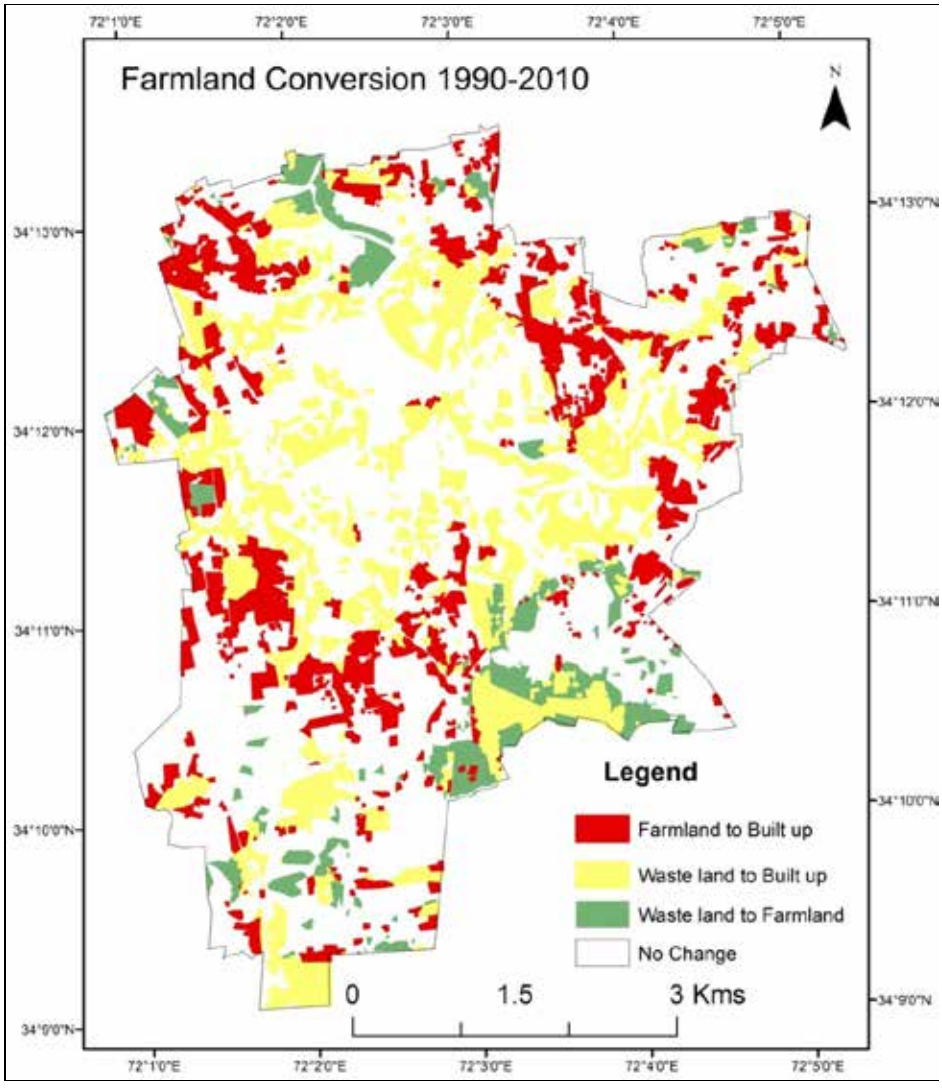

Fig. 5. Mardan city: Farmland conversion, 1990-2010.

increase in population, the demand of food consumption has increased, which has put immense pressure on the food supply chain. The food producers at the same time are experiencing tough competition for the land, water and energy, and are trying to minimize the negative impacts of food production on the environment [29]. Therefore, the urban food production is generally under estimated. Mouget [30] has conservatively estimated that 15-20% of the world food is produced in urban areas. According to Nelson [31], approximately 200 million urban dwellers are engaged in urban farming and providing food to almost 800 million people. Case studies point out significant degree of self-sufficiency in fresh vegetable, poultry production and other animal byproducts.

In south Asia, the population tripled from 588 million in 1990 to 1621 million in 2010. During the same period, with the increasing population, there is decline in per capita farmland. Kumar et al. (2012) found that during 1980 to 2010, in Bangladesh per capita agricultural land decreased from 0.11 ha to 0.05 ha, in India 0.23 to 0.13 ha, in Nepal 0.15 to 0.08 ha and in Pakistan 0.24 to 0.12 ha. It is predicted that by the year 2015, South Asian population will escalate to 2.2 billion people and their cereal requirement will rise from 241 million tons in 2000 to 476 million tons in 2025 [32].

According to Veni and Alivelu [33] in India the rapid growth in population has put immense pressure on the farmland, which has decreased the production of food grains crops. The land under built environment has been doubled over a period of past 50 year, which was 9.36 million hectares in 1951 and climbed to 22.97 million hectares in 2001 [34]. Tan et al. [35] identified a dramatic decreased in per capita farmland during 1998-2009 in Beijing from 0.05 to 0.03 ha, Zhejiang 0.06 to 0.04 ha, Shanghai 0.06 to 0.02 ha, and Guangdong 0.06 to 0.02 ha. It is mainly due to conversion of food basket land into built-up uses. Rauch and Morrison [36] pointed out that about 922 ha of fertile agricultural land in District Sleman (Indonesia) has been converted into housing complexes, with the conversion rate of about 33% (3.3% annually) during 10 year. Due to conversion of arable land, there has been remarkable decrease in production of rice in districts of Sleman and Bantul.

Pakistan is a developing country. Here, agriculture is considered as the backbone of country's economy. Its rapidly growing population largely depends on agricultural sector. The country's growth rate has declined from over 3% in 1980 to 2.09% in 2010. With the existing growth rate, the estimated population will get doubled by the year 2050 [37]. However the availability of per capita cereals has increased from 120 kilograms to 137 kilograms from 1961 to 1991, and then climbed to 154 kilograms in 2008-09.In spite of considerable improvements in food supply, the malnutrition is still a serious challenge in Pakistan [38].

Studies of urban encroachments of agriculture land and its impacts on food production in Pakistan have remained limited to large cities like Lahore or Peshawar. According to Zaman [39], in Lahore the production of wheat, maize, sugarcane, vegetables and fruit has been reduced and about 114,630 ha of cultivated land has been eaten up by built-up uses during 1987-2008. Samiullah [18] has studied the expansion of built-up area and its impacts on agricultural land in Peshawar City District and has stated that this district had lost most of its best agricultural land to urbanization. Residential land use was the largest consumer of arable land followed by the brick kilns during the period 1991-2009. Bhalli et al. [40] applied GIS and remote sensing technologies to monitor and assessed urban sprawl in Faisalabad, and pointed out that during the 30 years (1980-2010), there had been a rapid increase in the urban built environment. This expansion has taken place on the primary or fertile agricultural land in urban fringe and as a result the city area has doubled from 102 sq km in 1980 to 213 sq km in 2010 and, consequently, has drastically reduced the agricultural acreage.

In the Mardan study area, with the increase in population over the last two decades, the availability of per capita farmland has declined from 0.006 hectare in 1990 to 0.002 hectare in 2010. The per capita agricultural land in the city

limits will continue to decrease with continuing population growth. The Table 3 depicts the situation of food production in Mardan City. The yield per hectare has increased over the last 20 years. Also over the last two decades (1990-2010) the production of wheat has improved from 1,744 kg per hectare in 1990 to 2,104 kg per hectare in the year 2010. Likewise, the production of rice has increased from 1,538 kg to 1,890 kg, sugar cane from 39,987 kg to 48,550 kg per ha, maize from 1,918 kg to 3,324 kg and barley from 910 kg to 885 kg per hectare.

Table 3 Change in yield of major crops in Mardan city, 1990-2010.

Crops	Yield (kg/ha) 1990	Yield (kg/ ha)2010
Wheat	1744	2103
Rice	1538	1890
Sugar Cane	39987	48550
Maize	1918	3324
Barley	885	910

Source: Development Statistics of Khyber Pakhtunkhwa, 1990, 2010.

4. CONCLUSIONS

The present study demonstrated the effectiveness of remote sensing and Geographic InformationSystem as a tool in analyzing land use changes. The analysis revealed that Mardan city has experienced a rapid growth during the last two decades (1990-2010). The area under built environment has increased from 953 ha to 1,994 ha over the study period. The city has expanded in all directions over the peri-urban farmland. Most of this expansion is haphazard and unplanned mainly following ribbon sprawl along the roads and radiating out of the city. Over the period, the agricultural land has been lost to built-up area. The cultivable waste land which covered a big chunk (827 ha) in 1990 has been reduced to mere 16 ha in 2010. During 1990-2010 about 613 ha of cultivable waste land and 371 ha of farmland were lost to built-up uses. Though, there was also a gain in farmland, about 141 ha of cultivable waste land was transformed to farmland. Escalation in land value, housing demands, improvement in transport was the main factors responsible for the conversion of farmland into built-up areas. Consequently, the per capita farmland has been reduced, gradually. However, the production of cereal crops have been increased due to better irrigation facilities and utilization of improved type of seeds and application of fertilizers over the study period.

In order to sustainably utilize the urban land, the government must promote agriculture and encourage, establish and reinforce farmer organizations in Mardan city and or in peri-urban areas. Also, effective land use zoning may help to contain the urban encroachment over the farmland. The urban authorities may restrict construction on the available farmland. It could also develop alternate sites for development of new townships on the barren land. Monitoring urban expansion is also necessary to make the city's expansion is accordance with the urban planning standard. GIS and Remote Sensing techniques could be used to monitor urban expansion in and around Mardan city. The government should also initiate awareness programs on the negative implications of haphazard urban sprawl.

