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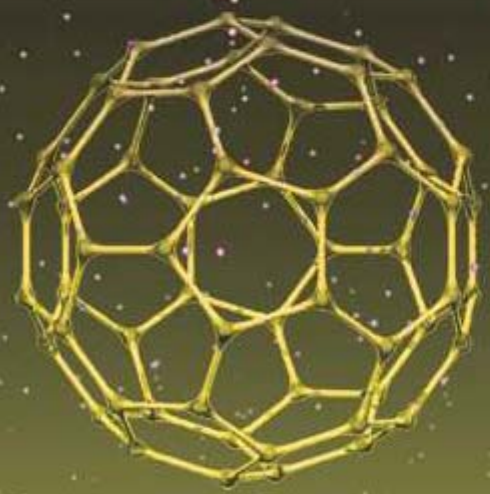

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Research Article

Towards a Framework for Scrum Handover Process

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Abstract: In the software industry Agile development methods are in practice at very large scale because of their ability to accommodate the change, yield quick results and provide high level of customer's satisfaction. Scrum is an agile methodology which is used for project management and software development. But release planning, documentation and scrum ceremonies are major challenges while practicing the scrum. At the completion stage of software systems, it is ready for the handover (transition) process, which is a process of transfer of responsibilities of the software system from developers to the maintainers. It is very crucial phase because smooth handover is required to avoid future problems related to the software system. This area needs to be explored more because the very little work has been done in this area. The main aim of this paper is to highlight the need of the handover process model and to suggest a framework which supports the planning and organization of handover, also provides and smooth handover process for scrum practices. We have opted the action research methodology to explore the knowledge and for developing framework. The proposed framework is evaluated through industrial experimentation and results show that the proposed solution is providing the baseline for the smooth handover of system in scrum.

Keywords:handover, transition, agile, scrum

1. INTRODUCTION

Organizations are now using the agile methods more as compared to the previous years. Most are using Scrum methodology. According to the survey conducted by Version One, 90% of respondents agreed that the agile development methods improve their abilities [1]. Scrum is the well-known way to adopt the agility because of the flexible nature and its straightforwardness [2].

The software projects are now heading towards a greater success rate than the past years. According to the research conducted by Standish group, the project resolution from CHAOS research 2012 is showing a great difference among the successful projects, failed projects and the challenged projects.

The graphical representation in figure 1 depicts the results. This clearly shows improvement in the software industry.

Challenged projects are tough in terms of time, environment, tool, budget, decisions and internal and external factors [3]. All the challenged areas that are identified have a great influence on any software system. The success of the software system is highly dependent on them. There is a clear decrease in the waterfall approach. The success percentage of the software has been increase since 2004 according to data given in [3]. It is clearly mentioned that there is no doubt in an increased use of agile development methodologies so it can be linked to the success factor of the software systems. Agility of software system has improved the success rate in recent years [1].

Agile is now considered as the universal software failure remedy. The main reason behind it is that, it allows doing small projects in an efficient way and allows the big projects to break down into

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small scalable modules, which are easily managed [3]. As agile continuously traces the progress of the team in the form of burn down charts, it helps the teams manage their velocity and complete the project successfully [4].

Scrum is a method of agile development and it is an iterative, incremental framework for development which put emphasis on the cross-functional teams working in short development bursts called "Sprints" to regularly produce a complete increment of product [5]. It is getting increasingly popular among the agile methodologies [6].

The closure of project is as much significant as its commencement. An enormous amount is invested in a software system. Clear evidence shows that the major amount of the project budget is consumed in developing, very less in project opening and project closing [7]. The major portion of a budget is normally spent on the maintenance if the project is poorly designed and developed.

Handover is a transition of a project from development phase to maintenance phase [8]. The project is now no more the responsibility of the development team, but the responsibility of the maintenance team. This is an important phase and can save the portion of a budget if performed well. Successful handover is highly dependent on the transferring party or teams [9]. It is still an uncovered area. Not much work is done in this field [10].The models described are either too old or generic, that cannot be applied to this age development methodology such as scrum. So, there is a clear need of the handover process for scrum practices which support the successful transition of the software system.

2. RELATED WORK

Handover is a research area which is still undercover. Very little work has been done in this area. Only few process models are designed, which are either too old or too generic and cannot be applied on new development methodologies like Scrum [10]. There are only three process models that describe the handover process [8]. These models are described in [11, 12, 13]. Another model which has been defined for handover process is specialized only for the one specific mid-sized organization [2]. One more handover framework has been devised by Khan [14], which describes the handover process in general and suggests the guidelines to perform handover successfully.

The publications [8, 9, 10] describe the handover process in general and also discussing the overall flow of the handover process by using its phases.

In the research area under the handover process, there exists a handover taxonomy, which is described in the publication [10].

So far, up to our best efforts, we have not found any publication that describes the model or framework for successful handover process in agile methodologies like Scrum. However, some publications are found in the perspective of evolution, maintenance and release of the software system in agile [15, 16].

Fig. 2 shows the activities of the handover process identified by the author in publication [8]. Basically, this process is divided into three sub-activities named pre-delivery , transition and post-delivery. The transition is the basic handover of the system which is performed to transfer thesystem and the set of responsibilities to the maintainers. However, this phenomenon is highlydependent on the organization's definition of the maintenance process [17].

To conduct successful transition or handover process the organization must actively participate in this process, because future maintenance is greatly hooked on the successful handover of the system.

There are several challenges that are being faced during the software handover process. They are insufficient system knowledge [8], lack of domain knowledge [8], insufficiency and lack of communication [7, 8], inadequate and improper end user documentation [7, 8], difficulties in tracking changes [8], lack of training [7] and knowledge

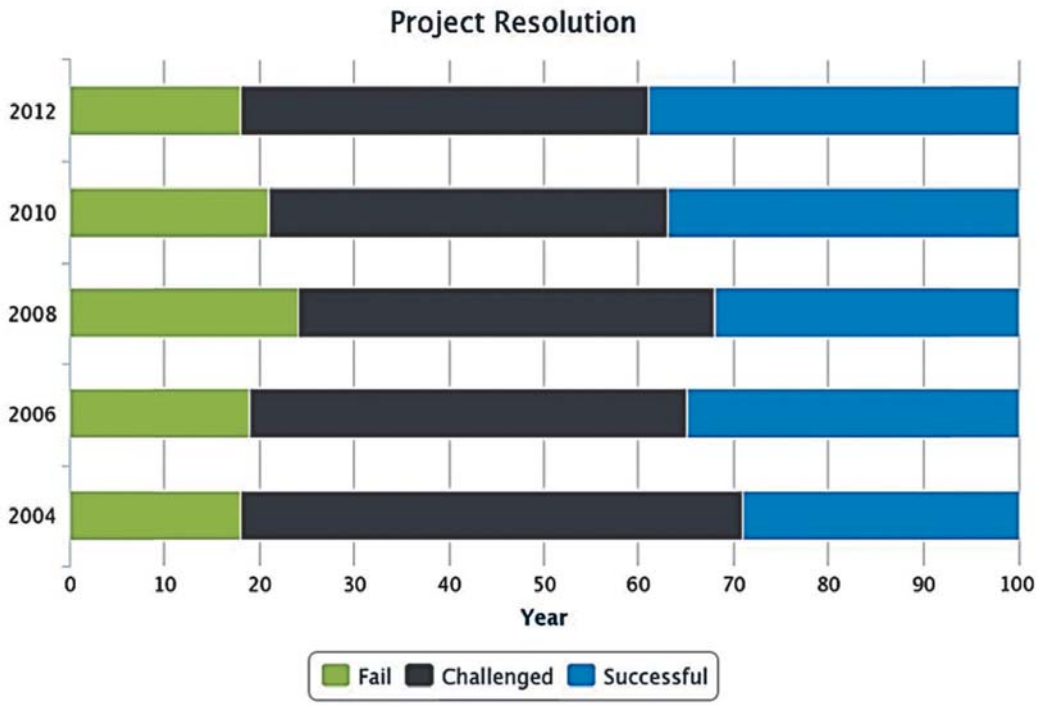

Fig. 1. Project resolution chaos 2012.

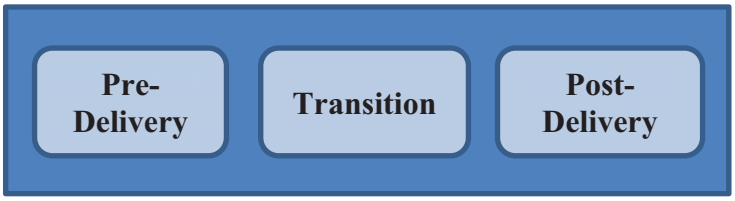

Fig. 2. Handover process.

sharing [7].

There is a need of a model or framework which handles these issues. The initial handover taxonomy has provided the base for the creation of the model or framework for the handover process [9, 10].

This initial handover taxonomy consists of seven process components, where each component comprises the set of logically akin activities mentioned in Fig. 3.

According to the 8th annual agile state survey report of Version One, 55% companies are using a pure scrum methodology. Rest of the 45% companies are using either scrum with other agile methodologies or pure agile methodologies [1].

Scrum has been a popular methodology under research since the last few years in the industry. A lot of work has been done in the scrum and it is still under discussion [5, 6, 18, 19, 20]. The work flow of scrum is described in Fig. 4.

The vision of the product comes from the stakeholders or product owner. This product vision is then transformed into the product backlog in the form of the set of requirements.

After the sprint planning the sprint backlog is prepared which contains the tasks to be done in a sprint. Every sprint has the duration of 3 to 4 weeks,

Fig. 3. Handover taxonomy process.

daily scrum meetings are the part of the sprint. Right after the completion of the sprint, a review is conducted which decides whether the product is finished or has to be improved further. When it is done, release planning is to be done after the demo meeting; if some improvements are required, then the cyclic process of scrum is re-performed [21].

3. MATERIALS AND METHODS

Scrum is the most commonly used agile methodology which provides the higher level of customers' satisfaction and easy to adopt and follow [5, 6, 18]. The authors in [22] have identified the few challenges and issues in the implementation of scrum. The major identified challenges are scrum ceremonies, documentation and the release process, which are directly related to the poor transition of the system. These may origins the ineffective handover of the system and may leads to the long run maintenance cost. The identified issues can be minimized by the well-planned handover process; this will include the proper release planning, documentation and ensures the communication required for the shifting of the software system from developers to the maintainers. This recognizes the clear need of the proper handover process for scrum practices.

The research methodology we have adopted is Action Research. The reason behind the selection of action research is the approach of learning by doing. It provides an iterative way to solve a problem and gives quick results. The detailed methodology is described in Fig. 5. At every stage of action research we have used different methods to execute action research successfully. Literature survey has been done to diagnose the problem. In action planning exploratory study issued to get the maximum knowledge about the handover process and scrum. After studying the problem, data are systematically gathered and arranged by performing a systematic literature review (SLR). A review protocol for SLR was opted as mentioned by Verner et al [23]. The motivation behind conducting SLR is to highlight the reasons and needs of the framework. At the stage of action taking a framework has been proposed to conduct successful handover process for scrum practices.

The process flow of activities with respect to the handover and agile is described in Fig. 7.

The process activities of the handover process are described in Fig. 3 and the process activities of agile with respect to the scrum phases are mentioned in the Table 2.

These activities are divided into three sub-activities according to the handover process flow. Pre-delivery, transition and post-delivery as described in Fig. 2. This division is done according to the flow of handover as described by Khan and Mattson [24]. The belonging of the activity to the phase (pre-delivery, transition, post-delivery) is done according to the activity definitions given by Khan and Mattson [10] for handover and by Mattson and Nyfjord [15] for a scrum.

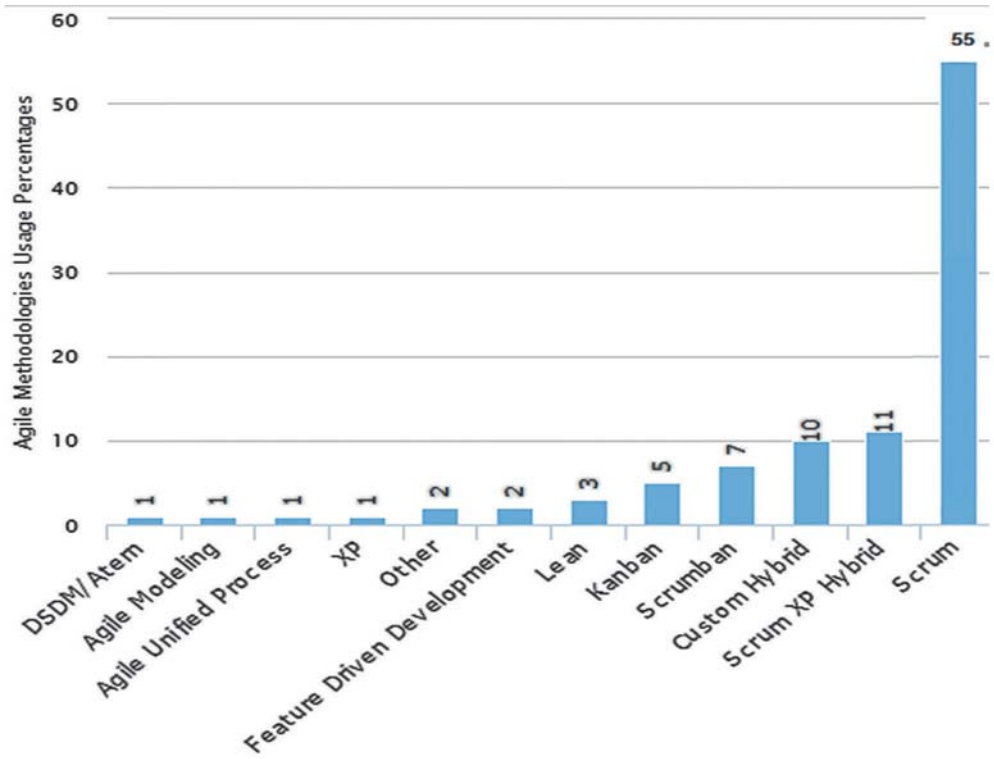

Fig. 4. Popularity of scrum.

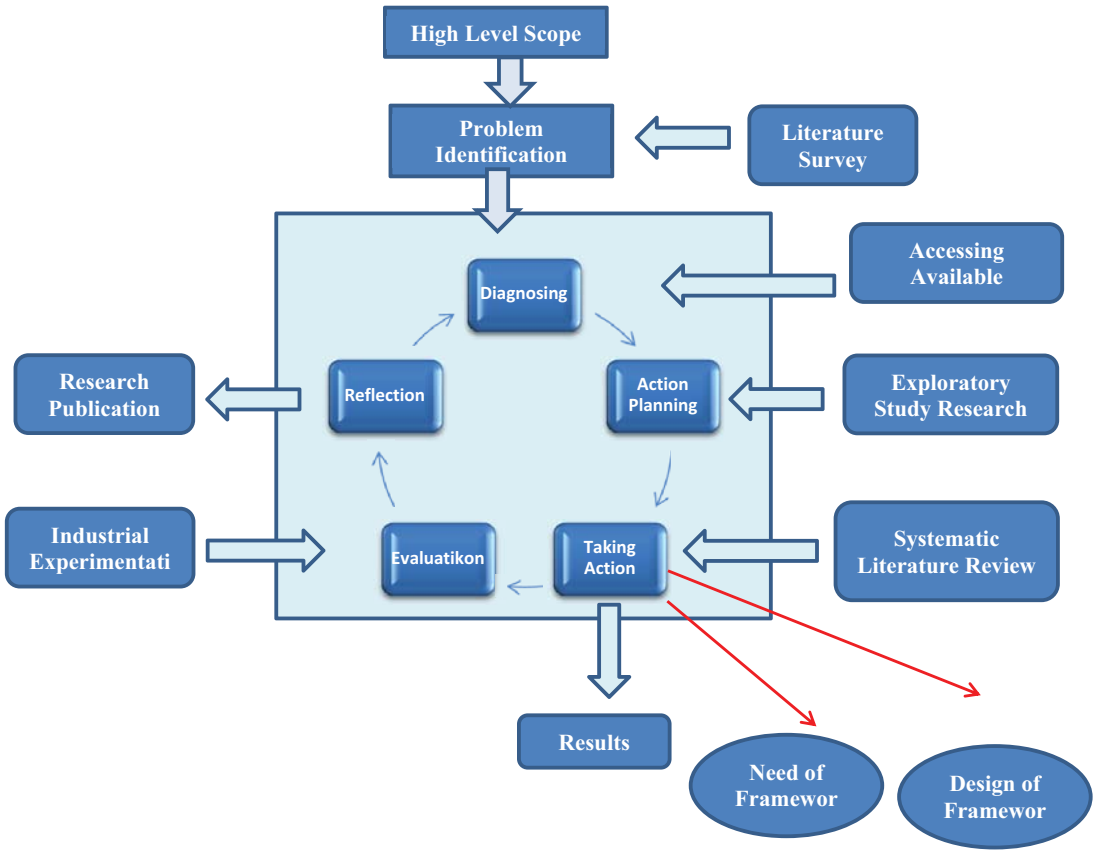

Fig. 5. Action research.

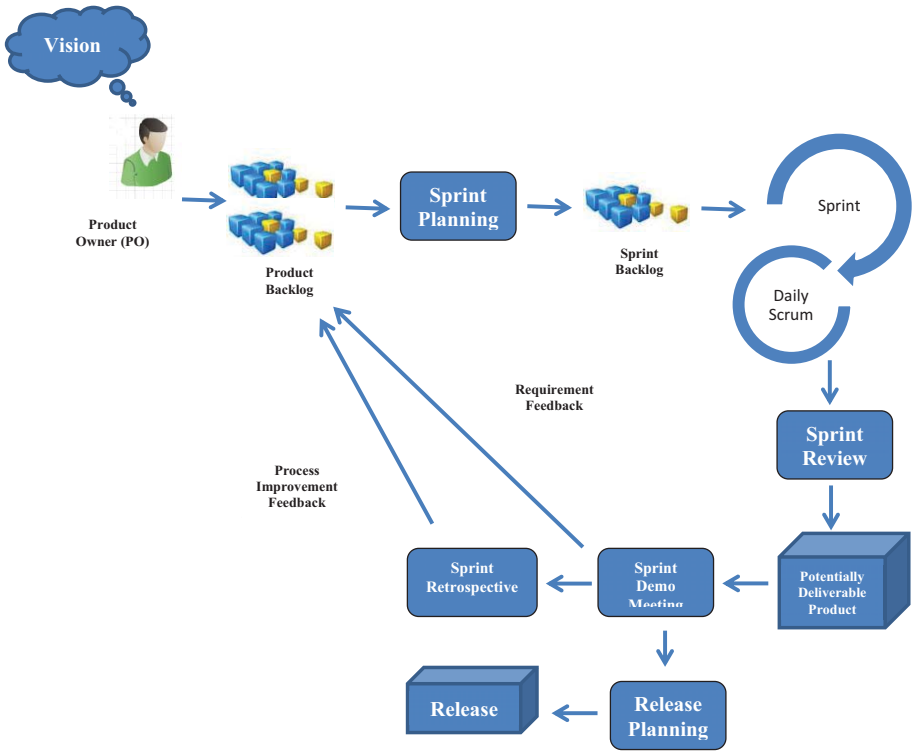

Fig. 6. Scrum process flow.

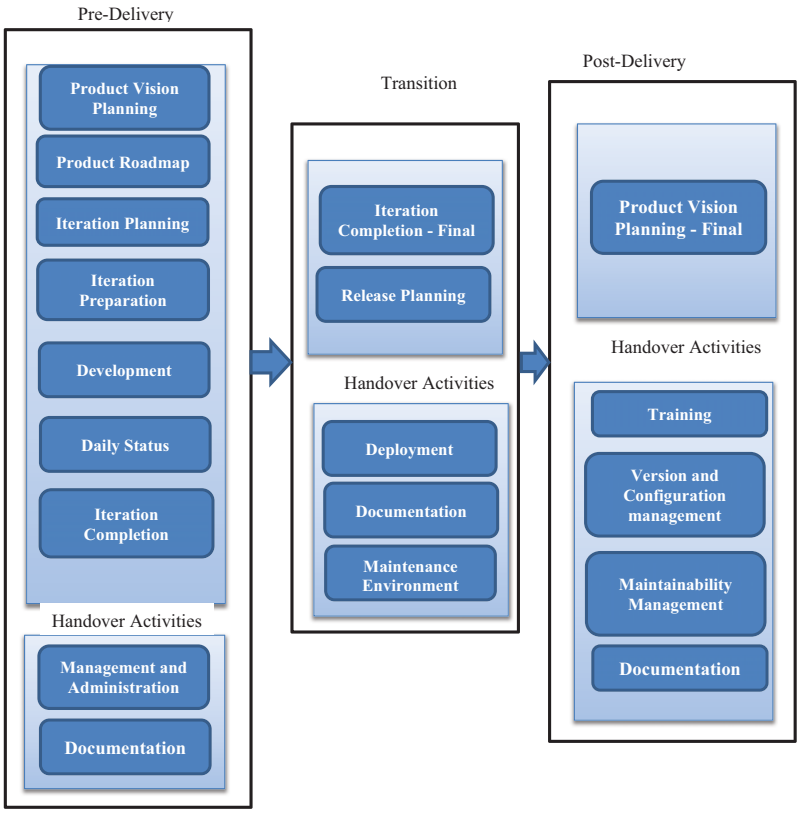

Fig. 7. Activities division in handover process.

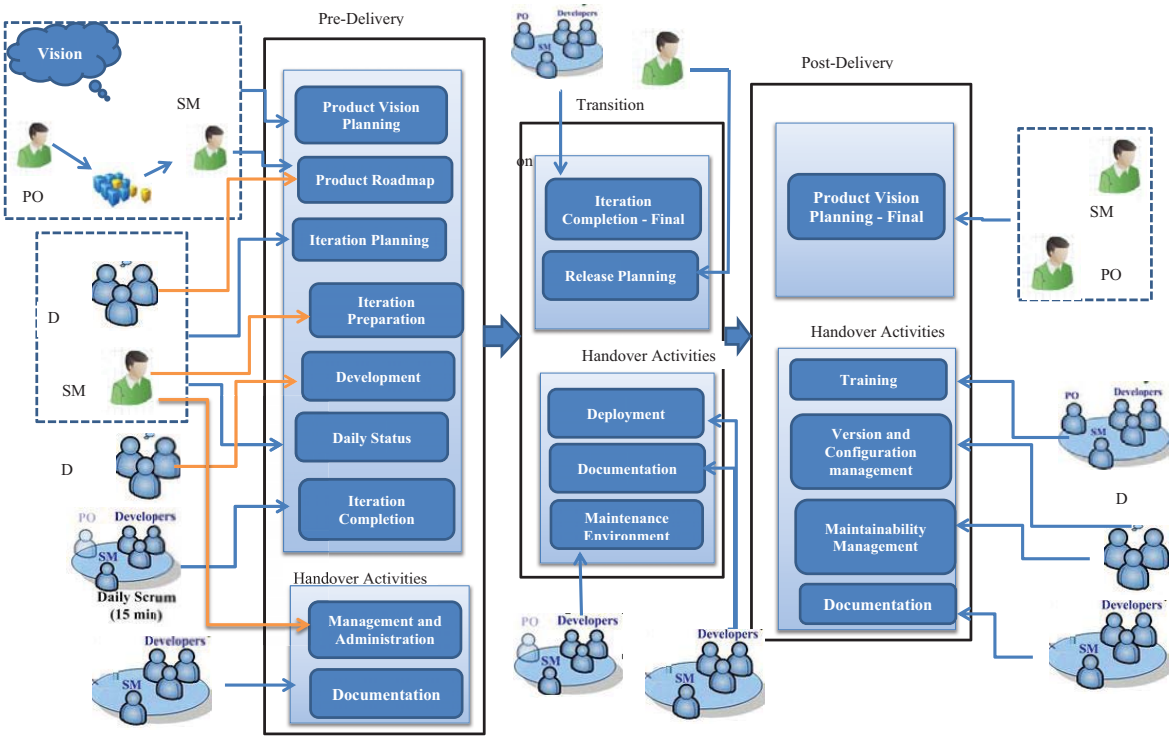

Fig. 8. Proposed framework.

A.	**Interviewee's Information**
1	What is your name, email and phone number?
2	What is the name and address of your company?
3	How many employees work in your organization?
4	What is your position and role in the company?
B.	**Handover Usage**
5	Are you familiar with the term 'handover'?
6	How a project does moves from developers to maintainers in your company?
C.	**Development Methodology**
7	Which development methodology does your organization use? (Scrum, XP, Waterfall etc.)
8	Does your company dealing with global software development?
D.	**Activities included in Handover**
9	Which activities are included at product owner's side?
10	Which activities are included at scrum master's side?
11	Which activities are included at development team's side?
E.	**Proposed Framework's Evaluation**
12	Does the proposed framework is feasible?
13	Did it help the organization to successfully handover the software system?
14	Is there any activity missing in the framework? If yes, please name it.
15	Do you feel that this framework is adoptable by industry?
16	List down the area of improvement in the framework

Fig. 9. Questionnaire.

Table 1. Project resolution from chaos 2012.

	2004	2006	2008	2010	2012
Successful Projects	29%	35%	32%	37%	39%
Failed Projects	18%	19%	24%	21%	18%
Challenged Projects	53%	46%	44%	42%	43%

Table 2. Agile process phases [15].

S. No.	Agile Processes
1	Product Vision Planning [15]
2	Product Roadmap and Release Planning [15]
3	Iteration Planning [15]
4	Iteration Process [15]
5	Daily Status [15]
6	Development [15]
7	Iteration Completion [15]

According to the proposed model as shown in figure 8, scrum master and team are carrying out their scrum responsibilities and in addition they are assigned three activities of handover in pre-delivery phase. Scrum Master is responsible for the management and administrative activity, scrum master with his team is responsible for developing and managing the maintenance environment. The role of product owner in this activity is either visible or invisible depending upon the nature of the product being in the sprint. In this phase documentation is also the responsibility of the scrum master and his team.

The second stage is the transition. During this phase scrum master is responsible for release planning and scrum master along with his team is accountable for the iteration completion, deployment and documentation.

The last phase is post-delivery. Scrum master and the team are responsible for the checking of the product according to the product vision. They also have to maintain the documentation for this stage. For maintenance, proper version and configuration management are also handled by them. For the training's activity product owner has to be with scrum master and team.

4. RESULTS AND DISCUSSION

To evaluate the work done is an important phase in the action research. After proposing the framework we have validated it through the expert reviews and participatory research through industrial experimentation.

4.1 Industrial Experimentation

The evaluation has been done through the industrial experimentation. For this purpose, Assessment Factors (AF)were defined to measure the satisfaction level of the participants. These factors were AF1-System Knowledge, AF2- Domain Knowledge, AF3-Efficient Communication, AF4- Maintenance of Documentation, AF5- Tracking Changes, AF6-Proper Change Planning, AF7-Training and AF8-Knowledge Sharing.

Total participants in this experimentation were sixteen. The threat for the validation was the selection of participants and their poor background or domain knowledge about the handover and

Table 3. Evaluation on basis of assessment factors.

Assessment Factor		Strongly Agree	Agree	Satisfactory	Disagree	Strongly Disagree	Satisfaction %
System Knowledge	AF1	7	4	3	1	1	87.5 %
Domain Knowledge	AF2	7	5	2	2	0	87.5 %
Efficient Communication	AF3	8	2	5	1	0	93.7%
Maintenance Documentation	AF4	5	6	4	0	1	93.7%
Tracking Changes	AF5	4	5	4	2	1	81.2%
Proper Change Planning	AF6	4	5	6	1	0	93.7%
Training	AF7	4	8	2	1	1	87.5%
Knowledge Sharing	AF8	6	4	2	1	3	75.0%

Table 4. Comparisons with old models.

Contributions	Proposed Model	Existing Models					
	Handover for Scrum	EM3: Handover Framework	Laine Markus	Thomas Vollman	Thomas Pigoski	ISO/IEC 14764	ISO/IES 15288
Handover Roles	✓	✓	✓	✓	✓	X	X
Handover Practices	✓	✓	✓	P	P	X	X
Handover Activities	✓	✓	✓	✓	✓	P	✓
Handover Lifecycle	✓	✓	X	X	X	X	X
Handover Guidelines	✓	✓	✓	X	X	X	X
Agile Practices	✓	X	X	X	X	X	X
Scrum Activities	✓	X	X	X	X	X	X
Division of handover activities for each phase	✓	✓	X	X	X	X	X
Division of agile activities for each phase of Handover	✓	X	X	X	X	X	X

✓= "exists" X = "does not exists" P = "partially exists"

scrum. To avoid this problem, we assured the right selection of participants by measuring their background knowledge. Also, they were provided with the sound knowledge about the handover process by a conducting short duration session of the meeting.

Predefined questionnaire templates were used to keep the uniformity and maintain the standard of the reviews about the proposed framework.

Figure 9 shows the questionnaire. Part A, B, C and D include the questions for the understanding of the handover process in industry. The part E contains the questions for the evaluation of the proposed frame work.