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Research Article

Comparative Studies on Flour Proximate Compositions and Functional Properties of Selected Pakistani Rice Varieties

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Abstract: The aim of this study was to evaluate the proximate and functional properties of flours obtained from various rice varieties, i.e., Super Basmati Shaheen, Super Basmati Kainat, Super Fine, JP5, Basmati 385, Fakhr-e-Malakand grown in different areas of Pakistan. Moisture content, ash content, crude protein, crude fat, water absorption index, water solubility and swelling power varied significantly ($P<0.05$) from each other. Moisture content, ash content, crude protein and crude fat of rice flour ranged from 5.46–7.08%, 0.48–1.23%, 8.02–9.85% and 0.43–1.5%, respectively. While, functional properties such as water absorption index, water solubility index and swelling power were found in the range of 5.38–6.26 (g/g), 1.95–4.94% and 5.74–7.64 (g/g), respectively. The results showed that the functional properties of rice flour were greatly influenced by the compositional variations of various rice varieties.

Keywords: Rice flour, proximate composition, water absorption index, water solubility index, swelling power

1. INTRODUCTION

Rice is an important cereal grain and is one of leading food crops in the world. Rice is a member of the family Poaceae (Gramineae) and is generally considered as annual grass, semi aquatic plant. Rice is a staple food in many Asian countries. South Asia alone produces about 30 % of global rice production [1]. The functional properties of rice grains such as, pasting profiles, gel texture and water holding capacity and cooking characteristics are important factors which determine the end use and marketing of rice grains, Rice has many beneficial properties as it is easy to digest, has mild taste and hypoallergenic properties [2-3]. Rice provides 45% of calories and 40% total protein requirement of an average population [4].

Different rice varieties are grown all over the world and have showed compositional variations in term of protein, lipid, starch content (amylose and amylopectin), minerals, vitamins, thermal properties, texture profile and pasting profile [5-7]. The variation in composition of rice depends on the genetic and environmental factors [8-9]. It is also observed that the properties of rice flour are also influenced by particle sizes, pasting properties and texture properties [10-12]. Rice grain quality is also influenced by various physicochemical characteristics [13-14]. The chemical and physical properties of rice flour such as starch gelatinization, water absorption and viscosity differ due to the compositional variation of rice flour [5-15].

A wide range of rice varieties are reported and their physicochemical and functional characteristics vary from one another [6]. The characterization of rice flour would facilitate the understanding of its possible applications [16]. Similarly, different rice varieties are grown in

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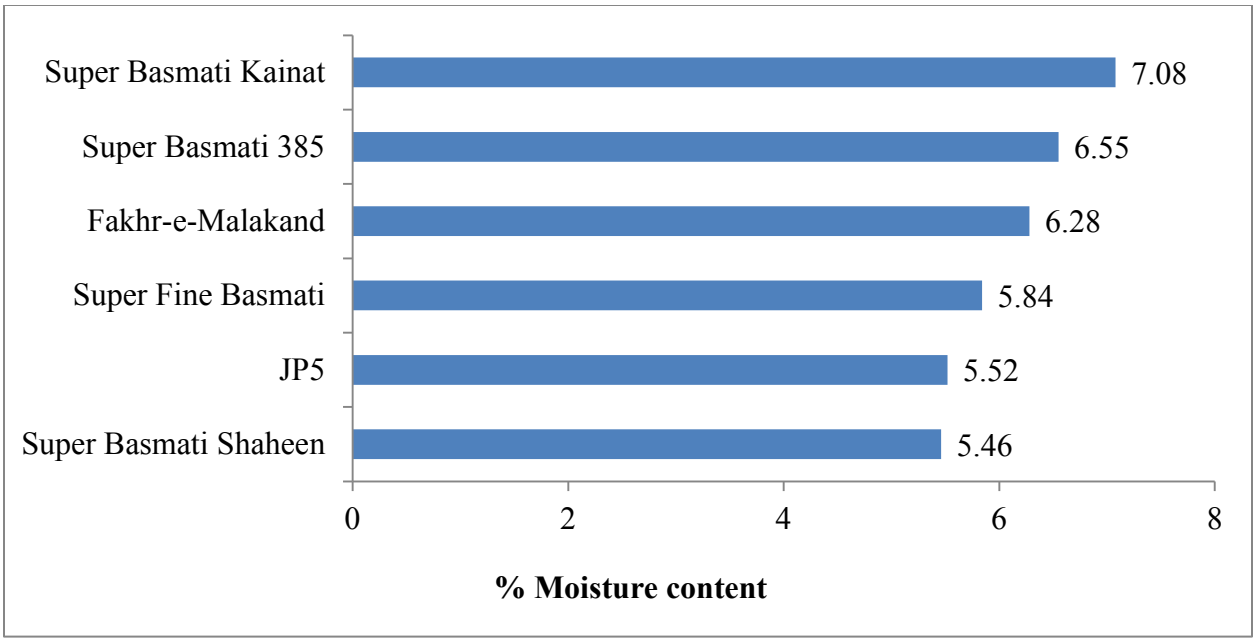

Fig. 1. Moisture content (%) of flour of selected rice varieties grown in Pakistan.

different part of Pakistan. However, no scientific work is reported in literature on the composition and especially on the functional properties of these varieties. Primarily, the aim of this study is to determine and compare the physicochemical and functional properties of selected rice varieties namely, Super Basmati Shaheen Rice, Super Basmati Kainat Rice, Super Fine Rice, JP5, Basmati 385, Fakhr-e-Malakand, cultivated in different areas of Pakistan.

2. MATERIALS AND METHODS

In this study, experiments were performed in the laboratory of Food Science and Technology Department, The University of Agriculture, Peshawar. The rice varieties were selected from various places for the study purpose. Fakhr-e-Malakand and Basmati 385 were procured from Agricultural Research Station Chitral. Super Fine and JP5 were procured from The University of Agriculture Peshawar. While two varieties

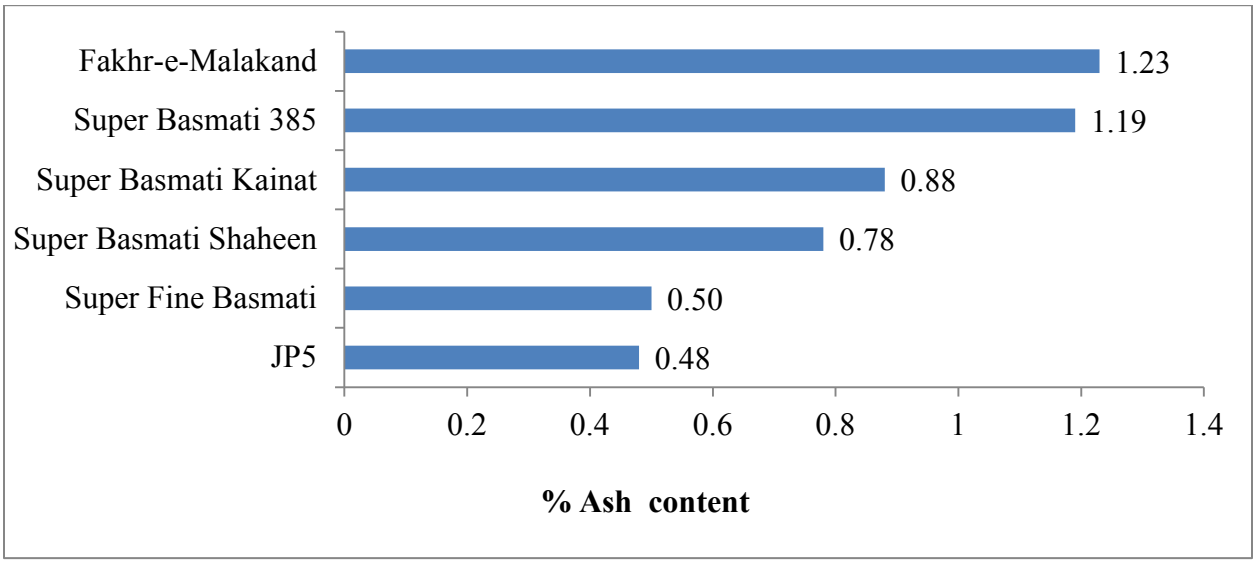

Fig. 2. Ash content (%) of flour of selected rice varieties grown in Pakistan.

namely, Super Basmati Shaheen and Super Basmati Kainat were purchased from the main market of Peshawar, on 5th May 2014. Three samples (2 kg) of each variety were collected from the respective sources and brought to the laboratory of Food Science and Technology Department for further research work. Rice varieties were properly cleaned and brought to mill house to obtain the flour. The chemicals and reagents used in the research work were extremely pure with analytical grade.

2.1 Preparation of Rice Flour

Rice was grinded to flour by the method as suggested by Fari et al [6] using manual laboratory mill (Thomas Wiley, Model 4 USA). The flour was sifted through 100 mesh sizes at Food Technology Laboratory, Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan. Rice flours were packed in plastic bags and stored at 4 °C for further study. Results were collected in three replications for each sample.

2.2 Proximate Composition of Rice Flour

The moisture content, ash content, crude protein and crude fat content of rice flour were determined by the method of AOAC [17].

2.3 Functional Properties of Rice Flour

The functional properties including water absorption index (WAI), water solubility index (WSI) and swelling power (SP) of the rice flour were analyzed by the method of Thumrongchote et al [18]. For the determination of water absorption and water solubility index 1 g of rice flour sample was weighed and placed in a centrifuge tube. Then 6 ml of distilled water was added for suspension. The tubes along with the samples were heated in shaking water bath at the temperature of 80 ^{0}C for 30 minutes. The solution was centrifuged at 2500 rpm for 10 minutes. After the centrifugation, the supernatants were carefully poured into Petri dish for drying at 105 ^{0}C for 10 hours in an oven, while the sediments were weighed as such. Water absorption index was calculated on the basis of wet sediments, while water solubility index was calculated on the basis of difference in the weight of dried supernatant and initial sample (equation 1 and 2).

$$WAI = \frac{wt.\ of\ wet\ sediment}{dry\ wt.of\ flour} \quad \text{Eq. 1}$$

$$WSI = \frac{wt.\ of\ dried\ supernatant}{dry\ wt.of\ flour} \quad \text{Eq. 2}$$

On the other hand, 0.5 g flour sample were taken in a centrifuge tube (pre-weighed) and diluted with 15 ml of distilled water in order to determine swelling power. The suspension was heated in water bath at a temperature of 80 ^{0}C for 30 minutes and then centrifuged at 4000 rpm for

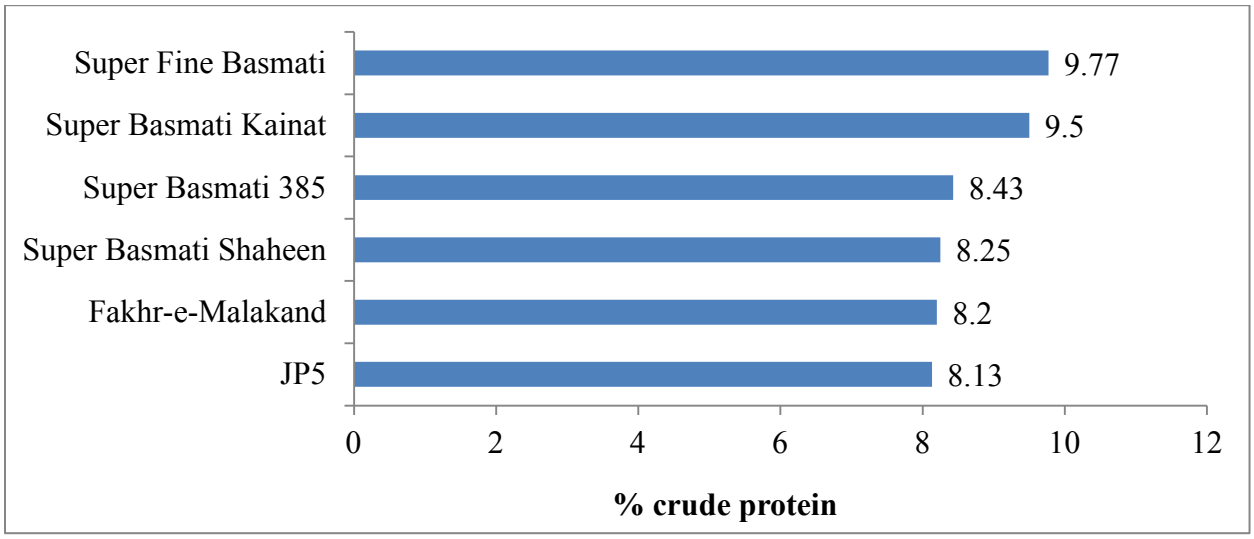

Fig. 3. Crude protein (%) of flour of selected rice varieties grown in Pakistan.

20 minutes. After centrifugation, the supernatants were poured into Petri-dish of known weight and dried at 105 ^{0}C for 10 hrs. After complete dehydration, the supernatants were weighed and the sediments from the centrifuge tube were also weighed. Swelling power was calculated by wet sediment and dried supernatant of rice flour (equation 3).

$$SP = \frac{wt.\ of\ sediment}{wt.of\ flour - wt.of\ dried\ supernatant} \quad \text{Eq. 3}$$

2.4 Statistical Analysis

Statistical analyses were performed via the software Stitistix 8.1. Data regarding the rice flour were analyzed by complete randomize design (CRD) one way ANOVA with three replications. Mean values were separated by Least Significant Difference Test (LSD) as described by Steel and Torrie [19].

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis of Rice Flour

Proximate composition including moisture content, ash content, crude protein and crude fat of rice flour are presented in Table 1. The moisture content of selected rice varieties showed significant (P<0.05) difference. The moisture content of rice flour ranged from 5.46% (Super Basmati Shaheen) to 7.08% (Super Basmati Kainat). The data revealed that moisture content of Super Basmati Kainat flour is significantly different than rest of the rice samples. The present findings are in agreement with the results reported in previous literatures [18, 20-21]. Thumrongchote et al [18] found moisture content of 5.00-5.88 % among non-glutinous rice varieties. In contrast, Islam et al [20] and Nura et al [21] observed comparatively higher moisture content (8.00-9.61%) in rice flour. Milling method, processing procedure and temperature affect the moisture content in rice flour [22]. Similarly, fiber content affects the moisture content of the flour. As the percentage of fiber content is higher in flour, the moisture content would be higher [23]. Moisture content above 20 percent accelerates microbial growth, so moisture content below this limit is preferable [24].

Table 1 shows the ash content of rice flour of different varieties which are significantly (P<0.05) different from each other. The ash content of rice flour of these selected varieties ranged from 0.48% (Fakhr-e- Malakand) to 1.23% (JP5). The present results are in accordance with literature reported previously. According to Han et al [25], the ash content ranged from 0.40-0.80% in rice flour of different Korean rice varieties. Similarly, Thumrongchote et al [18] studied that the ash content of rice flour varied from 0.30-0.40%. The ash content of rice flour of three selected varieties was in the range of 0.17-0.48% [21, 26]. Similarly, Islam et al [20] reported that rice flour has 1.5% ash content. Rice bran contain higher ash content (6.73%) as compared to other cereal bran [20]. It was observed that the variations in ash content of rice flour are due to varietal differences, sources and milling methods [25].

Table 1 illustrated that the protein content of rice flour of selected rice varieties varied

Table 1 Proximate composition of rice flour of selected rice varieties grown in Pakistan.

Varieties	Moisture %	Ash %	Protein %	Fat %
Super Basmati Kainat	7.08 ± 0.166 a	0.88 ± 0.06 b	9.50 ± 0.55 b	0.8 ± 0.05 c
Super Basmati Shaheen	5.46 ± 0.12 c	0.78 ± 0.06 b	8.25 ± 0.05 d	1.5 ± 0.2 a
Super Fine Basmati	5.84 ± 0.35 c	0.5 ± 0.05 c	9.77 ± 0.06 a	1.06 ± 0.15 b
Fakhr-e-Malakand	6.28 ± 0.22 b	1.23 ± 0.10 a	8.20 ± 0.02 e	0.43 ± 0.05 d
JP5	5.52 ± 0.13 c	0.48 ± 0.02 c	8.13 ± 0.07 f	0.58 ± 0.05 d
Super Basmati 385	6.55 ± 0.37 b	1.19 ± 0.07 a	8.43 ± 0.02 c	0.53 ± 0.05 d

Mean values following different letters are significantly (P < 0.05) different from each other.

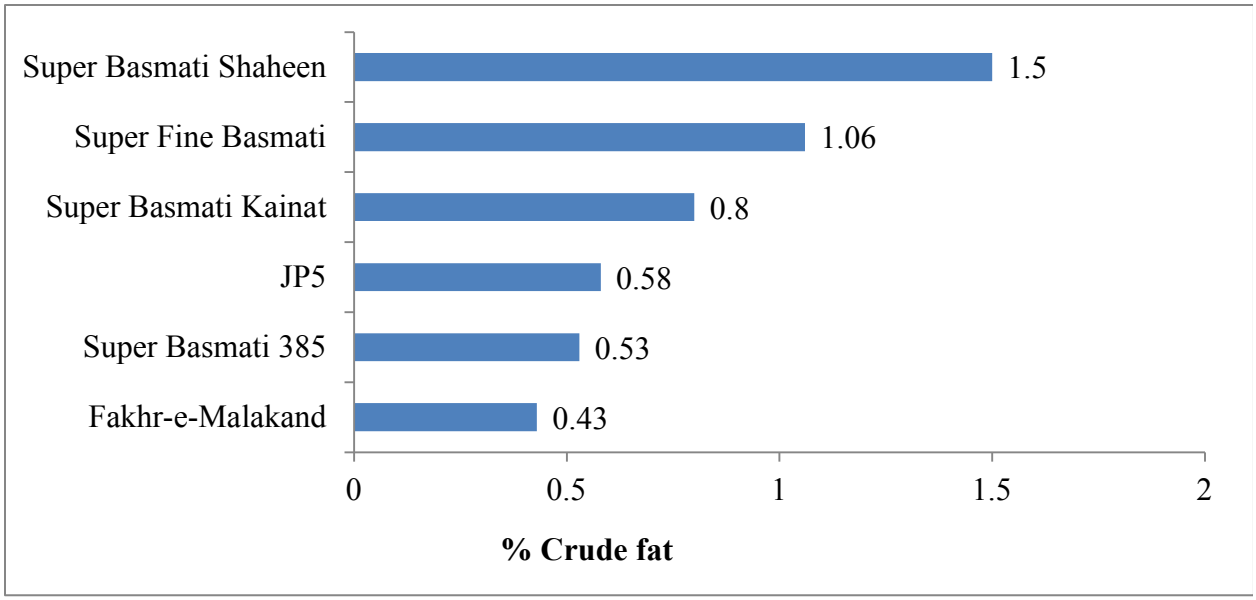

Fig. 4. Crude fat (%) of flour of selected rice varieties grown in Pakistan.