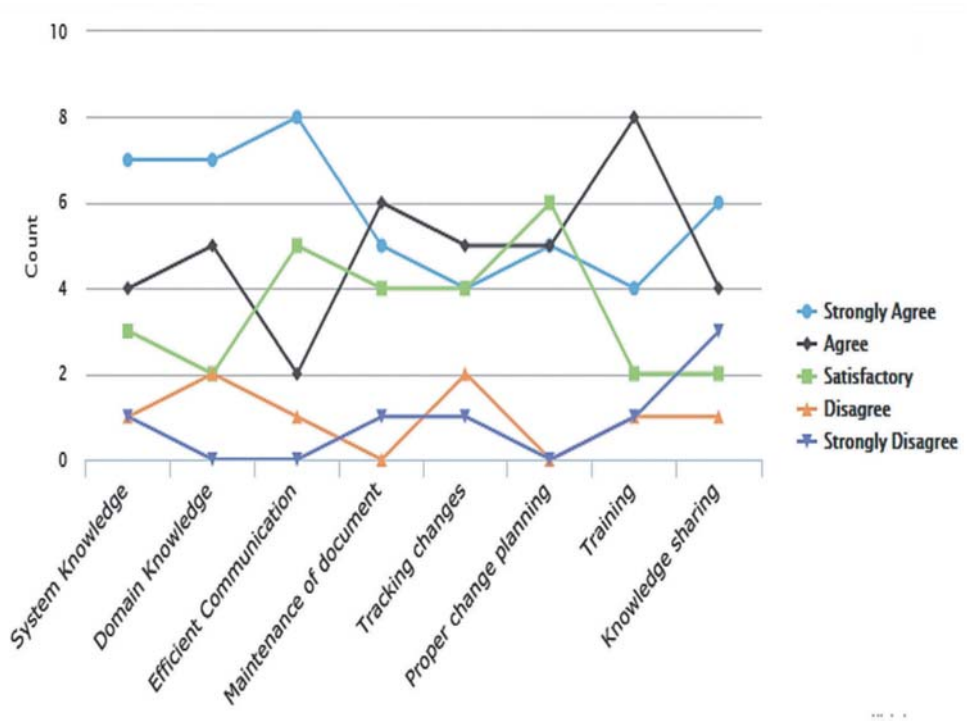

Fig. 10. Trend of satisfaction level.

For the evaluation purpose our primary focus was on the evaluation on the basis of assessment factors; defined above. Questions of part E were considered for the improvement in the proposed framework. The results for the evaluation of proposed framework on the basis of assessment factors are shown in the table 4. Each assessment factor is evaluated by sixteen participants. The number of participants against each factor shows their satisfaction level.

The results clearly show that the majority of participants were strongly agreed, agreed or satisfied for all the assessment factors; however, few disagreed against each assessment. The trend is shown in the figure 10.

From the trends of results of this industrial experimentation; explained in figure 10, it can be evidently seen that the participants have shown their confidence on the proposed framework of the handover process for scrum practices.

4.2 Comparison with Existing Models and Frameworks

A comparison with the existing models, framework and ISO/IEC standards is given in Table3. The comparison illustrates that the previous models or frameworks defined were generic and did not incorporate scrum practices in them but the proposed framework describes the handover process practices with incorporation of scrum practices. This will help the software industry to adopt the proposed solution for successfully performing the handover process.

5. CONCLUSION

The scrum development method is widely used in the software industry for gaining the high level of customer's satisfaction. On the contrary handover process is in its initial stages. The piece of research done in this area is very small. Up till now, no model had been devised which deals with the handover process for scrum practices. In this paper, we have highlighted the deficiency of scrum that leads towards the poor transition or transition failure. Our main contribution is the basic framework of the handover process for scrum practices. This will help the industry to plan and conduct the smooth transition of the software system. It provides the overall flow of the transition or handover process, which will assist to organize the handover process from the very beginning of the system till its end. However, this framework has the tendency to improve and incorporate detailed guidelines.

In future this framework is intended to be enhanced with more details in order to perform handover more appropriately and successfully. Post-delivery phase needs to be defined more precisely so that the roles and responsibility at this phase can be demarcated more clearly.

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Research Article

Simulation and Parametric Study of Urea Decomposition Section

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Abstract: In this study, the simulation model for the decomposition section of the urea plant was developed by using Aspen Plus. The decomposition reaction in the physical equipments such as heat exchanger, separator and stripper was handled with the use of the combination of the equilibrium reactors and built-in physical equipments in Aspen Plus. The thermodynamic model SR-Polar was used for the estimation of phase and chemical equilibria, density and other thermodynamic properties of the system. The results of the simulation were compared with the existing plant data and a good agreement was observed. Effects of the important parameters such as temperature, pressure and CO_2 stripping stream flow rate on the liquid distributor and 1st separator were also discussed in the decomposition section of urea plant.

Keywords: ammonium carbamate, simulation, Aspen Plus, urea

1. INTRODUCTION

Urea is one of the most important fertilizers, which is produced by the reaction of NH_3 and CO_2 at high pressure and temperature [1, 2]. The energy demands and environmental challenges for urea process are very high. The need of optimization and energy conservation has increased the interest in simulation of urea plant [3]. There are three different production processes of urea; once through process, partial recycle process and total recycle process. The most widely used process is total recycle process among these processes because this process is most flexible and energy efficient [4]. This process requires the separation of NH_3, H_2O and CO_2 from the product stream. These gases are recycled back in the form of ammonium carbamate to the synthesis reactor to increase overall conversion of the reactor. The removal of NH_3 and CO_2 from the solution could be either carried out by stripping or by decomposition process. The efficient decomposition and removal of ammonia carbamate from urea rich solution in the evaporation section leads to large energy savings. Hence the quality of the product can be increased economically [5]. Several studies have been done to investigate thermodynamic modeling and simulation of urea process [1-4, 6-8]. Most of the research studies have been carried out concerning the most suitable fluid package for urea synthesis section, modeling of synthesis section and the properties estimation of ammonium carbamate (the intermediate product in urea synthesis) [1-4, 8, 9] It has been observed from the literature that there is a knowledge gap for the study of urea decomposition, concentration and recovery section. A very little work has been done published for the simulation of these sections.

Bernardis et al [8] used the extended UNIQUAC equation for simulation of the high-pressure urea synthesis loop. Irazoqui and Isla et al [1, 2] treated isothermal urea reactor as an internal coil and

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main reactor and used the extended UNIQUAC equation to calculate the VLE of NH_3–CO_2–H_2O–urea system. Hamidipour et al [3] performed the modeling of synthesis section of an industrial urea plant and also carried out dynamic study of some of the parameters (e.g. pressure and liquid level in the synthesis reactor) using tuned Willson equation. Xiangping et al [4] used the extended UNIQUAC method to describe the non-linearity of urea system under high pressure and the vapor fugacity coefficient were determined using Perturbed-hard-sphere (PHS) equation of state. Brouwer [10, 11] provided the thermodynamics and phase equilibrium for the urea processes. Goharrokhi et al [12] carried out the urea synthesis reactor modeling based on the electrolytic system and studied the effects of N/C ratio and the temperature changes. Zendehboudi et al [13] proposed an efficient artificial neural network (ANN) technique for the simulation and optimization of the urea plant. The developed technique deals with complex vapour–liquid equilibria for the NH_3–CO_2–H_2O–$(NH_2)_2CO$ system and considers the CO_2 conversion in terms of temperature and the molar ratios of NH_3/CO_2 and H_2O/CO_2 in the liquid phase for urea reactors. Saima [7] performed the simulation of urea reactor in the Aspen Plus considering a plug flow reactor as a series of CSTRs and used the SR Polar method for estimation of thermodynamic properties for capacity enhancement.

In the present research study, the urea decomposition section of an industrial plant is simulated by using Aspen plus. The model development of the decomposition section is carried out using equilibrium reactors to ensure continuous decomposition in the decomposition section. The interaction parameters and binary coefficients are provided to apply SR polar thermodynamic model. This model provides a good approximation for (Vapor Liquid equilibrium) VLE of NH_3–CO_2–H_2O–urea system. The present research work provides simulation approach for decomposition section and simulation results are validated to real plant. Further this model is studied for sensitivity analysis.

2. PROCESS DESCRIPTION

The production of urea commercially occurs at high temperature (170 -200 ^oC) and high pressures (13 to 25 MPa) by reaction of NH_3 and CO_2. Fig. 1 shows the process flow diagram of urea plant. Ammonia and carbon dioxide from ammonia unit and ammonium carbamate from recovery section are fed to urea reactor. In the reactor two consecutive reactions such as the formation of ammonium carbamate and dehydration of ammonium carbamate take place to produce urea and water. The formation of ammonium carbamate is an exothermic reaction and this heat of reaction is used to drive the endothermic dehydration of carbamate.

$$2NH_3 + CO_2 \rightleftharpoons NH_4COONH_2$$

In recycle process, the synthesis mixture from the reactor is sent to decomposition section where

$$NH_4COONH_2 \rightleftharpoons NH_2CONH_2 + H_2O$$

carbamate decomposes to NH_3 and CO_2. The NH_3 and CO_2 are separated by flashing and the stripped off gases are absorbed in absorber in the recovery section with a small amount of water to form ammonium carbamate and this is recycled back in to synthesis reactor. Heat generated in the absorption process is utilized by the decomposers. While urea rich solution leaving decomposer is sent to the filtration unit prior to further concentration and then prilling of urea takes place in the prilling tower. Ultimately the urea is sent either for marketing or for storage.

The main emphasis of this research is to model the decomposition section of urea plant. Therefore decomposition section of urea plant is discussed in detail.

2.1 Decomposition Section

The decomposition section consists of

- High Pressure Decomposition
- Low Pressure Decomposition

In high pressure decomposition, decomposition is achieved by reducing the pressure and increasing

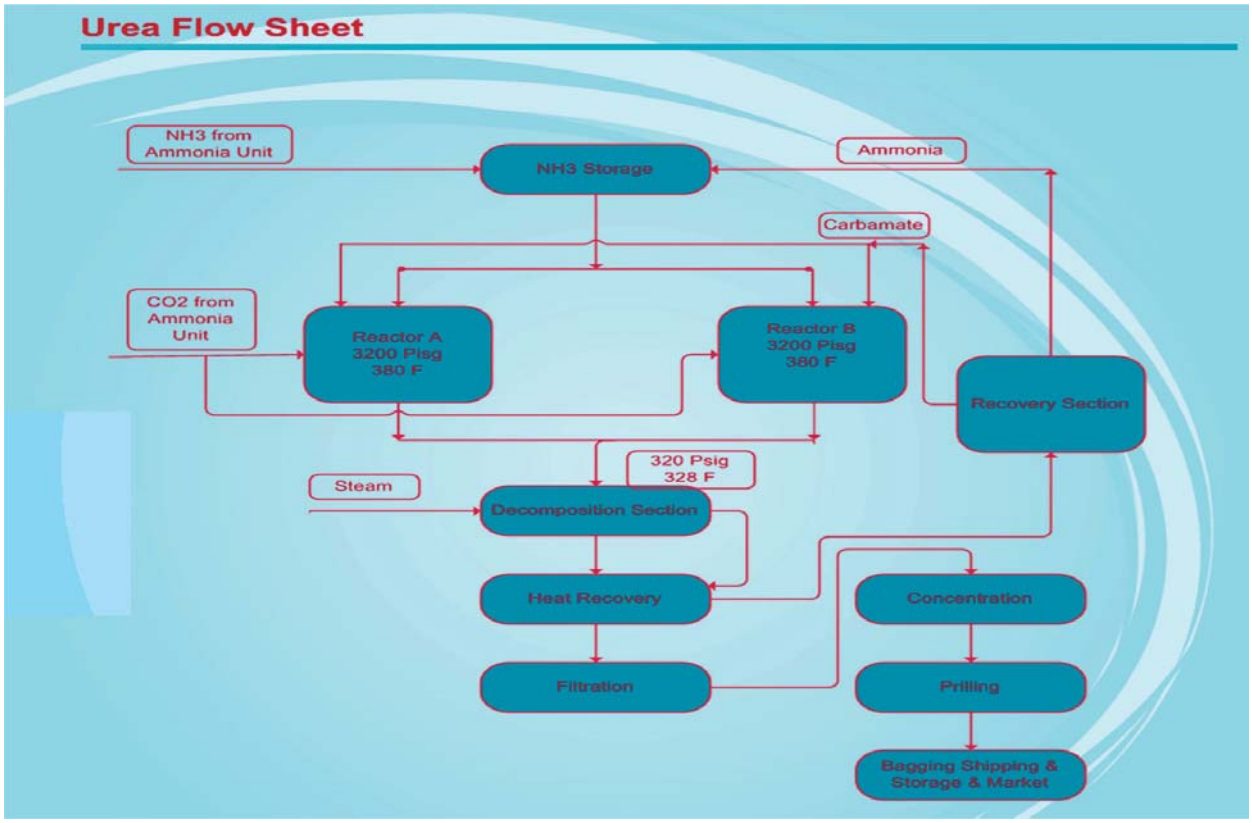

Fig. 1. Process flow diagram for urea plant.

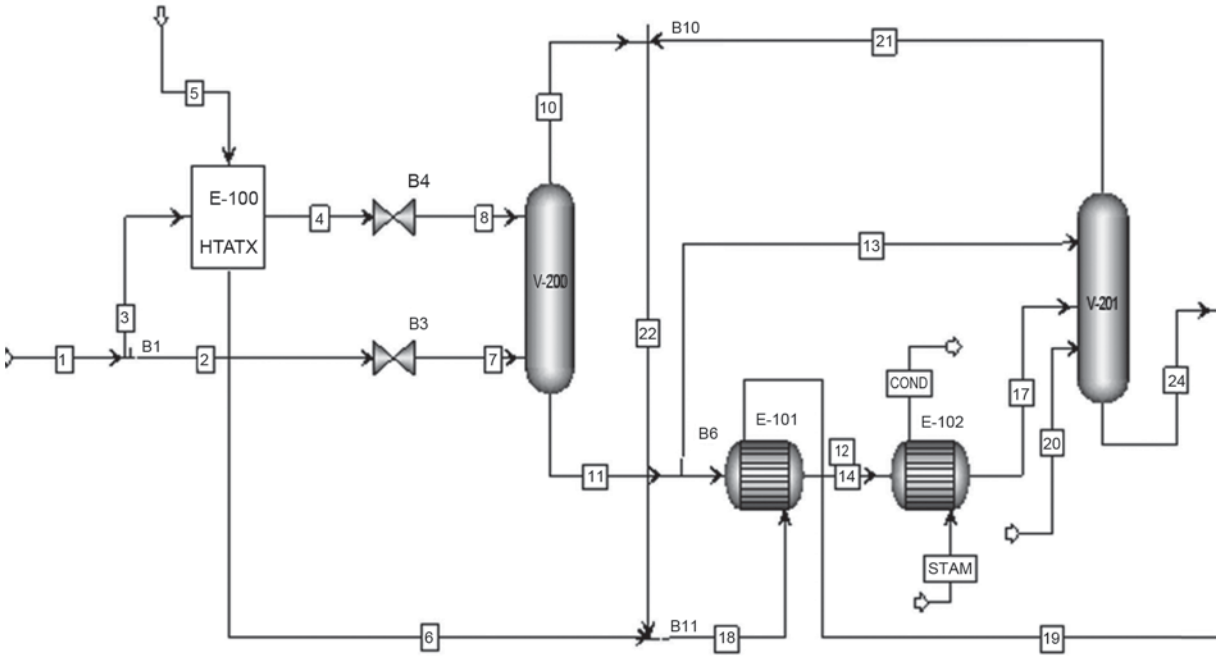

Fig. 2. High pressure decomposition section of urea plant.

the temperature in following equipment as shown in the (Fig. 2).