significantly ($P<0.05$) from each other. The protein content of rice flour of these selected varieties ranged from 8.13% (JP5) to 9.77% (Super Fine Basmati). The data revealed that protein content of Super Basmati Kainat and Super Fine basmati is significantly higher than rest of the varieties. The results are in agreement with the findings of Islam et al [20], Nura et al [21], Han et al [25] and Wadchararat et al [26]. They reported that protein content of rice flour was in the range of 6.0% to 9.0%. Similarly, Fari et al [6] analyzed the protein content (6.84-11.18%) of rice flour obtained from selected eight varieties of Sri Lanka. The variation of protein content within varieties is influenced by the method of milling, as protein is mostly found in embryo tissue (aleurone layer), which is removed during milling of grain [27]. However, the physicochemical properties of

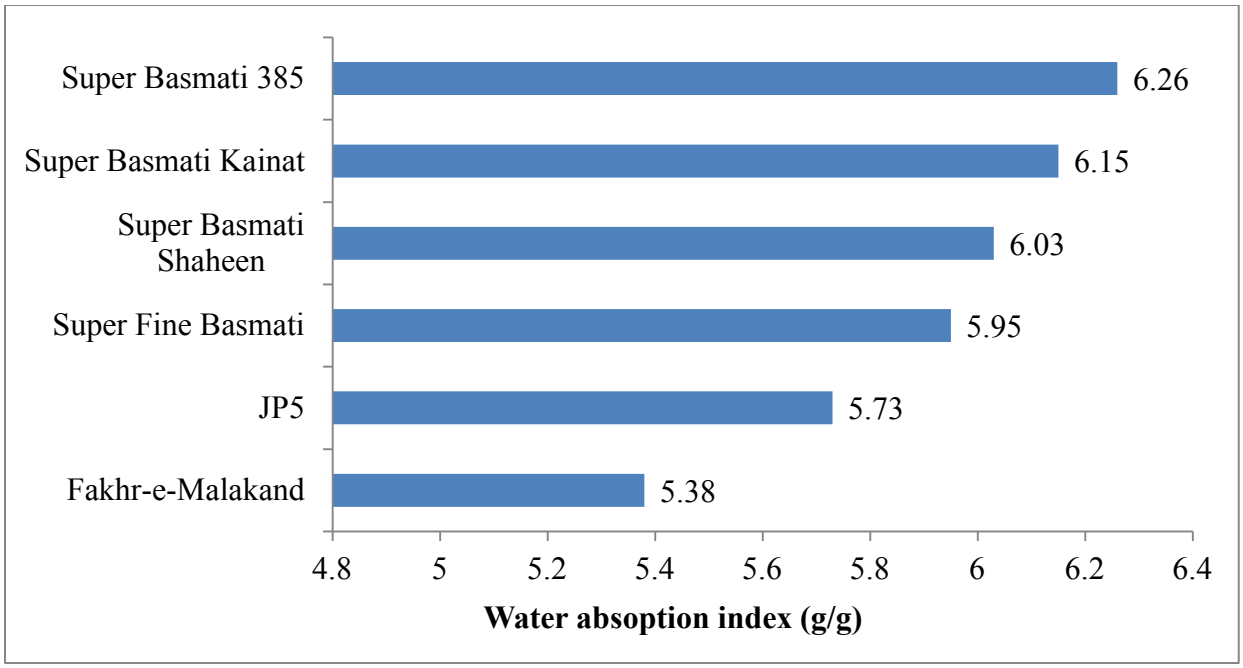

Fig. 5. Water absorption index of flour of selected rice varieties grown in Pakistan.

rice flour is also influenced by various environmental factors, genetic modification, storage condition (time and temperature) and equipment used. The changes may directly or indirectly affect the properties of rice flour [28-30]. Protein and starch molecules are bound together to form a strong network, which substantially affect the pasting profile of flour. Higher protein content in flour will be able to form bonds with greater starch content, thereby increasing the water holding capacity of the flour [31-32]. High protein content improves the nutritional and textural profile of product prepared from such flour [26].

Statistical analysis showed that crude fat content of rice flour of selected varieties differed significantly ($P<0.05$) from each other (Table 1). It was found that Fakhr-e-Malakand has lowest (0.43%) crude fat content, while Super Basmati Shaheen has highest (1.5%) crude fat content among the varieties. Similarly, Fari et al [6], Thumrongchote et al [18], Han et al [25] and Islam et al [33] observed that fat content of rice flour of different varieties were in the range of 0.56-1.36%, 0.63-2.17%, 0.27-0.30% and 2.80% respectively. The fat content varies significantly among different rice varieties, which might be due to varietal differences, environmental factor, sample processing and milling methods [25-26, 28]. Storage condition (temperature and storage time) also influence the physicochemical properties of rice flour. These variations may directly or indirectly influence the properties and composition of rice flour [28]. Fat content was found in greater amount in rice bran as compared to other cereal bran. However, some portion of bran is removed during milling which substantially reduce the fat content. So milling process must be optimized, if higher fat content is desired in flour [25-26].

3.2 Functional Properties of Rice Flour

The functional properties of flour have great importance in the manufacturing of products and it is the basic property that reveals the relations between the structure, composition and molecular arrangement of food components with the nature of environment where it is measured and associated. Functional properties provide useful information for industrial purpose determined by their chemical, physical and organoleptic properties [33]. The functional properties of rice flour such as water absorption index; solubility and swelling power of different rice varieties grown in Pakistan were studied and are presented in Table 2.

3.2.1 Water Absorption Capacity (WAI)

Water absorption capacity of rice flour of different selected rice varieties showed significant ($P<0.05$) difference (Table 2). The water absorption capacity of Super Basmati 385 is higher (6.26 g/g) than rest of the rice varieties. While, lower (5.38 g/g) water absorption index was recorded for Fakhr-e-Malakand. The difference in water absorption index of rice flour might be due to various factors such as methods of milling (dry or

Table 2 Functional properties of rice flour of different selected rice varieties grown in Pakistan.

Varieties	Water Absorption index (g/g)	Water Solubility %	Swelling Power (g/g)
Super Basmati Kainat	6.15 ± 0.05 b*	1.95 ± 0.046 c	7.64 ± 0.03 a
Super Basmati Shaheen	6.03 ± 0.015 c	4.94 ± 0.045 a	6.72 ± 0.02 d
Super Fine Basmati	5.95 ± 0.01 d	1.98 ± 0.005 c	6.94 ± 0.04 c
Fakhr-e-Malakand	5.38 ± 0.01 e	1.95 ± 0.046 c	5.74 ± 0.045 e
JP5	5.73 ± 0.015 f	3.92 ± 0.03 b	6.71 ± 0.015 d
Super Basmati 385	6.26 ± 0.01 a	1.96 ± 0.01 c	7.26 ± 0.01 b

*Mean values followed by different letters are significantly different from each other ($P < 0.05$).

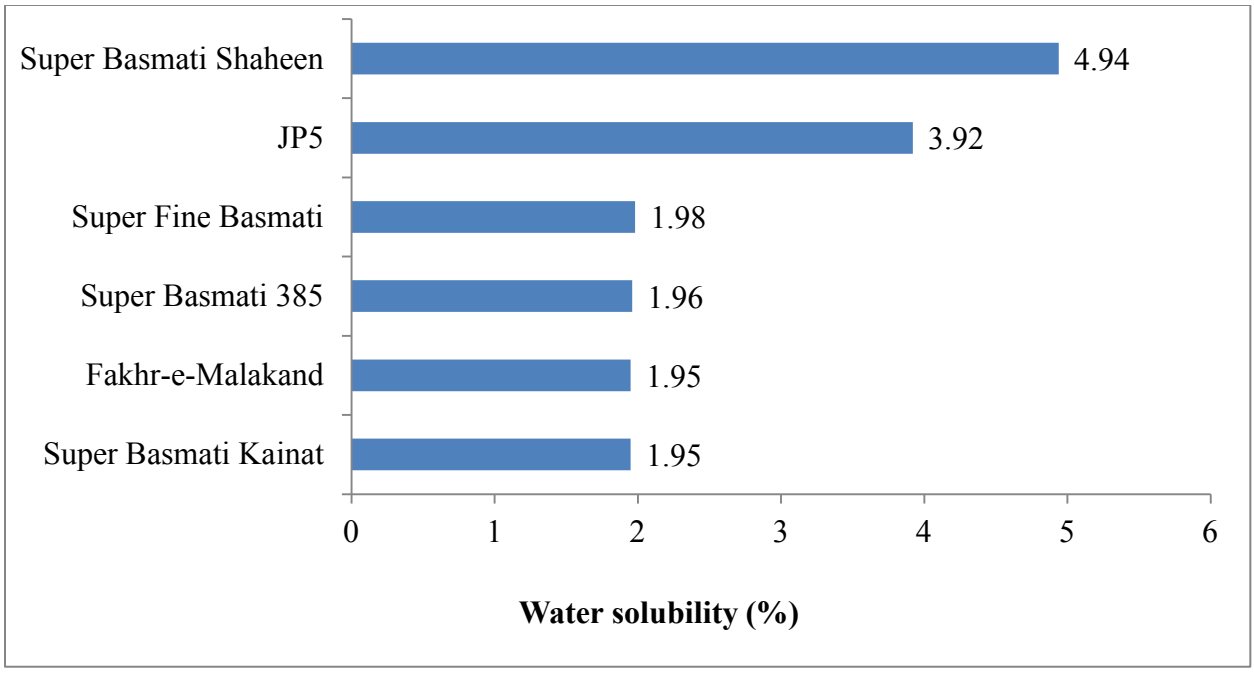

Fig. 6. Water solubility (%) of flour of selected rice varieties grown in Pakistan.

wet), level of starch damaged and processing temperature [34]. Similarly, the results presented in this study are in agreement with the findings of Thumrongchote et al [18] and Heo et al [34], who observed WAI of 6.0–8.0 and 3.3–5.0 g/g, respectively, in flour obtained from different rice varieties. Water absorption is the ability of flour to associate with water under specific conditions where water is limited [35-36]. The composition of flour such as carbohydrate, fiber, protein and amylose content are the major factors influencing water absorption index [18, 21, 36]. Particle size of flour is another important factor which effect water absorption capacity. Flour with smaller particle size has higher surface area for flour hydration [18, 21]. The WAI is also dependent upon pore size, capillary and protein charges. This is due to strong correlation of extent of protein hydration with polar constituents along with the interaction of hydrophilic components by hydrogen bonding. The higher protein content lead to strong hydrogen bond, which subsequently increase the water absorption capacity of rice flour [35]. The difference in variety and starch granule structure significantly influence the hydration capacity of the flour [21, 37].