- Reflux Cooler (E-100)
- Liquid Distributor (V-200)
- 1st Pre Decomposer (E-101)
- 1st Decomposer (E-102)
- 1st Separator (V-201)

Stream (1) from outlet of the reactor is splitted into two streams; stream (2) and (3). Stream (3) is sent to the reflux cooler (E-100) for cooling by a heat recycle stream(5). After that its pressure is reduced to 300 psi by a mini let down valve (B4) and then it is introduced to the top of liquid distributor (V-200). Stream (2) is sent to the bottom of the liquid distributor (V-200) by reducing pressure to 300 psi through main let down valve (B3). This causes the considerable cooling and flashing of NH_3, CO_2 and H_2O. Stream (3) coming from the top of liquid distributor reduces H_2O and CO_2 contents in the off gas stream (10) from V-200. The stream (11) from liquid distributor is divided into two streams (12) and (13). Stream (12) is sent to the 1st pre-decomposer (E-101) where it is heated by a heat recycle stream (18).Which is further heated by a 185 psig steam in the 1st decomposer (E-102) and then urea rich solution stream (17) is introduced to the bottom of V-201. Stream (13) is used as a reflux to 1st separator (V-201). The CO_2 stream (20) is introduced from the bottom of 1st separator for stripping and decomposition of ammonium carbamate. CO_2 rich stream (21) from the top of V-201 is mixed with ammonia rich stream (10) from V-200 and is mixed with heat recycle stream (6) to recover its heat completely in E-101.

The low pressure decomposition includes the following components (Fig.3):

- Second Flash Separator (V-202)
- Second Decomposer (E-103)
- Second Separator (V-203)

The product stream (24) leaving the bottom of 1st separator is sent to the flash separator (V-202) and decomposition is achieved by flashing. The gaseous phase is separated from liquid and concentration is further increased. The residual carbamate solution (26) from V-202 is sent to the second decomposer (E-103) where it is heated from the low pressure heat recycle stream (19) coming from the shell side of E-10. It is then sent to the second separator (V-203). The low pressure CO_2 stream (29) leaving the top of the second separator (V-203) is mixed with low pressure gaseous stream (25) from V-202 and then sent to the recovery cycle. Urea rich solution (30) is sent to evaporators where excess water is removed and the concentrated urea solution is then sent to prilling tower and urea granular product is stored in the storage vicinity.

3. SIMULATION MODEL DEVELOPMENT

The simulation of decomposition section of urea is a challenging research area because of the unavailability of thermodynamic properties of urea, ammonium carbamate and biuret and solid handling in commercial simulators such as Aspen Plus & HYSYS etc. [14, 15].It requires a critical approach in simulating urea production process in Aspen Plus. The solid handling and VLE properties of urea and ammonium carbamate are estimated in Aspen Plus using a FORTRAN code, linker file urea.dll and urea.opt based on a pilot plant data provided by Aspen Tech [15]. The binary coefficients are added for urea and ammonium carbamate for the estimation of other thermodynamic properties like phase & chemical equilibria. The formation of biuret is normally controlled by process conditions, so plant is operated at the conditions where biuret formation is negligible. The unavailability to account for reactions in the physical separation units and heat exchangers is handled by using Redrac model for strippers [16]; and equilibrium reactors before separators and heat exchangers. The analysis of ammonium carbamate in plant is available in the form of CO_2 and NH_3 only, so the carbamate is estimated from the literature based on assumption that the free CO_2 is present to a minimum extent in liquid phase.

3.1 Assumptions

The simulation of urea decomposition section is based on the assumptions:

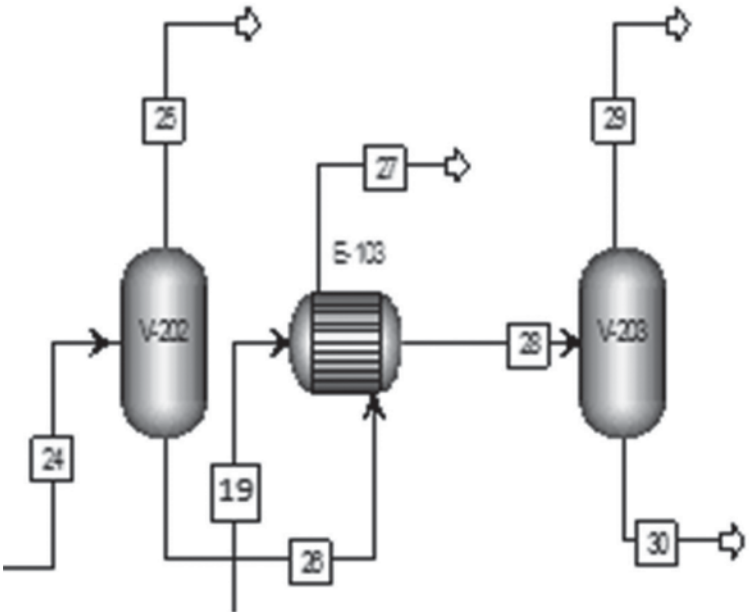

Fig. 3. Low pressure decomposition section of urea plant.

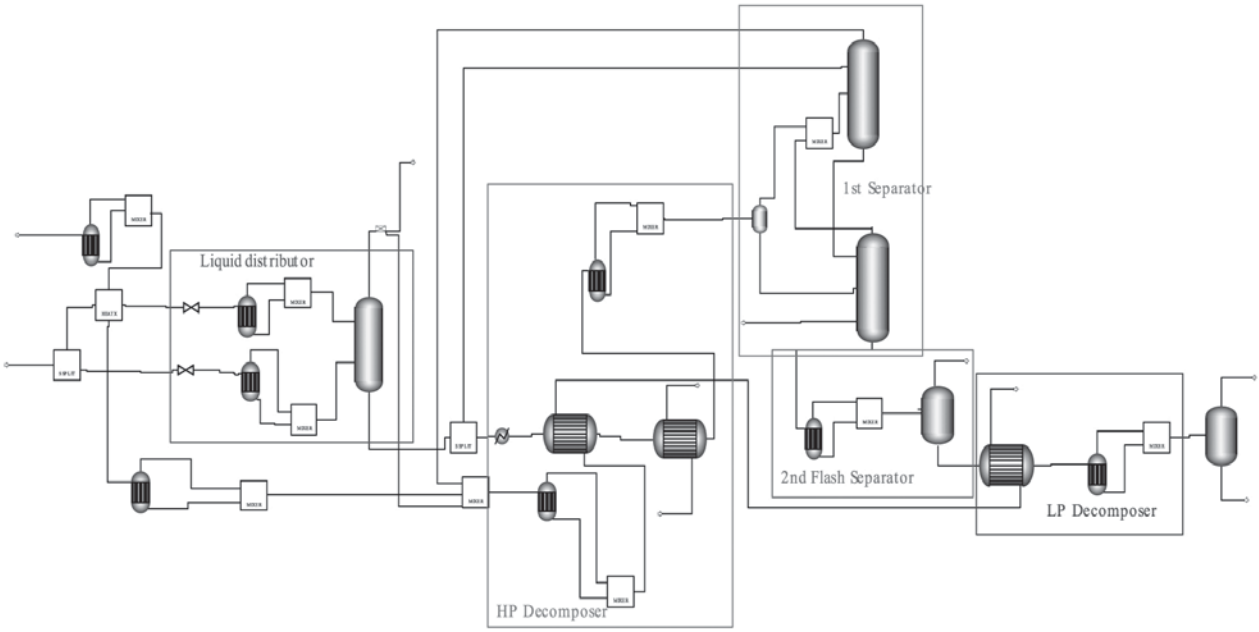

Fig. 4. Aspen Plus Flow sheet of decomposition section of urea plant.

i. The process is at steady state;
ii. Formation of biuret and other by-products has been neglected;
iii. Free CO_2 is present to a minimum extent in the liquid phase and present only in the form of ammonium carbamate;
iv. No carbamate and urea is present in gas phase; and
v. All Inert gases are considered only in the form of air.

In P & ID there are two reactors and two reflux coolers, and in the aspen plus the single reflux cooler is modeled.

3.2 Thermodynamic Property Package

The model for thermodynamic properties is based upon SR-POLAR. The model uses an equation of state and is suitable for the non-ideal mixtures as

well as for even high temperature and pressure. Further, the model contains extensions that enable an accurate description of the phase and chemical equilibria, the density and other thermodynamic properties (e.g., enthalpy).

The UNIQUAC thermodynamic package is used in previous studies [1, 2, 8], but in the present study it is not used because it assumes that under high temperature (160 to 200°C) and relatively low water concentration, the extent of ionization will be small. Also, modern equations of state such as SR-POLAR model are well suited to the description of thermodynamic properties of non-ideal systems.

The SR-POLAR property method is based on an equation-of-state model by Schwarzentruber and Renon, which is an extension of the Redlich-Kwong-Soave equation of state. SR-POLAR method can be applied to both non-polar and highly polar components. It is also applicable to highly non ideal mixtures and mixture of light gases with polar and non-polar compounds. Reasonable results at any condition can be achieved, provided UNIFAC interaction parameters are available. But results are least accurate close to the critical point. The simulation case is beyond the critical range thus this model can be used with UNIFAC interaction parameters. The system of ammonium carbamate is highly polar solution and there are some light gases along with the mixture. So the SR-POLAR is used for the estimation of phase equilibrium for that purpose the temperature dependent binary parameters are used to accurately represent phase equilibria.

3.3 Simulation Model

The reactor effluent composition (Table 1) is estimated from the assumption mentioned in section 3.1. The different equipments at the real plant are modeled using built in aspen plus model described in Table 2, simulation flow diagram is shown in Fig. 4 and the stream input specifications are given in Table 3. The simulation approach for the individual units is described below.

The reactor effluent is introduced to S Split to split the stream to be used as a reflux in liquid distributor and main feed for the liquid distributor. It divides the stream only on the basis of flow rate. The composition, temperature and pressure remain constant. The reflux to liquid distributor is passed to the double pipe heat exchanger which is modeled as a Heatx model. Only one heat exchanger is used instead of two to give the total surface area for the simulation.

RedFrac model is used for the simulation of liquid distributor. The purpose of liquid distributor is to separate the free NH_3 from the urea solution. The decomposition in liquid distributor due to reduction in pressure is accommodated by the introduction of

Table 1. Reactor effluent composition in terms of carbamate.

Components	% (Wt)	lb/hr
Urea	31.06	145305.07
CO_2	0.10	471.10
NH_3	31.07	145382.03
H_2O	20.09	93985.15
Carbamate	17.67	82677.23
Total	100.00	467820.57

Table 2. Real plant units and model developed in Aspen Plus.

Real Plant Units	Aspen Plus Model
Liquid Distributor V-200	**Liquid Distributor Model** o Equilibrium Reactor(B1,B2) o Mixers(B3,B5) o RedFrac Model (Liquid Dist)
1st Pre Decomposer E-101	**1st Pre Decomposer Model** o Heater o Shell and Tube Heat Exchanger
1st Decomposer E-102	**1st Decomposer Model** o 1st Dec o Equilibrium Reactor(B11) o Mixer(B12)
1st Separator V-201	**1st Separator Model** o Rectifying Section(1ST-SEPT) o Flash separator(B24) o Mixer(B14) o Stripping Section(1ST-SEP)
2nd Flash Separator V-202	**2nd Flash Separator Model** o Equilibrium Reactor(B16) o Flash separator(2ND-SEP) o Mixer(B17)
2nd Decomposer E-103	**2nd Decomposer Model** o Shell and Tube Heat Exchanger (2ND-DEC) o Equilibrium Reactor(B13) o Mixer(B10)
2nd Separator V-203	**2nd Separator Model** o Flash separator(2ND-SEP)

Table 3 Stream input data.

Stream Name &Number	Reactor Effluent (1)	Heat Recycle (5)	Steam	CO2 stream (20)
Temperature (°F)	382	162.5	395	246
Pressure (psig)	3199	292.5	185	296
Total Flow (lb/hr)	467820.57	48847.35	52356	20709
Composition	Wt%	Wt%	Wt%	Wt%
Urea	0.311	0.132	-	-
CO_2	0.001	0.213	-	0.999
NH_3	0.311	0.341	-	-
H_2O	0.200	0.314	1.000	-
Carbamate	0.177	-	-	-
Air	-	-	-	0.001
Total	1.000	1.000	1.000	1.000

equilibrium reaction. The ammonium carbamate decomposes to form NH_3 and CO_2 by reduction in pressure and increase of temperature.

$$2NH_3 + CO_2 \leftrightarrows NH_4COONH_2$$

This reaction is highly endothermic and reaches to chemical equilibrium very quickly. So the decomposition reaction is included in the simulation as React1 to account for the reaction in the RedFrac Models. The reaction is added as the equilibrium reaction and model estimates the K_{eq} from the Gibbs free energy.

Four equilibrium stages are used in the Redfrac model to separate the free ammonia from the urea solution. The decomposition occurring because of let-down valves is accommodated by the use of the equilibrium reactors after let-down valves. The pre-decomposer is designed as a combination of a heater and a shell and tube heat exchanger. The heater accounts for transferring of exothermic heat of reaction of the ammonium carbamate formation in the heat recycle stream and then the remaining sensible heat transfer is carried out in shell and tube heat exchanger.

1st Decomposer is a shell and tube heat exchanger and the decomposition reaction of ammonium carbamate also takes place in tube side. In Aspen Plus, reaction cannot be accommodated to the heat exchangers so an equilibrium reactor is used to account for the decomposition in 1st decomposer.

The first separator is modeled as a combination of two RedFrac models and a flash separator between them. As actual separator has three trays at the top and three trays at the bottom and a larger space for the flashing at the center. Top section acts as a distillation column with a reflux stream from the top. The bottom section acts like a stripping section where medium pressure (MP) CO_2 stream is used as a stripping agent. The purpose of separator is to remove most of the CO_2 and NH_3 by introduction of MP CO_2 as stripping medium.

So the unit is designed as a combination of three units (Fig. 4):

- Rectifying section
- Flash separator
- Stripping column

Flash Separator is a simple vessel and operating temperature and pressure are 228 °F and 43.5 psig respectively. The decomposition in the flash separator is considered by employing an equilibrium reactor before it. The 2nd decomposer is a shell and tube heat exchanger with an equilibrium reactor. Second separator is also designed as a flash separator to separate the decomposed ammonium carbamate in the 2nd decomposer. The temperature and pressure of 2nd flash separator are 230 °F and 28.45 psig, respectively.

4. MODEL VALIDATION

The licensors' material balance of the liquid distributor, 1st and 2nd separator is compared with the simulation results. The results in Aspen Plus are given in the form of carbamate, for the sake

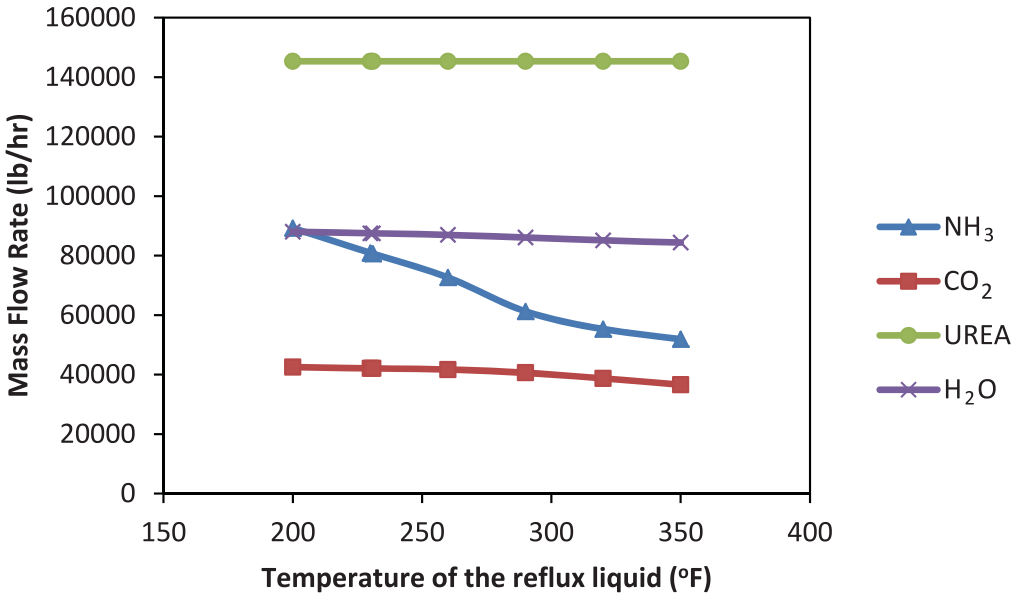

Fig. 5. Effect of temperature on the mass flow rate of the liquid stream of liquid distributor.

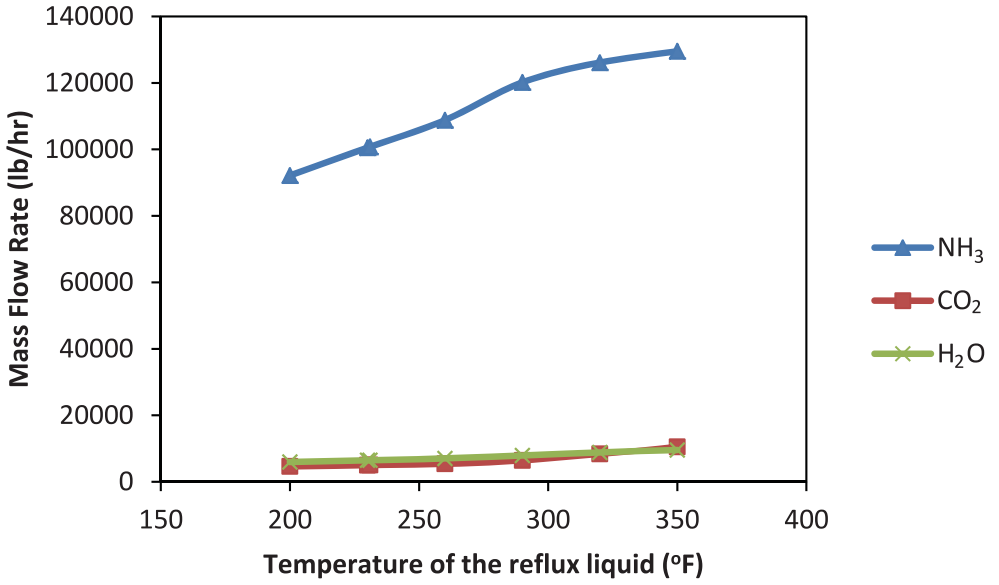

Fig. 6. Effect of temperature on the mass flow rate of the vapour stream of liquid distributor.

of composition; the carbamate is mentioned as ammonia and carbon dioxide. The results of Aspen plus model are shown in Table 4 for validation.

1st separator is a single unit in actual plant but it is simulated as a combination of three units because of large disengagement region between the top and bottom three plates. When the material balance of the real plant is compared with the Aspen plus model the results showed in Table 4 are more close to the plant model as compared to modeling a single separator (for details, see [18]). The material balance is compared for the purpose of data validation across the 2nd separator as well. The comparison of the results with the industrial plant data showed a close agreement with the plant data and the % error is ranging from 0 to 15%. Hence the model can be extended for the purpose of further studies.

Table 4. Model Validation of simulation results.

Stream	Liq. from Liquid Distributor			Gas from Liquid Distributor			Liq. from 1st Separator			Gas from 1st Separator			Liq. from 2nd Separator			Gas from 2nd Separator		
	Mass Fraction		Error	Mass Fraction		Error	Mass Fraction		Error	Mass Fraction		Error	Mass Fraction		Error	Mass Fraction		Error
	Plant Data	Aspen	%	Plant Data	Aspen	%	Plant Data	Aspen	%	Plant Data	Aspen	%	Plant Data	Aspen	%	Plant Data	Aspen	%
Urea	0.412	0.407	1.00	0.000	0.000	0.00	0.571	0.572	0.19	0.000	0.000	0.00	0.661	0.655	0.92	0	0.001	100.00
CO_2	0.116	0.117	0.86	0.053	0.049	7.54	0.064	0.058	10.74	0.380	0.388	1.95	0.023	0.02	13.04	0.225	0.243	8.00
NH_3	0.229	0.233	1.72	0.874	0.883	1.09	0.072	0.072	0.13	0.527	0.527	0.04	0.024	0.023	4.35	0.48	0.418	14.81
H_2O	0.242	0.243	0.41	0.073	0.067	8.81	0.293	0.299	1.97	0.093	0.085	9.18	0.292	0.302	3.31	0.295	0.338	12.78
Air	-	-	-	-	-	-	-	-	-	0.000	0.000	1.84	-	-	-	0.000	0.000	0.00
Total	1.000	1.000		1.000	1.000		1.000	1.000		1.000	1.000		1.000	1.00		1.00	1.00	
Mass Flow (Ton/hr)	176.51	178.24	0.87	57.40	55.67	3.01	127.19	126.95	0.19	59.67	61.41	2.83	09.86	110.31	0.40	8.79	9.01	2.45
Phase	Liq	Liq		Gas	Gas		Liq	Liq		Gas	Gas		Liq	Liq		Gas	Gas	
P (psig)	317.40	317.40	0.00	317.40	317.40	0.00	287.60	287.60	0.00	287.60	287.60	0.00	28.45	28.45	0.00	28.45	28.45	0.00
T (°F)	250.00	250.90	0.36	263.00	263.00	0.00	280.00	279.40	-0.21	274.40	274.39	-0.004	30.00	230.00	0.00	230.00	230.00	0.00

5. SIMULATION RESULTS

5.1 Analysis of Liquid Distributor

The purpose of the liquid distributor is to separate the free ammonia present in effluent from the reactor so that the decomposition of ammonium carbamate in the next sections is not hindered by free ammonia present in urea solution. The effect of temperature and pressure of liquid distributor is also studied.

5.1.1 Effect of Temperature on the Liquid Distributor

Fig. 5 shows the effects of temperature of the reflux liquid stream (4) on the mass flow rates of NH_3, CO_2, H_2O and urea. The mass flow rate of NH_3 and CO_2 deceases with the increase in temperature of stream (4) while that of H_2O and urea remains constant. There is only a slight decrease in CO_2 as compare to NH_3 as CO_2 in liquid stream condenses with ammonia to form ammonium carbamate. This slight decrease in CO_2flow rate indicates that the decomposition of ammonium carbamate is very small. The decrease in mass flow rate of ammonia is more pronounced than CO_2 in the liquid stream of liquid distributor due to ammonia present in free form and ammonia produced by decomposition of ammonium carbamate.

In Fig. 6 the effect of temperature on the gaseous stream is also shown in the liquid distributor. Free ammonia is released to a maximum extent in the liquid distributor. Increase in the temperature of reflux liquid increases the mass flow rate of NH_3 in gaseous phase appreciably, showing the decomposition of the ammonium carbamate. While the water vapor and CO_2 does not change appreciably as the liquid from the top of liquid distributor suppresses H_2O and CO_2 flow in vapor phase. Urea composition is not shown in Fig.6 as urea remains in the liquid phase.

5.1.2 Effect of Pressure on the Removal of NH_3 from Liquid Distributor

The amount of ammonia decreases with increase in pressure of the liquid distributor (Fig. 7). High pressure section of decomposition acts as the heat source for the decomposition in the low pressure section, so the pressure is maintained in such a way that the removal of NH_3 and CO_2 is sufficient to meet the heat loads of low pressure decomposition section.

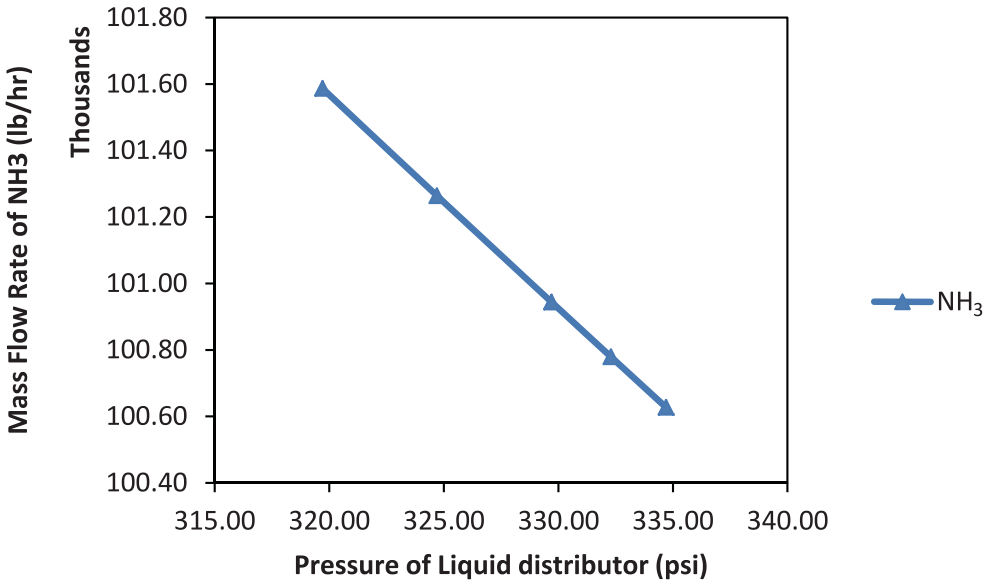

Fig. 7. Effect of pressure on ammonia removal in Liquid Distributor.

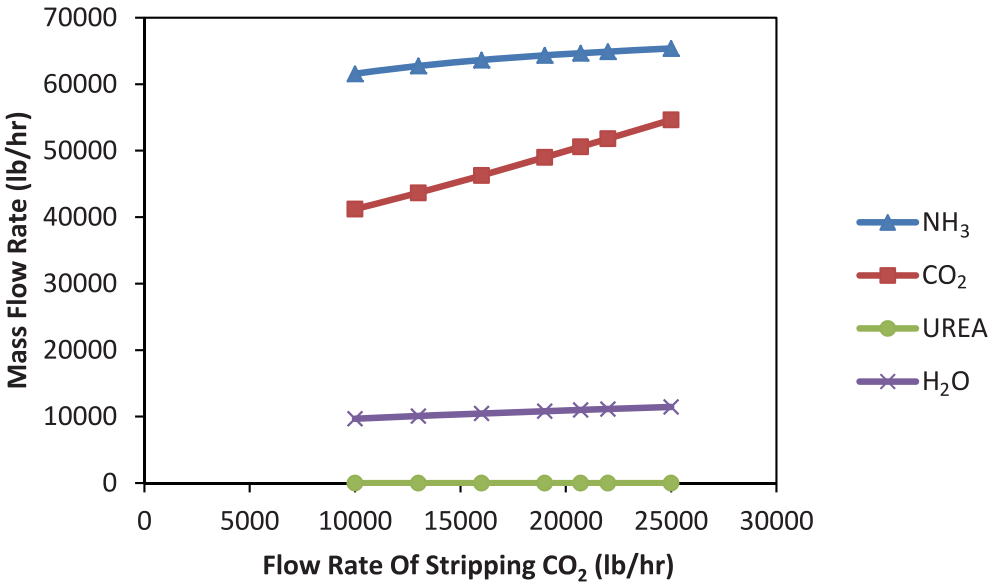

Fig. 8. Effect of CO_2 stripping gas flow in the vapor stream of 1st separator.

5.2 Analysis of 1st Separator

1st separator is one of the critical equipment of decomposition section. The purpose of separator is to remove NH_3 and CO_2 from liquid stream leaving from 1st decomposer. The relatively colder stream is delivered as a reflux solution to the top of the tray. The purpose of reflux stream is to decrease water vapor contents in the gaseous phase. High temperature leads to hydrolysis of urea and that is why sensitivity analysis is considered in the temperature range of 270 -275 °C.