3.2.2 Water Solubility Index (WSI)

Water solubility index of rice flour of different selected rice varieties are significantly ($P<0.05$) different from each other (Table 2). The present findings revealed that solubility of rice flour ranged from 1.95% (Super Basmati Kainat) to 4.94% (Super Basmati Shaheen). Similarly, Thumrongchote et al [18], Wadchararat et al [24] and Heo et al [30] investigated the water solubility of rice flour of different rice varieties which ranged from 0.6% to 7.94%. Data also shows that water solubility of rice flour of Super Basmati Shaheen and JP5 are highly significant than rest of the rice varieties. The WSI of flour depends on the temperature and amylose content of rice flour. However, relationship of solubility with temperature was directly related, while amylose content has inverse relation to solubility of rice flour [26]. Other factors which affected water solubility are the presence of protein and starch lipids complex, which reduces solubility [18, 26]. One of the major factors effecting water solubility is the methods of milling and damaged starch content [34]. The degradation of starch granules led to higher water solubility [38].

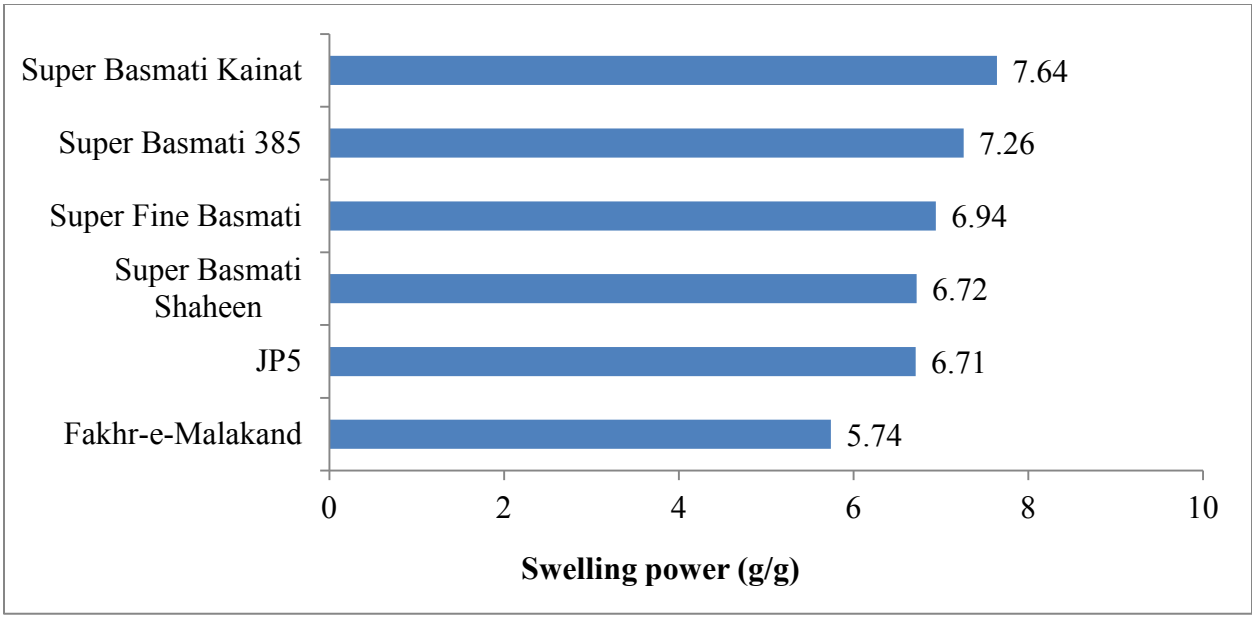

Fig. 7. Swelling power of flour of selected rice varieties grown in Pakistan.

3.2.3 Swelling Power (SP)

The SP of rice flour of selected rice varieties differed significantly ($P<0.05$) from each other, except JP5 and Super Basmati Shaheen. The observed values ranged from 5.74 g/g (Fakhr-e-Malakand) to 7.64 g/g (Super basmati Kainat) among the varieties. The present findings are in agreement with previous literature that found values ranging 4.7–16.23 g/g in rice flour of different rice varieties [6, 18, 26, 36]. The SP of rice flour might be affected by amylose and protein content, which inhibit the granular swelling due to disulphide and intermolecular bonding in protein that result in extensive and strong network [6, 18, 26, 39]. Protein is one of the most important macronutrient, which has the ability to bind starch and form starch granules, which affect the pasting properties of rice flour. The protein and starch content in rice flour are embedded tightly in the lipid matrix and form an amylose lipid complex that influences the pasting properties [40]. Similarly, the ratio of amylopectin and amylose as well as their structural confirmation in a starch granule substantially effect flour swelling power [41]. The SP of wet and dry milled rice flour at 25 ^{o}C and 100 ^{o}C were 3.8 g/g, 12.6 g/g, and 4.7 g/g, 10.9 g/g, respectively [34] that reveals that milling method and processing temperature also influence the swelling capacity or rice flour. Starch degradation also resulted in the reduction of SP or rice flour [42].

4. CONCLUSIONS

In Pakistan different rice varieties are grown and grades as second staple food amongst the crop and as an important cash crop of the country. It was found from the present study that flour of different rice varieties (Super Basmati Kainat, Super Basmati Shaheen, Super Basmati 385, Fakhr-e-Malakand, Super Fine Basmati and JP5) obtained from different sites of Pakistan showed variation in composition and functional properties. Lowest values regarding percent moisture, ash, protein and fat content were observed in Super Basmati Shaheen, JP5, JP5 and Fakhr-e-Malakand, respectively. While highest values regarding all these parameters were recorded in Super Basmati Kainat, Fakhr-e-Malakand, Super Fine Basmati and Super Basmati Shaheen, respectively. In case of water absorption index, water solubility and swelling power, minimum values were noted in Fakhr-e-Malakand, Super Basmati Kainat and Fakhr-e-Malakand, and Fakhr-e-Malakand, respectively, while maximum values were found

in super Basmati 385, Super Basmati Shaheen and Super Basmati Kainat, respectively. The present findings provide the basic information regarding properties of rice flour obtained from various sources and are recommended for the preparation of various rice based products concerning nutritional and health aspects.

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Research Article

Impact of Terrain Slopes and Aspect on the Natural Regeneration of the Coniferous Forest in the Northern Pakistan - A Case Study of Ayubia National Park

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Abstract: Natural regeneration of forest is an important factor for sustainable forestry in Northern Pakistan. In natural forests, the coniferous forest has higher regenerative capacity which enhances their significance for developing countries like Pakistan with rapid depleting forest areas and lack of resources to fund any afforestation campaign. Ayubia National Park is taken as case study to study natural regeneration of forests. The paper explores the status of natural regeneration of conifers forest in Ayubia National Park, Abbottabad, Khyber Pakhtunkhwa, which lies in the moist temperate northern zone of Pakistan. Main objective of the paper is to find out the impacts of two vital factors, i.e., slope and aspect, on regeneration of coniferous forests in the study area. One hundred, fixed circular plots on varying terrain slopes, aspects and accumulated snow were selected randomly as sample representation of the whole Ayubia National Park. Five species were selected to ascertain the density, frequency ratio, abundance and distribution pattern of the regeneration in the six forest blocks. There were better growth status and survival rate of regeneration at the south aspect (56%) than the north (44%). As much as 60.8% regeneration was recorded on the rolling slopes followed by 25.2% and 13.98% at steep and normal slopes, respectively. The present natural regeneration was well distributed at south aspects than its growth on north aspects. This phenomenon indicates a negative impact of the anthropogenic activities on forest regeneration at normal terrain. Similarly, steeper slopes also have a negative impact on natural regeneration of the conifers forest.

Keywords: Anthropogenic activities, coniferous forest, regeneration, terrain, slope

1. INTRODUCTION

Forest regeneration is an important natural process that helps in restoring the forest over large areas. Natural regeneration can successfully occur only with sufficient amount of growing space, understory light, supportive soil composition and ample ground litter that help for seed germination and subsequent growth of seedlings. Natural regeneration is a valuable mean for moving towards forest types that are more efficient in providing valuable forest products and assuring multipurpose forest functions such as recreation, site protection, water conservation and preservation of the habitats of endangered species of plants and wildlife [1, 2] .