5.2.1 Effect of CO_2 Stripping Gas on the Stripper

A stream of CO_2 is introduced through the 1st separator bottom tray for stripping of some of the residual ammonia from urea product solution. Fig.8 shows increase in CO_2 stripping gas flow rate increases the mass flow rate of NH_3 and

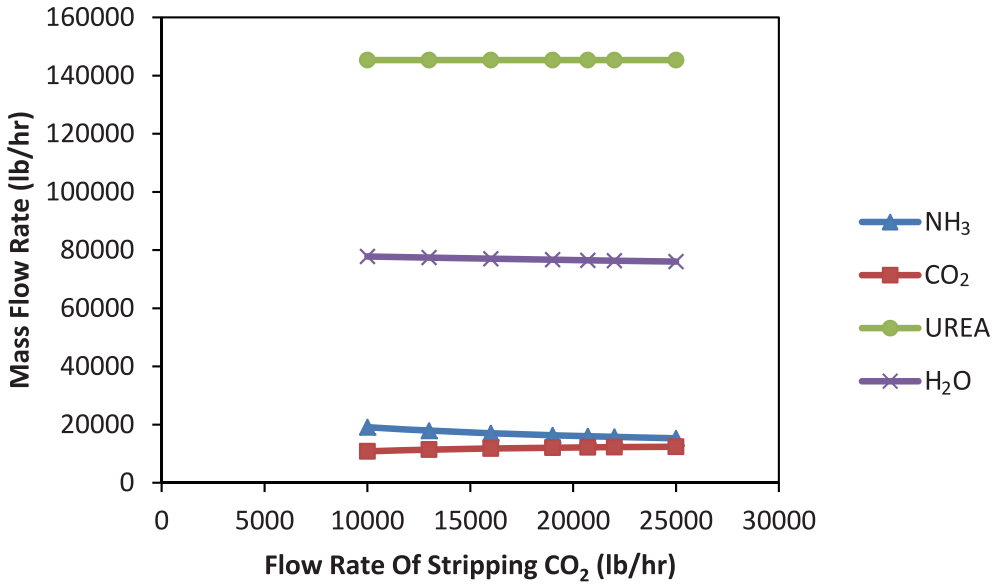

Fig. 9. Effect of CO2 stripping gas flow in the liquid stream of 1st separator.

CO_2 in vapor stream. The sharp increase in CO_2 shows the flow of the stripping gas as well as the decomposition of ammonium carbamate. While there is a slight increase in mass flow rate of H_2O that shows the evaporation of water in vapor phase and urea remains constant as it does not strip off.

Fig.9 presents the effect of CO_2 stripping gas flow on liquid stream of 1st separator. The quantity of ammonia and water is decreased with the increase in mass flow rate of stripping CO_2. While the mass flow rate of CO_2 is increased with the increase in mass flow rate of stripping CO_2. It has been observed that there is a little formation of ammonium carbamate along with the decomposition of ammonium carbamate. The amount of urea remains constant in the liquid phase.

6. CONCLUSIONS

Simulation model developed for the decomposition section of urea plant can be used for optimization purposes. It can also be used to investigate the effects of operating conditions on the decomposition of carbamate. Hence the model is suitable for conservation of energy and optimized plant performance. The model is fine tuned to the operating plant and the error (%) is small which makes it acceptable for further studies.

7. ACKNOWLEDGEMENTS

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Research Article

Breeding Efficiency of Indigenous × Jersey, Indigenous × Jersey × Friesian Crossbred Cows at Livestock Development Research Centre, Muzaffarabad, Azad Jammu and Kashmir

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Abstract: The primary objective of this study was to improve the reproductive efficiency of indigenous cattle of Azad Jammu and Kashmir at Livestock Development Research Center (LDRC) by crossing with European breeds. The indigenous heifers were impregnated with Jersey semen and F_1 crossbred were produced. The F_1 offspring were crossed among themselves (inter *se* mating) to obtain the F_2 offspring and simultaneously the F_1 cows were crossed with Friesian bull to produce three-breed crossbred cows. The number of cows for each group were 37 for indigenous, 25 for F_1 (Indigenous × Jersey) cross, 8 for F_2 ($F_1 \times F_1$) cross and 14 for F_1 × Friesian cross. The highest breeding efficiency was observed in F_1 (93.68 ± 1.85 %) and F_2 (93.71 ± 2.74 %) and increased highly significantly ($P < 0.0001$) compared to indigenous cows (73.46 ± 2.50 %). The mean breeding efficiency of F_1 × Friesian (65.62 ± 3.05 %) did not differ significantly from that of indigenous cows ($P = 0.0870$). The Jersey breed was found to be suitable for improving the breeding efficiency of indigenous cows of hilly areas of Azad Jammu and Kashmir.

Keywords: Subtropical, highland type, breeding efficiency, indigenous cows, crossbred cows

1. INTRODUCTION

The productivity of indigenous cattle of Azad Jammu and Kashmir is very low. This may be because of poor genetic makeup. Reproductive performance in dairy cattle is of paramount importance. To maintain efficient production, it is necessary that cows reproduce regularly [1]. It has been reported that lowered breeding efficiency may be associated with high production [2, 3] and contradictorily, that there is little relationship between production and breeding efficiency [4, 5, 6, 7]. The economic returns from dairy animals are not only based on milk production alone but also on their reproductive efficiency [8]. Everett et al [9] reported that breeding efficiency and production were essentially interdependent. Reproductive efficiency is proposed as a measure of the net biological accomplishment of all reproductive activities and phenotypic expression of the interplay of genetic and environmental factors [10]. Indicators of reproductive efficiency are service period affecting in turn, the calving interval. However, the breeding efficiency in addition to accommodating the number of calving also takes care of age at first calving and total number of days from first to last lactation. Reproductive efficiency represents the overall performance of the herd with respect to age and reproductive traits [11]. Age of dairy cows at first parturition and the lengths of her subsequent calving intervals are usually considered of primary importance in measuring breeding efficiency [12]. Low reproductive efficiency due either to delayed first service, missed estrus, or multiple services per conception continues to be

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a major problem in dairy herds. Poor reproductive performance results in excessively late age at first calving and long lactations. Both are costly to the dairy producers because of the veterinarian breeding expense, high reproductive replacement costs and fewer calves being born [13]. Several reports had indicated that poor reproductive performance, manifested as prolonged calving intervals, can result in reduced milk yield and increased culling rates and replacement cost [14–16].

Although the crossbreeding has been adopted as a tool to improve traits of economic importance of indigenous cattle in canal irrigated areas of Punjab and other part of Pakistan, however, such adoptability studies are missing in AJK, therefore this study was planned to improve the overall productivity of indigenous cattle along with reproductive efficiency traits by crossing with European breed of Jersey and Friesian and to assess the adoptability of crossbred dairy cattle in local environmental conditions.

2. MATERIALS AND METHODS

2.1 Animals and Farms

LDRC is located at the bank of river Jhelum 6 kilometers away from the main city of Muzaffarabad which is the capital of Azad Jammu and Kashmir. This farm was established in 1990 by the Government of Azad Jammu and Kashmir by purchasing of 66 indigenous cattle. The animals were maintained in brick closed sheds throughout the year. The milking cows, dry cows and young calves were kept in separate shed with roof constructed from asbestos sheet and iron bar, the floor is of concrete. During the summer months the animals were showered with cold water and electric fan were provided in the shed to beat the heat.

2.2 Breeding Program

A breeding program with the introduction of Jersey and Holstein Friesian was started in July 1990. In first cross F_1 offspring from crosses between indigenous and Jersey were produced. Calving of F_1 offspring occurred from July, 1991 to April, 1998. In second type of cross F_1 female were crossed with F_1 male, as a result of which $F_1 \times F_1$ (F_2) offspring were produced during the period of May, 1994 to April, 1999. In third type of cross the F_1 female were crossed with pure Friesian bull to produce 25 % indigenous + 25 % Jersey + 50 % Friesian offspring during May 1994 to April 1999.

The diagrammatic presentation of breeding program is illustrated below:

1. Indigenous × Jersey
   ↓
   F_1 (Indigenous 50 % + Jersey 50 %)

2. F_1 (Indigenous 50 % + Jersey 50 %) × F_1 (Indigenous 50 % + Jersey 50 %)
   ↓
   F_2 (Indigenous 50 % + Jersey 50 %)

3. F_1 (Indigenous 50 % + Jersey 50 %) × Friesian
   ↓
   Indigenous 25 % + Jersey 25 % + Friesian 50%

2.3 Feeding Regime

All the animals were stall fed on farm raised green fodder. The ration was formulated to provide the recommended quantity of nutrients according to body weight and status of animals as given in Table 1. The composition of the feed varied according to the fodder crop available during the year. Elephant grass and maize were mainly fed during the months of May to October and from November to April green berseem and wheat straw were fed to these animals. Green fodder was chaffed and offered to these animals. Roughages comprised of wheat straw and stoves of maize. The concentrate mixture composed of wheat bran, oil seed cake (rape seed cake and cotton seed cake) and molasses. Lumps of common salts (sodium chloride) were placed in mangers and cows were free to lick with accessibility of clean drinking water.

2.4 Data Collection

It was a retrospective study, carried out over a period from 1990–2010. The data regarding

Table 1. Daily nutrient fed to cows per 500 kg body weight and according to their productive and reproductive status maintained at LDRC, AJK.

Status	Total Dry Matter (kg)	Nutrients (kg)			
		TDN[a]	CP[a]	Ca[a]	P[a]
Early Lactation	11.91	7.05	1.25	0.04	0.02
Lactating and Pregnant	11.41	6.27	0.99	0.03	0.02
Dry Non Pregnant	8.41	4.23	0.60	0.02	0.01
Pre-calving (60-90 days before calving)	10.32	5.59	0.88	0.03	0.02

[a]TDN = Total Digestible Nutrients; CP = Crude Protein; Ca = Calcium; P = Phosphorus

reproductive records of 84 cows out of which 37 were indigenous, 25 were F_1 (Indigenous × Jersey), 8 were $F_1 \times F_1$ (F_2) and 14 were F_1 × Friesian cows. Among reproductive parameters service period, calving interval and breeding efficiency were studied in present research work. Although, the other parameters of reproductive performance such as sex ratio, age at first calving, number of services per conception were also recorded for indigenous and crossbred groups in this breeding program. However, the data for these traits has not been included in this research paper.

2.5 Service Period

Service period of each cow was calculated by the difference between the date of calving and the date of subsequent fertile conception.

2.6 Calving Interval

Calving interval was calculated by the interval between the dates of two successive calving.

2.7 Breeding Efficiency

The breeding efficiency of each cow was calculated by using the following formula suggested by Wilcox et al [17].

$$\text{Breeding Efficiency (\%)} = \frac{365 \times (N-1)}{D} \times 100$$

Where N= Total number of parturitions, D= Number of days from first to last parturitions.

2.8 Statistical Analysis

The difference in the mean breeding efficiency among the four breed groups were worked out through analysis of variance. Graph Pad Prism 5 package was used for statistical analysis.

3. RESULTS

Mean breeding efficiency of indigenous and crossbred dairy cow is given in Table 2. Mean breeding efficiency increased highly significantly in F_1 ($P<0.0001$) and F_2 ($P=0.0007$) hybrid cows compared to that of indigenous cows. Crossing of F_1 females with Friesian bull decreased the breeding efficiency highly significantly in F_1 × Friesian cows compared to that of F_1 ($P<0.0001$) and F_2 ($P<0.0001$) hybrid cows. Mean breeding efficiency of F_1 and F_2 cows did not differ significantly from each other ($P=0.9933$). Statistically no significant difference of mean breeding efficiency was observed in F_1 × Friesian and indigenous cows ($P=0.087$).

Mean service period of F_1 × Friesian cows was highest (266.7 ± 16.56 days) and the lowest (81.81 ± 11.19 days) mean service period was observed in $F_1 \times F_1$ (F_2) cows. Crossbreeding of indigenous cows with Jersey decreased the service period highly significantly in F_1 ($P < 0.0001$) and F_2 ($P < 0.0001$) hybrid cows compared to that of indigenous cows and service period of F_1 and F_2 did not differ significantly ($P = 0.37$) from each other. Crossing of F_1 female with Friesian bull increased the service period in F_1 × Friesian cows and it was similar to that of indigenous cows ($P = 0.549$). Mean calving interval of indigenous and crossed dairy cows are given in Table 2.

Mean calving interval of indigenous and

Table 2. Mean breeding efficiency, service period and calving interval of indigenous and crossbred cows.

Breed Group	Breeding Efficiency (%)	Service Period (days)	Calving Interval (days)
Indigenous	73.46±2.50[1] (37)[2]	256.0±8.67 (102)	518.6±9.54 (102)
Indigenous × Jersey (F_1)	93.68±1.85***a (25)	92.60±5.04***a (121)	368.8±5.32***a (121)
$F_1 \times F_1$ (F_2)	93.71±2.74***a (8)	81.81±11.19***a (26)	359.8±11.68***a (26)
F_1 × Friesian	65.62±3.05***bc (14)	266.7±16.56***bc (34)	540.9±22.39***bc (34)

[1]Mean ± SE; [2]Values in parenthesis ( ) are Number of cows

a = Indigenous vs F_1, F_2 & F_1 × Friesian ; b = F_1 vs F_2 and F_1 × Friesian; c = F_2 vs F_1 × Friesian

P ≤ 0.05*, P ≤ 0.01**, P ≤ 0.001***

crossbred dairy cows is given in Table 14. Mean calving interval of indigenous cows was highest (518.6 ± 9.543 days) and lowest (359.8 ± 11.68 days) was observed in F_2 cows. Crossbreeding of indigenous cows with Jersey decreased the calving interval highly significantly in F_1 ($P < 0.0001$) and F_2 hybrid cows ($P < 0.0001$) compared to that of indigenous cows. Calving interval of F_1 hybrid did not differ significantly ($P = 0.482$) compared to F_2 hybrid cows. When F_1 hybrid cows were crossed with Friesian bull, then in F_1 × Friesian cows the calving interval increased to that of indigenous cows ($P = 0.289$).

4. DISCUSSION

In present study long service periods and subsequently long calving intervals of indigenous and F_1 × Friesian cows might have contributed to the low breeding efficiency. The long service period might be due to delayed resumption of ovarian activity after calving. The breeding efficiency varied among indigenous and crossbred cows in this study.

The breeding efficiency of indigenous cows (73.46 ± 2.50 %) in this study increased as a result of their crossbreeding with Jersey in F_1 and F_2 crossbred cows. The high breeding efficiency of F_1 (93.68 ± 1.85 %) and F_2 (93.71 ± 2.74 %) crossbred cows was due to their short service period and calving interval. Mean breeding efficiency of F_1 and $F_1 \times F_1$ (F_2) was higher than that of breeding efficiency of Jersey cows in different countries as 87.01 ± 1.73 % in Pakistan [18] and in India it was 88.20 ± 0.55 % [19]; 91.66 ± 1.25 % [20] and 83.98 ± 9.90 [21].

The breeding efficiency of Holstein Friesian cows was 73.12 ± 2.29 % [18] in Pakistan, 74.9 % in Sudan [22]; 87.28 % in USA [17]. In this study when F_1 crossbred cows were crossed with Friesian bull the breeding efficiency decreased in F_1 × Friesian crossbred cows (65.62 ± 3.05 %) compared to F_1 and F_2 crossbred cows. This decrease in breeding efficiency attributed to long service period and calving interval. The long service period of F_1 × Friesian cows might be due to the reason that the these cows did not resume the ovarian cycle at an early time after calving. The breeding efficiency of 50 % Friesian inheritance cows in this study was similar to that of 50 % Friesian inheritance cows (66.3 ± 0.49 %) in Ethiopia [23].

5. CONCLUSIONS

The high breeding efficiency of Indigenous × Jersey (F_1) crossbred cows compared to F_1 × Friesian crossbred cows in present study is an indicative of better adaptation of Jersey crossbred cow to climatic conditions of Muzaffarabad, Azad Jammu and Kashmir.

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Research Article

Cobalt-induced Alteration in Hematology and Reproductive Organs of Rhode Island Red Chickens (*Gallus gallus domesticus*)

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Abstract: The purpose of this study was to assess the effect of cobalt on the reproductive structure and hematology of adult male Rhode Island Red chickens *(Gallus gallus domesticus).* Sexually mature chickens were given single dose of 30 mg/kg body weight cobalt chloride intraperitoneally for 48 hours. The gain in body weight was not significantly different between control and treatment group, however the testicular weight was significantly reduced ($P<0.001$). Histopathological evaluation of the testes revealed several abnormalities including degeneration of spermatogonial cells spermatocytes, spermatids and necrosis of seminiferous tubules. Analysis showed that the diameter of seminiferous tubules was significantly decreased ($P<0.05$). Evaluation of the number of RBCs and WBCs detected significantly ($P<0.0001$) increased in experimental group. Biochemical analysis of plasma showed that there was no significant difference in glucose, cholesterol and urea concentration. Contrary to this, the biochemical analysis of reproductive organs showed significant ($P<0.0001$) decrease in glucose concentration while cholesterol and DNA level increased significantly ($P<0.0001$) and urea concentration remain unaltered. Results of this study report a negative effect of cobalt on hematology and histology of testes.

Keywords: Cobalt toxicity, Rhode Island Red chicken, reproductive organ, hematology

1. INTRODUCTION

The environmental pollution and contamination of animals including game with cobalt is a serious problem in most countries. It is an essential element, but at high concentrations is toxic [1]. By blood circulation cobalt could be delivered and subsequently accumulated in different organs like – liver, kidneys, hematopoietic organs, brain, reproductive organs [2]. Experimental treatment with cobalt exerts negative effect on male reproductive organs and fertility when applied chronically [2–5] whereas acute administration has minor effect [6]. Cobalt chloride produced hepatic and renal damage, characterized by increased activity of alanine and aspartate transaminases like GPT (glutamic-pyruvate transaminase), GOT (glutamic oxaloacetic transaminase) and alkaline phosphatase. However lactate dehydrogenase activity (LDH) was decreased. In addition, serum urea, serum creatinine, serum total protein and serum bilirubin concentrations were significantly elevated [7].

It is assumed that the erythrocytosis of miners working at altitude is partially caused by inorganic cobalt inhalation [8]. Biochemical analysis of mouse blood samples show that hemoglobin content was increased in a time-dependent manner in mature mice (day 45 to day 90), while it was reduced in immature mice (day 18 to day 30) [9]. Increased Co concentration in plasma changes plasma iron (Fe) concentration which indicates that the solubility of the compounds is an important factor for bioaccumulation in blood plasma and in the organs respectively [9].

The literature about the concentrations of

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biochemical components in plasma, organ and histomorphological changes in the gonads of male birds caused by cobalt treatment is not well studied therefore the present study was designed to investigate the information about the adverse effect of single dose of cobalt chloride on body weight, testicular weight, total count of red blood cell (RBCs), white blood cell (WBCs) and others biochemical components in Rhode Island Red chicken

2. MATERIALS AND METHODS

A total of 10 male birds at 240 days of age were used in this study. The birds were divided into two groups, and each group comprised of 5 birds. All the birds were maintained under normal day light arrangement, fed on standard poultry feed and tap water ad libitum. One group served as control while other group was given single dose of cobalt chloride (30 mg/kg b.w.) for 48 hours intraperitoneally. After 48 hours the body weight was measured and the blood samples were taken in 3 mL disposable syringes from wing vein. The blood samples were transferred into EDTA tubes and kept at 4 °C. Blood was used for hematology like RBCs and WBCs count by using hemocytometer and for biochemical analysis. After blood collection, the birds were slaughtered and reproductive organs were taken immediately. Each tissue was divided into three parts. One part was fixed in buffered formalin for one week for histological studies. The tissues were dehydrated by passing through increasing grade of alcohol (30%, 50 %, 70 %, 90 % and 100 %) for two hours in each grade. The tissues were impregnated in the mixture of xylene and paraffin wax for 2 hours at 54^0C and then in Paraffin wax for 2 hours at 54^0C. The dehydrated tissues were embedded in paraffin wax by using cavity boxes. The sections (5 µm thick) were scratched in rotary microtome and then transformed to albumenized slides. The sections were double stained with haematoxylin and eosin. Tissues were cleared in xylene and mounted with DPX.

The second part was used for the preparation of saline extract for glucose and urea estimation by O-toluidine method of Hartel et al[10] and Natelson et al [11] respectively, while the third part was used for the preparation of extract for the estimation of cholesterol and nucleic acid .The nucleic acids were extracted according to the method as described by Shakoori and Ahmed [12] while the cholesterol, RNA and DNA contents were estimated according to Zak [13], Schneider [14] and Burton [15] respectively. The morphometric study was carried out by fixation and staining of tissues. The blood cells were counted according to Natt and Herrick's method as described by Natt and Herrick [16].

2.1 Statistical Analysis

The values are presented in Mean ± SEM. Differences were compared by student t-test using computer program GraphPad Prism 6.04 version. As probability (P) value less than 0.05 was regarded as significant difference.

3. RESULTS

The mean body weight measured during the experimental period in both control and treatment group is given in Table 1. Statistical analysis showed that cobalt had no significant ($p>0.05$, $t_{(8)}=2.121$) effect in the body growth, while the testicular weight and diameter reduced significantly ($P<0.001$, $t_{(8)}=5.949$; $P<0.0001$, $t_{(8)}=7.645$) compared to control group. The results indicates that there was a significant ($P<0.05$, $t_{(46)}=2.410$) decrease in the diameter of seminiferous tubules in treatment group compared to control group (Table 2).

Table 1. Effect of cobalt on body weight gain during the 48-hours period in male Rhode Island Red chickens.

Group	Initial body weight (g)	Final body weight (g)	Increase in body weight (g)
Control (5)	1208 ± 10.20	1272 ± 7.34	64.00 ± 6.78
Treated (5)	1036 ± 10.30	1100 ± 10.49	64.00 ± 5.09

Mean ± SEM
Values in parenthesis are Number of birds

Table 2. Comparison of weight and diameter of testes and seminiferous tubules in Rhode Island Red chickens after administration of single dose of cobalt chloride.

Group	Testis		Seminiferous Tubules
	Weight (g)	Diameter (cm)	Diameter (µm)
Control	18.16 ± 0.56 (5)	7.71 ± 0.18 (5)	170.4 ± 4.54 (24)
Treated	13.72 ± 0.48*** (5)	5.09 ± 0.28**** (5)	154.3 ± 4.93* (24)

Mean ± SEM
Values in parenthesis are number of samples
*P<0.05, ***P<0.001, ****P<0.0001

Table 3. Effect of cobalt chloride on weight, length and diameter of vas deferens in Rhode Island Red chickens after 48 hours.

Group	Weight (g)	Length (cm)	Diameter (µm)
Control (5)	0.83 ± 0.022	12.16 ± 0.14	111.3 ± 0.21
Treated (5)	0.66 ± 0.036**	10.60 ± 0.48*	78.62 ± 3.75***

Mean ± SEM
Values in parenthesis are Number of samples
*P<0.05, ** P<0.01, *** P<0.001

Similarly, weight, length and diameter of vas deferens significantly (P<0.01, $t_{(8)}$=4.027; P<0.05, $t_{(8)}$=3.086; P<0.0001, $t_{(46)}$=8.690, respectively) decreased in cobalt treated group as shown in Table 3. Histological sections of the testis were examined to determine the direct effect of cobalt chloride on the structure of testis in treated group. In all sections of testis necrosis, occurrence of empty spaces in seminiferous epithelium and in interstitial tissues, congested blood vessels and degeneration of spermatogonial cells, spermatocytes and spermatids were detected. Control group did not have these histological abnormalities (Fig. 1).

The effect of cobalt chloride on concentration of different biochemical components in plasma, testis and vas deferens is given in Table 4. Statistical analysis showed that concentration of glucose, cholesterol and urea in plasma did not change significantly (p>0.05, $t_{(8)}$=0.4809;$t_{(8)}$=0.4992; $t_{(8)}$=0.5751). Whereas, the concentration of glucose in testis and vas deferens decreased (P<0.0001, $t_{(8)}$=8.918; P<0.01, $t_{(8)}$=4.977) significantly. Moreover, a significant increase in the concentration of cholesterol (P<0.0001, $t_{(8)}$=36.68; P<0.0001, $t_{(8)}$=9.891) and DNA (P<0.0001, $t_{(8)}$=13.3; P<0.0001, $t_{(8)}$=9.331) was observed in treatment group compared to control group. No significant (p>0.05, $t_{(8)}$=0.9810 p>0.05, $t_{(8)}$=0.4931) effect of cobalt on urea level was noted in testis and vas deferens. The statistical showed that the mean number of RBCs and WBCs significantly (P<0.0001; $t_{(8)}$=10.25; P<0.0001; $t_{(8)}$=33.02) increased in treatment group compared to control group (Table 5).

Table 4. Concentration of different biochemical components in plasma, testis and vas deferens after administration of single dose of cobalt chloride in Rhode Island Red chickens.

	Group (5)	Glucose	Cholesterol	DNA	Urea
Plasma (mg/mL)	Control	343.0 ± 7.29	641.0 ± 33.92	-----	83.69 ± 9.43
	Treated	332.1 ± 21.40	662.9 ± 27.82	-----	90.57 ± 7.34
Testis (mg/mL)	Control	347.4 ± 6.83	1964 ± 33.22	2053 ± 17.00	471.3± 13.06
	Treated	244.6 ± 9.28****	3456 ± 23.48****	2378 ± 17.48****	451.1 ± 15.87
Vas deferens (mg/mL)	Control	245.3 ± 7.32	3135 ± 47.40	2024 ± 9.20	270.0 ± 4.37
	Treated	201.4 ± 4.89**	3742 ± 39.01****	2145 ± 9.20****	266.8 ± 4.79

Mean ± SEM
Values in parenthesis are number of samples
** P<0.01, **** P<0.0001

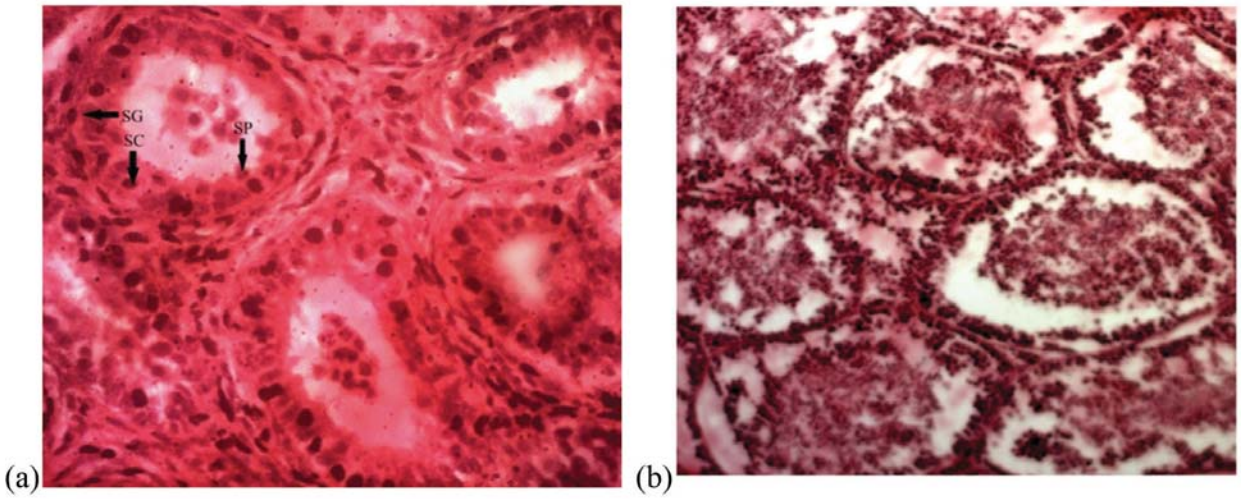

Fig. 1. Photomicrographs of Rhode Island Red chickens testis. **(a)** This high magnification of H&E stain section of a of control group showing several seminiferous tubules and population of leydig (interstitial) cells that occurs in small clusters in the space between adjoining tubules X 400. **(b)** Cobalt treated group showing necrosis of seminiferous tubules, interstitial tissues. There is a degeneration of spermatogonial cells, spermatocytes and spermatids X 400. (SG:*spermatogonium*, SC: *spermatocyte* SP: *spermatid).*

Table 5. Mean number of RBCs and WBCs in control and cobalt treated chickens after 48 hours.

Groups	RBCs ($x10^3 mm^3/dl$)	WBCs (mm^3/dl)
Control (5)	862 ± 10.67	4,660 ± 29.15
Treated (5)	978 ± 3.74****	7,740 ± 88.60****

Mean ± SEM
Values in parenthesis ( ) = Blood samples
**** P<0.0001

4. DISCUSSION

The results of present study showed that the treatment with single dose of cobalt chloride given intraperitoneally induced abnormalities in *Gallus gallus domesticus*. Induction of cobalt chloride showed statistically non significant (p>0.05) effect on the mean body weight. Diaz et al [17] reported a significant decrease in body weight of broiler chickens when cobalt chloride was given at a rate of 116, 251 and 472 mg/kgb.w in feed for 14 days. In this study the testicular weight was significantly (P<0.001) decreased. This is an agreement with the results obtained by Elbetieha et al [2] who reported that testis weight decreased when cobalt chloride was given to mice at a rate of 200, 400 and 800 ppm for 12 week. Similarly, Madzhariva et al [18] also reported that the testis weight decreased when a dose of $CoCl_2$ 75 mg/kg and 125 mg/kg body weight was given in balb/c mice for 18 days and for 25 days. The single dose of cobalt chloride significantly decreased (P<0.05) the diameter of seminiferous tubule and caused necrosis and degeneration. This indicates that cobalt chloride caused degeneration of germ and sertoli cells and shrinkage of seminiferous tubules. Similarly, Pavlova et al [19] reported that the administration of cobalt chloride 75 or 125 mg/kg via drinking water for 60 days caused the necrosis and degeneration of seminiferous tubules in mouse. Our data also supported the finding of Bitner et al [20] and Elbetieha et al [2] who reported that the changes in the structure of testis including necrosis and degeneration of seminiferous tubules. But these finding are contrary to Lukac et al [2] who reported that the single dose of cobalt chloride at a rate of 5, 10 and 20 mg/kg body weight in hamster significantly increased the diameter of seminiferous tubule while volume of the seminiferous tubules significantly decreased [20].Present study showed that cobalt treatment affected the weight, length and size of vas deferens. A significant decrease in mean weight, length and diameter of vas deferens was observed in chickens. No report quoted in the

accessible literature about the changes induced by cobalt in vas deferens. The single dose of cobalt chloride statistically did not affect the concentration of plasma glucose and cholesterol levels. According to Freeman and Langslow [21] there was no hyperglycemic effect observed when Rhode Island Red chickens were treated with $CoCl_2$ and $NiCl_2$ with single intraperitoneal injection of 10, 20, or 40 mg/kg body weight. Ohmichi et al [22] reported that cobalt chloride produces a rise in blood glucose and cholesterol in rabbits given at a rate of 25 mg/kg body weight for three days. Deshmukh [23] observed in his study that treatment with $CoCl_2$ (10 mg/kg, i.p. for 30 days) significantly decreased the plasma glucose level in diabetic rats. Cobalt administration caused significantly ($P<0.0001$) reduction in testis and vas deferens glucose level while cholesterol and DNA level was significantly ($P<0.0001$) increased. No information is available in accessible literature regarding effect of cobalt on tissue biochemistry. After administration of single dose of cobalt chloride the number of RBCs and WBCs was significantly increased ($P<0.0001$) in present study. Similar finding were also obtained by Shrivastava et al [24] who reported that there was significant increase in red blood cells and white blood cells when rats were treated with cobalt chloride at a rate of 12.5 mg/kg body weight for 7 days orally via gastric canola.