Generally, in northern Pakistan the regeneration is abundant on northern and north eastern/ western aspects as compared to southern aspects. There is either inadequate regeneration or at times no regeneration on the southern aspect due to the fact that southern aspects are comparatively warmer because of more sun facing than the northern aspects [3].

Hardwick et al [4] worked on regeneration of forest in south-east Asia. They indicated four sets of factors which influence regeneration of forests,

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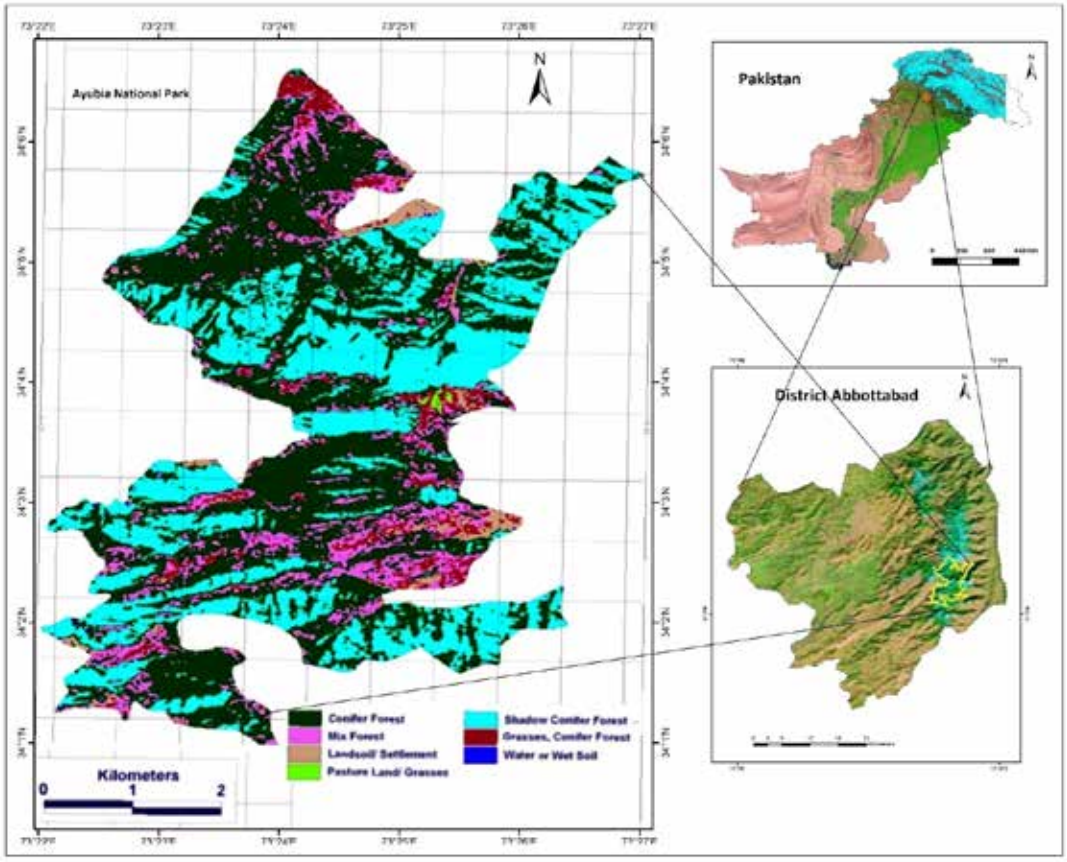

Fig. 1. Ayubia National Park, and its major land uses [15].

i.e., distribution, site sources that include seasonal disparity in moisture, weed competition and plant and propagule availability. Similarly, some scholars like Holland and Steyn [5] have worked on this aspect in relation with in solation period and strength. Numerous researches (e.g., Cantlon [6] in North America, Kutiel and Lavee [7] in Middle East, Kirkpatrick et al. [8] in Australia, Vetaas [9] in Africa, and Ghimire et al [10] and Paudel and Vetaas [11] in Himalayan region) have indicated that the difference among different aspects is the outcome of variation in solar radiation received. Similarly, Cantlon [6] and Pook [12] observed that conflicting gradients differ in intensity of light, top soil, temperature, moisture, and evaporation, as well as length of growing period. In northern hemisphere usually, the south-facing slopes obtain more daylight and thereby supporting drought-resistant vegetation, whereas north-facing slopes maintain humidity and therefore supporting moisture-loving plants.

Khattak [13] reported that in natural forests, the coniferous forest has higher regenerative capacity which enhances their significance for developing countries like Pakistan with rapid depleting forest areas and lack of resources to fund any afforestation campaign. The significance of natural regeneration is extremely high in retaining forest sustainability but its stand density and growth is affected directly or indirectly by the natural and anthropogenic factors due to the local interactions and conditions. Forest density, frequency ratio, abundance and distribution pattern are generally used in determining the status of natural regeneration [14]. This paper focuses on these parameters to explore the status of natural regeneration of coniferous forest in Pakistan using Ayubia National Park as a case study. The study aims to identify the correlation of slope and ground aspect in determining the stand density, abundance and distribution pattern of the regeneration of coniferous and broad leaved forest

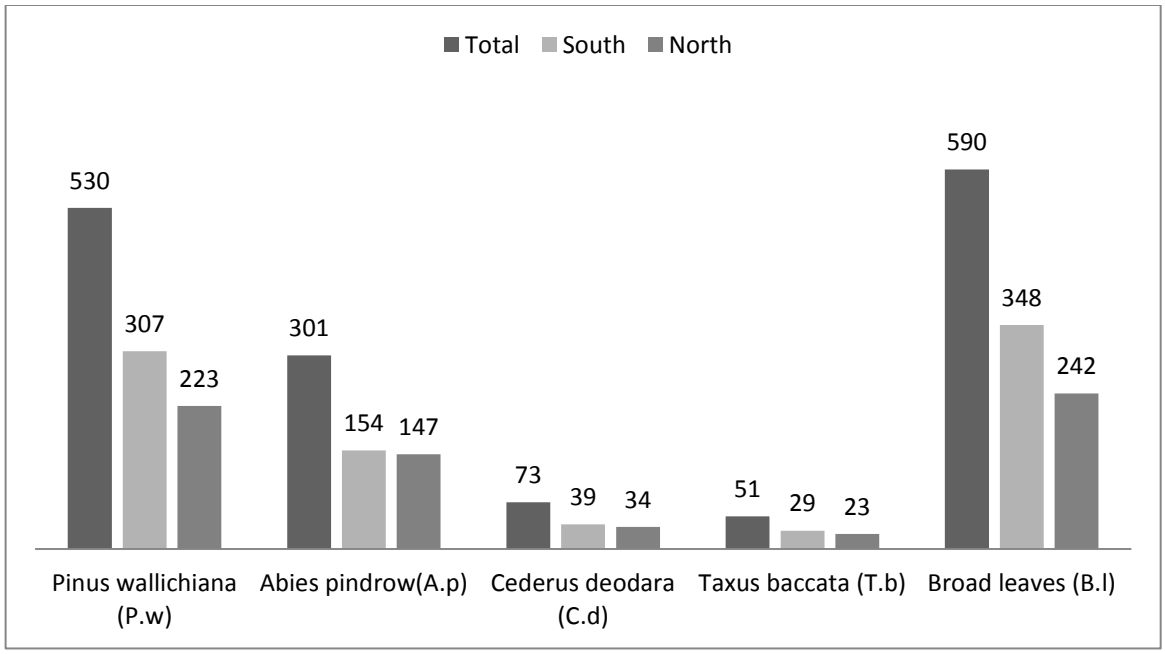

Fig. 2. Spread of regeneration on north and south aspects.

and its relation with the status of pole and mother trees.

1.1 Ayubia National Park

The Ayubia National Parklies between $34^0 01'$ to 34^0 3.8′ N latitude and $73^0 22.8'$ to $73^0 27.1'$ E longitude which are spread over a total area of 3312 hectares [15]. In relative terms, Ayubia National Park lies in the eastern side of District Abbottabad, while the Park is one of the 14 declared national parks in Pakistan that fall in IUCN category '*V*'. The Park is known as the hotspot in the moist temperate zone of Himalayan Range Mountains in District Abbottabad of Khyber Pakhtunkhwa province of Pakistan [16]. Coniferous forest is the major land use of the forest though other land uses like rangeland, agriculture land, shrubs and bushes can also be seen sporadically dispersed in small parcels of land (Fig. 1).

Topography of Ayubia National Park is rugged with slopes varying from normal to the steep which is the common features of the whole Galliat area. The Ayubia National Park occupies predominantly the eastern slope of the main mountain ridge running in north south direction between the town of Donga Gali and Ayubia. The average elevation of Ayubia National Park is 2440 meter above sea level while the highest peak in the Park is Mukshpuri (2813 meters) [17]. The climate of the area is moist temperate with very cold and snowy winters and pleasantly mild summer season. The minimum temperature during winter months falls below freezing points. The mean annual rainfall is well above 1500 mm, most of which is received during monsoon period from July to August, while the month of May, June, September and October are the driest. Rainfall, temperature and humidity conditions of the area are highly conducive for rich vegetation growth [18].

Shafique [19] reported that the Park is settled with 22 species of mammals, 154 species of birds and 08 species of reptilians and amphibians. Mammals in the Park include Asiatic leopard, black bear, yellow throated marten, Kashmir hill fox, red flying squirrel, Himalayan palm civet, masked civet and rhesus monkey. Birds in the park are golden eagle, Himalayan griffon vulture, honey buzzard, peregrine falcon, kestrel, Indian sparrow hawk, hill pigeon, spotted dove and collared dove [20]. According to Shafique [19], the Park is predominantly enriched with

coniferous forest species (*Abie spindrow, Cedrus deodar, Picea smithiana, Pinus wallichiana, and Taxus wallichiana)* mixed with broad leaved evergreen (*Quercus floribunda, Quercus glauca, Quercus incana, Quercus dialatata)* and deciduous broad leaved trees *(Acer caesium, Aesculus indica, Cornus macrophylla, Juglans regia, Prunus padus, Diospyros lotus, Ulmus wallichiana*).

The main objective of this research was to explore the impact of anthropogenic activities on regeneration in the study area and to answer the question that what is correlation between slope and ground aspect on the regeneration of coniferous forest.

2. MATERIALS AND METHODS

Fixed point sampling technique was used to select sample plots in the Park to equally represent the existence and growth of different species regeneration on different forest areas. The sample plots were randomly selected with the similar probabilistic representation of different vegetation type, terrain slope, southern and northern aspects. Besides, due considerations were also made to select the sample plots with the various natural and anthropogenic confronted threats to the regeneration process. Circular plots of an area of about 0.01 hectares were designated around various selected species using a rope to mark a circle of 6 meters diameter.

The study area was further sub divided into six blocks, namely, Bagan, Tajwal, Darwaza, Donga Gali, Kao and Bakot. One hundred sample plots were taken in these forest blocks. However, the number of plots taken in each block was differed due to the area strength. The detail of plots taken in each block is shown in Table 1.

2.1 Regeneration Measurement Standards

The regeneration status was measured through the process of manual enumeration. The circumference of every single tree in the sample plot was measured at the breast height of the observer using a measuring tape. With the application of simple geometric formula, the diameter of the tree at the breast height was ascertained. This was used to group the trees into three categories. The trees were considered as

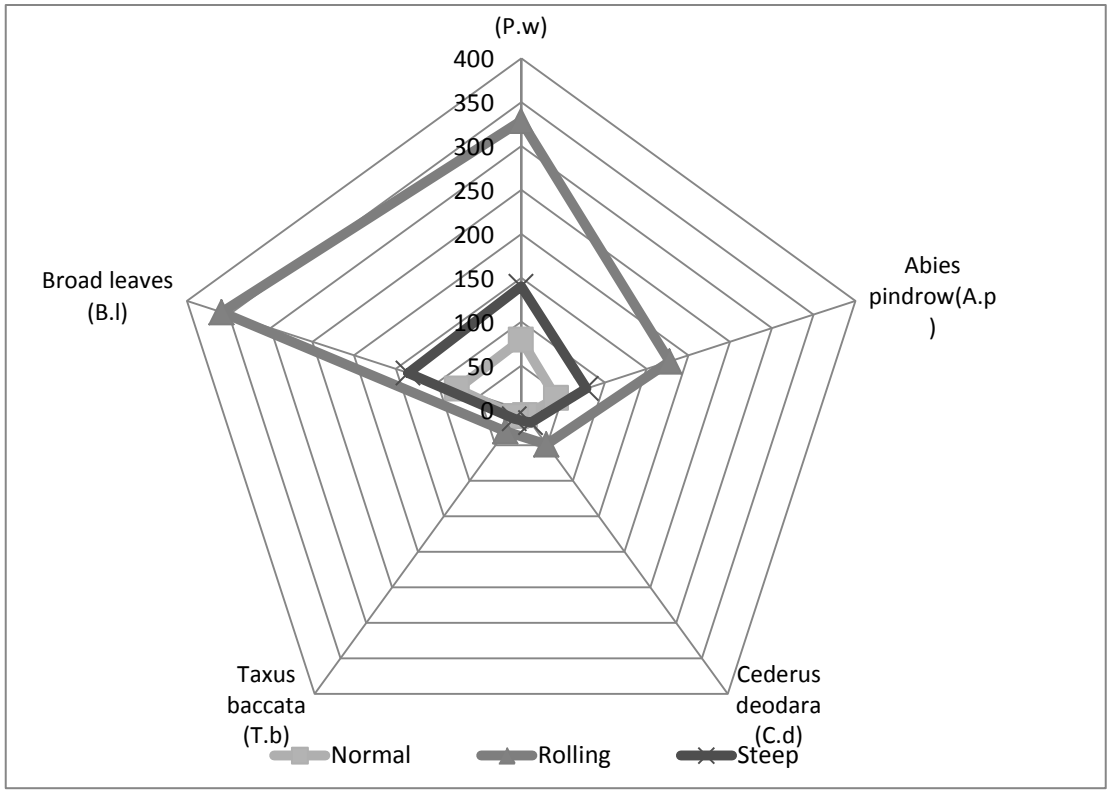

Fig. 3. Number of selected species on various categories of slope.

regeneration with the stand height less than 180 centimeters and diameter at breast height (DBH) less than 20 cm [22]. When height of a tree was greater than 180 cm and its diameter was between 20 cm and 24 cm, this growth stage of various species were labeled as *pole crop* and when diameter of a tree was greater than 24 cm it was categorized as *mother tree* (Table 2) [17].

3 RESULTS AND DISCUSSION

Five species were recorded in the selected sample plots. These species include *Pinus wallichiana* (P.w) *Abies pindrow* (A.p) *Cederus deodara* (C.d), *Taxus baccata* (T.b) and *broad leaves* (B.l). The data for the four selected indicators are presented in Table 3.

3.1 Frequency Ratio

The frequency ratio of regeneration in all 100 sampling plots was found in the range of 16% to 76% (Table 3). Minimum value was recorded for *Taxus baccata* and maximum value of frequency ratio was recorded for the *Pinus wallichiana*.

3.2 Density

The density (species / hectare) of regeneration was ranged from 0.51 to 5.9 ha^{-1}. It is evident from the Table 3 that Broad leaves specie was having a highest density followed by *Pinus wallichiana* (P.w) and *Cederus deodara* (C.d). Minimum value of density was recorded for *Taxus baccata*.

3.3 Abundance

Hubbell [21] was of the view that species abundance is a key indicator for determining biodiversity. Abundance is defined as total number individuals of specie in all sample plots per number of sample plots with that particular specie. According to Hussain [22], relative species abundance refer to how common (or rare) a species is relative to other species in a given location or community. The fields investigations (Table 3) clearly indicated that the regeneration of Broad leaves were in abundance (8.08) as compare to all other species. While the lowest value of abundance was found in *Cedrus deodara*.

3.4 Distribution Pattern

The vegetation in Ayubia National Park was mostly spread away in contiguous and somewhere in random pattern. From the results, it was evident that the *Pinus wallichiana*, *Abies pindrow* and *broad leaves* had the contiguous pattern of

Table 1. Number of plots taken in each forest block.

S. No.	Forest block	Area of each block (hectare)	%age of the total area	Number of selected plots from the block
1	Bagan	1006.83	30.40	31
2	Tajwal	35.21	1.06	1
3	Darwaza	108.87	3.29	3
4	Donga Gali	76.68	2.32	2
5	Kao	1763.27	53.24	53
6	Bakot	321.27	9.70	10
Total		**3312.13**	100	**100**

Table 2. Tree types and their measuring standard.

S. No.	Tree type	Measuring standards (cm)	
		Height	DBH
1	Regeneration	< 180	< 20
2	Pole crop	≥180	≥20 ≤ 24
3	Mother tree	>180	> 24

distribution whereas the *Cedrus deodara* and *Taxus baccata*was distributed in random. All the species were distributed in a random and contiguous pattern and no specie were recorded in regular pattern of distribution.

3.5 Comparison of Regeneration Density with the Densities of Pole Crops and Mother Trees

The regeneration of the forest is strongly correlated with the mother and pole crop trees as evident from Table 4. This correlation coefficient of regeneration density with the densities of pole is 0.6734 and coefficient of determination (R^2) indicates the fraction of the variance in the two variables is 0.4535. The coefficient of determination for the regeneration and mother trees is almost the same as that of pole tree yielding similar 'r' value compares densities of regeneration with pole crops and mother trees of the five selected species. Visual analysis of the table also indicates that the density per hectare of regeneration was significantly high for all type of species in the Park. The higher values of regeneration for broad leaves and *Pinus wallichiana* indicated good regeneration status for these species. The regeneration density of *Cedrus deodara (0.73)* and *Taxus baccata (0.51)* were comparatively lower but on the other hand it was higher than the Pole crops of *Cedrus deodara (0.08)* and *Taxus baccata (0.07). Abies pindrow* regeneration density (3.01) represented a dense canopy cover than density of mother trees (0.61) and Pole crops (0.27). *Cedrus deodara*was normally distributed and having relatively good regeneration (0.73) than pole (0.09) and mother trees (0.08). The regeneration density (0.51) of *Taxus baccata* had no major difference with the Pole crops and the Mother trees in collected densities. But in the overall results the high

Table 3. Scope of regeneration of selected species of coniferous forest in Ayubia National Park.

Species	Regeneration					
	Number of plots in which species occured	Number of individuals in all sample plots	Frequency ratio (Number of plots in which species occurred/ Total sample plots)*100	Density (Total individuals in all sample plots/ Total sample plots)	Abundance (Total individuals in all sample plots/number of sample plots with the species)	Distribution pattern (Abundance / Frequency ratio)
Pinus wallichiana (P.w)	76	530	76	5.3	6.97	0.092
Abies pindrow (A.p)	58	301	58	3.01	5.19	0.089
Cederus deodara (C.d)	24	73	24	0.73	3.04	0.127
Taxus baccata (T.b)	16	51	16	0.51	3.19	0.199
Broad leaves (B.l)	73	590	73	5.9	8.08	0.111

Table 4. Correlation analysis of density of regeneration against pole crop and mother tree.

Species	Regeneration	Pole crop	Mother tree	Correlation coefficient 'r'	
				Pole crop	Mother tree
Pinus wallichiana (P.w)	5.3	0.8	0.79		
Abies pindrow(A.p)	3.01	0.27	0.61		
Cederus deodara (C.d)	0.73	0.09	0.08	0.67	0.66
Taxus baccata (T.b)	0.51	0.07	0.07		
Broad leaves (B.l)	5.9	0.23	0.29		

regeneration status was recorded for Broad leaves (5.9) which were much denser than pole crops (0.23) and mother trees (0.29).

3.6 Impact of Aspect on Regeneration

The natural regeneration of the forest trees has a close relationship with the aspects, i.e., North and South of ground surface. The data were collected at both `North' `South' aspect. Data analysis revealed that the regeneration was slightly more at South aspect (56.61%) than the North aspect (40.38%) of the Ayubia National Park. Out of 40.38% at North aspect, 25.56% of regeneration was conifers and 14.82% were broad leaves. The regeneration of coniferous forest covered 35.73% and broad leaves 23.38% at south aspect out of 59.61% of vegetation density. The enriched natural regeneration of coniferous was observed at both aspects. In winter season, the north aspects are densely covered with snow and due to poor access to the sunlight snow cover melts slowly as compared to the south aspect where the snow layer melts early and rapidly. Hence at the south aspect, the ground became clear and provided a very fertile soil for good regeneration, so the south aspect was highly enriched with natural regeneration.

3.7 Slope and Regeneration

The rolling slope covers a high number of regeneration than normal or steep slopes respectively. The 60.8% of regeneration exists at rolling slope while 25.2% and 14.0%, at the steep and normal slopes respectively. Normally the vegetation grows at rolling slope because of balance water runoff, proper seed germination and control grazing activities. Most of the anthropological activities are concentrated at the normal slopes which is evident from existence of tracks and pathways. The snow accumulated mostly at Normal plains but little at rolling and steep slopes.

4 CONCLUSIONS

The research reveals that natural regeneration of the forest in Ayubia National Park is highly dense, frequent and has contiguous vegetation. All trees type (regeneration, pole crops and mother trees) of *Pinus wallichiana* are remarkably intense in terms of density, frequency and abundance followed by Broad leaves. *Taxus baccata* has measured in significantly poor status of natural regeneration because of the threats like forest fire and other anthropogenic activities. The natural regeneration of all trees of coniferous and broad leaves is good as compared to the growth of its Pole crop and Mother trees. The present natural regeneration is well distributed at South aspects than its growth on North aspects. Besides, a close relation is observed between the regeneration and terrain slope. The regeneration is frequent on the rolling (60.77%) followed by steep (25.24%) and normal slopes (13.98%). Different threats like forest logging and cutting, forest fire, grazing pressure, trampling, fuel wood, and severe climatic condition adversely affect the natural regeneration of Ayubia National Park forest. On these basis it is concluded that the overall status of regeneration hovers between good to moderate though some conifer species are facing various threats to its proper growth and nourishment. As regeneration of coniferous forest was rapid on the southern aspect, therefore anthropogenic activities needs to be monitored so as these does not affect the natural regeneration of the forest cover. The Wildlife and Forest Department must work in collaboration to maintain the existing ecosystem so that natural regeneration is not affected.

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Pakistan Academy of Sciences

Obituary

Prof. Dr. Muhammad Arshad
(1956–2016)

Prof. Dr. Muhammad Arshad was born on April 1, 1956 in Faisalabad, Pakistan. He did his PhD in Soil Microbiology and Biochemistry from the University of California, Riverside, CA, USA. Dr. Arshad had his entire professional career at University of Agriculture, Faisalabad (UAF) where he served as Director, Institute of Soil and Environmental Sciences; Director, Centre of Agricultural Biochemistry and Biotechnology; Director of Centre for Advance Studies in Applied Genetics and Saline Agriculture; and Senior Tutor. Dr Arshad also served as Editor-in-Chief of Pakistan Journal of Soil Science and as elected President of Soil Science Society of Pakistan, twice, and President of ECP.

Currently, Professor Arshad was serving as Dean, Faculty of Agriculture, University of Agriculture, Faisalabad. Overall, he had more than 37 years experience of teaching and research.

Dr. Arshad had authored/co-authored 4 books (3 published by international publishers and one by national publisher), 11 book chapters, and 155 research articles in foreign refereed journals – with over 300 Impact Factor and more than 2000 citations (ISI Web of Science). He produced 20 PhD scholars as major supervisor and executed 12 research projects, as Principal Investigator, funded by international and national funding agencies.

Innovative scientific contributions and effective writings of Dr. Arshad brought him many national and international honors and awards, like "Best Scientist in Agriculture for the year 2001" by the Pakistan Academy of Sciences; "Best University Teacher for the year 2003" by Higher Education Commission (HEC); National Civil Award *"Tamgha-i-Imtiaz"* 2005 by Government of Pakistan; and best performance shield by the President of Pakistan. Dr. Arshad was honored as Fellow of Soil Science Society of Pakistan in 2006 and by TWAS Prize in Agriculture Sciences for 2007. Recently, he was decorated by HEC as "Distinguished National Professor" for the year 2015-16. He was a recipient of Research Productivity Award throughout. In short, Prof. Arshad was a well recognized scientist in the field of Agriculture and Environment.

In November 2015, Prof. Arshad was elected as Fellow of Pakistan Academy of Sciences; however, he expired prior to his induction as Fellow, which was scheduled towards the end of year 2016. In entire history of the Academy, Prof. Arshad was the second Fellow-elect who left this world prior to induction as Fellow; incidentally, the earlier one was also an agricultural scientist, Dr Abdus Sattar, Chief Scientific Officer & Director of Nuclear Institute for Food & Agriculture (NIFA), Peshawar who was elected in year 2000.

Dr. Arshad was a kind person. He possessed a pleasing personality, and was an amicable colleague and supervisor. In his demise Pakistan has lost a statwart agricultural scientist.

May Allah Almighty bless the departed soul with eternal peace; and give fortitude to his family to bear this irreparable loss! Aameen.

Dr. Abdul Rashid, *FPAS*

Obituary

Dr. Agha Ikram Mohyuddin
(1932–2016)

We are grieved on the sad demise of an eminent scientist and a senior Fellow of the Pakistan Academy of Sciences, Dr. Agha Ikram Mohyuddin, who breathed his last in Islamabad on Wednesday, 10th February, 2016. He was elected Fellow of the Pakistan Academy of Sciences in 1992.

Dr. Agha Ikram Mohyuddin was born in Jullundur, British India on 21 September, 1932. He obtained his BSc in 1953 and MSc (Zoology) in 1956 from University of the Punjab, Lahore. Later, he earned PhD from Queen's University, Kingston, Ontario, Canada.

Dr. Ikram Mohyuddin was one of the founding members of Eco-Conservation Initiative (ECI). He served as Chief Consultant, Integrated, Integrated Pest Management (IPM) Project and Chief Executive, Integrated Pest Management Consultants; Principal Investigator and Coordinator of all the IPM Projects in Pakistan, appointed by Govt. of Pakistan, 1993-1994; Scientist-in-Charge / Director, PARC-IIBC Station, International Institute of Biological Control (IIBC), 1980-1994; Principal Entomologist IIBC, 1960-1979; Lecturer, Zoology Department, Government College, Lahore, 1956-1960; Adviser on IPM of Sugarcane Pests, Gunnung Madu Plantations Bandar Lampung, Sumatra, Indonesia, 1982-1995; Consultant, Malakand Fruit and Vegetable Development Project (Pakistan-Swiss Joint Venture), 1989-1990; Consultant, US-AID Funded Project on IPM of Horticultural Crops in Sri Lanka as Sta of Oregon State University/ Development Alternative Inc., MD, USA, 1990-1992.

Dr. Ikram Mohyuddin was Member, FAO/ UNEP Panel of Experts on IPM, 1982-1986; Member, Panel of Experts, University of Agriculture, Faisalabad ,Pakistan; Member Advisory Committee, Museum of Natural History, Islamabad, 1987-1991. He published Two Books and a Monograph; awarded several Patents and worked on 54 projects in Canada, Uganda, Turkey, Rumania, Bulgaria, Indonesia, Sri Lanka and Sultanate of Oman.

In recognition of his significant contributions in the field of agriculture, Dr. Ikram Mohyuddin was conferred President's Award for Pride of Performance, 1996; Ismail H. Zakria Gold Medal (by Pakistan Society of Sugar Technologists), 1997; Open Gold Medal, Pakistan Academy of Sciences, 1990; Dr. Norman Borlaug Award, by Govt. of Pakistan, 1988; and Ghulam Muhammad A. Fecto Gold Medal, 1986, 1983.

His areas of research were Biological Control and Integrated Pest Management.

Dr. Ikram Mohyuddin possessed a pleasing personality; he was a kind and humble person. In the death of Dr. Ikram Mohyuddin, Pakistan in general and the Pakistan's plant protection scientific community in particular have lost a committed and accomplished research leader.

May the Allah Almighty rest his soul in eternal peace and give fortitude to his family to bear this irreparable loss! Aameen.

Dr. Abdul Rashid, *FPAS*

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