5. CONCLUSIONS

The results of this study indicate that exposure to single dose of cobalt given intraperitoneally did not affect the body weight, but significantly reduced the growth of reproductive organs. However negative impact of cobalt was observed on testis biochemistry, histology and hematology. Cobalt might be considered as possible risk factor for male reproductive health in chicken. However, further research is warranted to study the negative effect of cobalt at molecular level and also the preventive effects of anticarcinogenic compounds against the carcinogenic activity of cobalt in this valuable species.

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Research Article

Efficacy of *Fagonia cretica* in Treating Hemolytic Anemias

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Abstract: In Thalassemia repeated transfusions and chelating agents besides certain other drug trials aimed to alleviate the agony of repeated transfusions and bone marrow transplant did show some credible results in a group of patients but eventually it goes a long way to achieve some convincing results. The role of *Fagonia cretica* in treatment of Thalassemia was a chance occurrence, when a patient suffering from Thalassemia Major, who was receiving weekly transfusions and chelation therapy, got seriously ill. She had huge spleen, fairly enlarged liver and ascites. Out of curiosity this herbal preparation was given to the dying patient, who had otherwise no chance of survival. Surprisingly, she showed miraculous results and was no longer transfusion dependent after about six months and after nine months her reports for Thalassemia were negative. It was expedient to have drug trial of this herbal plant that could possibly help patients suffering hemolytic anemia like Thalassemia. Our earlier trials of *Fagonia cretica* had shown convincing results in a number of patients suffering from Thalssemia; it not only reduced the transfusion needs but also improved the quality of life in a large number of patients. For this study, a total of 180 Thalssemia suffering patients, mean age 7 years (1 to 17 years), were selected randomly. Of the 180, 100 patents were allocated to the treatment group and 80 to the control group, who were put on placebo. Among the treatment group, 25 patients were suffering from Thalassemia Intermedia, 10 from Sickle cell and the rest 65 from Thalassemia Major. Of all these patients, only 19 Thalassemia Intermedia patients, six Sickle cell patients, 54 Thalassemia Major category patients and 50 control group patients completed the study. The patients belonging to Thalassemia Major treatment category were further divided into three sub-groups according to their response to the treatment of *Fagonia cretica*. The patients in the treatment group were given 120 mg/kg body weight *Fagonia cretica* over a period of 10 to 18 months. The patients of the control group were put on placebo. The primary and the clinical end points were: reduced transfusion needs with decrease in the HbF, HbA2 and HbS in Sickle Cell Thalassemia and increase in the total Hb and HbA. The secondary end points included the improvement of all the clinico-pathological events resulting from anemia, repeated transfusions, iron overload, i.e., frontal bossing, maxillary hyperplasia, swelling of joints with arthralgia and myalgia, frequent chest infections and fever, sleep disturbances, shortness of breath, bleeding of gums and epistaxis, etc. Data were analyzed by the intention to treat. The total hemoglobin and size of the spleen and liver palpable below costal margins in cm were recorded at the time of admission to the study and then at the end of study, whereas the HbA, HbA2, HbF and HbS were recorded when the patients were first diagnosed and finally at the end of study. We observed that there was significant ($P \leq 0.05$) increase in the total hemoglobin and HbA and decrease in the HbF, HbA2, HbS and also reduction in the sizes of spleen and liver ($P \leq 0.05$) in all categories of patients. The results of this study clearly defined two groups of patients according to their response to the treatment: The patients of Group-1 initially had HbA on electrophoresis present (it included all the 25 patients belonging to Thalassemia Intermedia, six patients belonging to Sickle Cell Thalassemia and nine patients of Thalassemia Major. The Thalassemia Major patients in this group presented with all the features of iron overload with enlarged liver and spleen, frontal bossing, maxillary hyperplasia, etc. This group of patients never needed transfusion after they started *Fagonia cretica* Treatment. The patients in Group-2 included 45 Thalassemia Major patients, who did not have HbA on electrophoresis at the initial stage of diagnosis. They were further divided into two categories: 33 patients belonging to Category-A showed evidence of iron overload with enlarged liver and spleen, frontal bossing and maxillary hyperplasia, etc. There was significant reduction in the transfusion needs in this group of patients. The 12 patients of Category-B were suffering with Thalassemia major. These patients looked quite normal. They had no evidence of iron overload. There was no enlargement of liver and spleen or other bony abnormalities. This group of patients did not show significant response to the treatment by *Fagonia cretica*. *Fagonia cretica* was given in doses of 120 mg/kg body wt to all these patients.

Keywords: *Fagonia cretica,* Thalassemia, sickle cell Thalassemia, Quality of life.

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1. INTRODUCTION

Hemolytic anemias present a real challenge in the treatment. These anemias are usually caused, when the lifespan of the red cells is shortened either due to genetically determined defects involving the structure or metabolism of the membrane, hemoglobin disorders or enzyme deficiencies involving the main metabolic pathways or it may be acquired as immune (iso- or auto) or non-immune like trauma, membrane defects, drugs and chemicals, bacterial or parasitic infections and due to hyper-spleenism. This results in reduced circulating red cell mass, which leads to relative tissue hypoxia that eventuates in many clinical manifestations [1, 2].

We have already reported [3] and in this study we further elucidate the role of dried aerial parts *of Fagonia cretica*-induced efficacy in different hemolytic anemias particularly Thalassemia Syndrome and Sickle Cell Thalassemia. Local medical practitioners use *Fagonia cretica* for treating a wide variety of ailments, including different malignant conditions [4]. This substance is well tolerated and does not exhibit adverse effects like vomiting, diarrhea or alopecia, which are common side effects of standard cytotoxic therapy [4]. To the authors' best knowledge this is the second of the series of our study that elucidates the genetic mutation activity in thalassemia. Herein, we have shown that dried aerial parts of *Fagonia cretica* is able to induce genetic mutation and increase the percentage of HbA, while reducing the HbF, HbA2 and HbS, besides exerting a substantial effect on primary Erythropoiesis. The sickle-cell anemia is another common hemolytic disorder often associated with Thalssemia [1, 2]. The clinical features of a sickling disorder are found in association with a peripheral blood picture with typical β^o-thalassemia red-cell changes, i.e., a low MCH and MCV. In the more secure forms of sickle-cell thalassemia there may be an elevated reticulocyte count, and sickled red cells are found on the peripheral blood film. The diagnosis can be confirmed by hemoglobin electrophoresis, which in sickle-cell β^+-thalassemia shows hemoglobin S together with 10 to 30 % hemoglobin A and an elevated hemoglobin A_2 value [1, 2]. In sickle-cell B-thalassemia the hemoglobin consists mainly of hemoglobin S with an elevated level of hemoglobin F and A_2 to be absolutely certain about the diagnosis it is necessary to examine the parents; one should have the sickle-cell trait and the other the β -thalassemia trait [5, 6].

During the last couple of years it has become clear that Thalassemia is extremely heterogeneous and that its clinical picture can result from the interaction of many different genetic defects , which result from a reduced rate of production of one or more of the globins chain (s) of hemoglobin [7-17]. Because Thalassemia occurs in populations in which structural hemoglobin carriers are common, it is not at all unusual for an individual to receive a Thalassemia gene from one parent and a gene for a structural hemoglobin variant from the other. These different interactions produce an extremely complex and clinically diverse series of genetic disorders, which range in severity from death in utero to extremely mild, symptomless, hypochronmic anemia and perhaps quite divide response to the treatment in different patients [18-22].

Convincing results of the herbal plant treatment were obtained, when the whole flowering plant was collected from mid February to mid May, besides providing congenial atmosphere while drying the plant under shade. Its aqueous extract gives satisfactory results, but is not palatable because of its bitter taste.

The roles of *Fagonia cretica* in different malignant conditions have already been described [3, 4, 20, 21] but our observations in hemolytic anemias present a new epoch of research in Thalassemia and the like conditions. There are quite a few chemical ingredients in *Fagonia cretica* , like Saponin-1 and Saponin-2, besides it contains beta-sitosterol; ceryl-alcohol; chivonic acid; water soluble Saponin, i.e., glucose rhamose; xylose; arabinose; fagogenine and lipids 0.3-1.14%: Campesterol; aglycone; fagonine; oleonolic acid; betulic acid the later four are derived from

Saponin fraction (18-20). However, it is not clear exactly which of the particular ingredient alone or in combination is effective against different clinical conditions [20-22].

2. MATERIAL AND METHODS

A total of 180 patients were picked randomly from the Dr A.Q. Khan Thalassemia Research Center, Multan, Pakistan. Among them, 100 patents were allocated to the treatment group and 80 to the control group, who were put on placebo. Clinical evaluation and classification of the patients in different categories was arranged according to Kazazian [11]. In all the patients, the detection of HbA, F A2 and S was done on high performance liquid chromatography (HPLC).

Among the treatment group, 25 patients were suffering from Thalassemia Intermedia, 10 patents from Sickle cell and the rest 65 belonged to Thalassemia Major Category; but only 19 in Thalassemia Intermedia, 6 in Sickle cell, 54 in Thalassemia Major Category, and 50 in control group completed the study. The patients belonging to Thalassemia Major Treatment category were divided into three groups according to their response to the treatment of *Fagonia cretica*. The patients picked for the study belonged to the following three categories: (i) Thalassemia Major; (ii) Thalassemia Intermedia; and (iii) Sickle Cell Thalassemia.

Category I. The *Thalassemia Major* patients were further divided into three groups according to their response to the treatment particularly the transfusion needs.

Group-1. Included nine patients, who never needed transfusion after they started the *Fagonia cretica* treatment.

Group-2. Included 33 patients, who showed significant decrease in the transfusion needs.

Group-3. Included 12 patients, whose transfusion needs did not quite changed.

Group-1: The data collected for the computation of the results: these patients were first diagnosed, and after 10 to 18 months of *Fagonia cretica* treatment, during which no blood was given to these patients.

Group-2: The data collected for the computation of the results: Patients first diagnosed and after 10 to 18 months of *Fagonia cretica* treatment, during which blood transfusions needs were significantly reduced.

Group-3: The data collected for the computation of the results: Patients first diagnosed and after 10 to 18 months of *Fagonia cretica* treatment, during which blood transfusions needs did not change significantly. The blood sampling in this group of patients was delayed as much as possible to avoid possible overlay of the donor's blood. This period was six to eight weeks after the last transfusion.

The patients belonging to Thalassemia Intermedia and Sickle Cell Thalassemia never needed transfusion after they started using *Fagonia cretica*.

The patients belonging to Group-1, 2 and 3 of Thalassemia Major were studied for the HbF, HbA, HbA2, (recorded when first diagnosed), total hemoglobin, size of the spleen and liver (at the time of admission to the study), and similar observations were made in all the patients belonging to Intermedia and in Sickle Cell Thalassemia. We also accounted for HbS at the end of study, i.e., 10 to 18 months after the treatment had started.

The paired t-test was used for the comparison of means to see the effect of the treatment of *Fagonia cretica* on various parameters of Thalassemia.

3. RESULTS

3.1. Thalassemia Major: Group-1

Clinical investigations of nine patients of Thalassemia major **Group-1** included total hemoglobin, the size of spleen and liver palpable below costal margin measured at the start of the study and again at the end of study (Table 1), whereas the values for HbA, HbF and HbA2 were recorded, when the patients were first diagnosed. These patients were given herbal medicine for 7 to 18 months and it was observed that they were no

longer in need of any blood transfusion.

The mean values of different parameters are listed in Table 2. We applied the paired t test to compare various factors of blood tests and sizes of the liver/spleen. The total Hb significantly improved from 1.79 to 4.28 g/dL (P≤0.01). The mean hemoglobin content in patients before treatment was 6.59 g/dL whereas after a period of 7-18 months treatment of the *Fagonia cretica* (no need of blood transfusion) it rose to 9.62 g/dL. We also observed that average Hb A before the treatment was 5.18% and 29.44% after treatment and the minimum improvement is above 13% (P≤0.01), whereas the reduction in HbF was from 8.69% to 52.51% (P≤.0.01). The HbA2 also reduced significantly (P≤0.01). The value before treatment was 2.85 and reduced to 2.19 after the treatment. The mean values for spleen before and after treatment were 5.25 cm and 0.50 cm and the reduction to size of spleen is 2.07 cm to 7.42 cm (P≤0.05) which is quite significant. The liver size reduced significantly in the range of 0.40 cm to 4.60 cm (P≤0.05). The mean values of the size of the liver before and after the start of treatment were 2.50 cm and 0.00 cm, respectively.

3.2. Thalassemia Major: Group-2

In this group there were 33 patients. The Interval between the two transfusions increased from 10±3 days to 60±10 days (Electrophoresis after treatment was done just before the subsequent transfusion after about 50-70 days).

Their total hemoglobin, the size of spleen and

Table 1. Group-1 Patients of Thalassemia Major: No transfusion was needed after *Fagonia cretica* treatment was started; follow up period varied from 10 to 18 months.

Age (years)	Sex	Hb (g/dL)		% Hb A		% Hb A_2		% Hb F		Palpable below Costal Margin (cm)			
										Liver		Spleen	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
8	M	5.5	6.6	15.0	22.3	2	1.5	83.0	76.2	3	0	**6**	2
14	M	5.5	6.8	12.7	18.2	1.7	1.0	85.6	79.8	6	0	**12**	2
7.5	M	6.5	10.9	0	17.4	3.3	2.1	96.7	80.5	0	0	**2**	0
3.0	M	6.9	09.1	3.7	23.8	3.1	2.6	93.2	73.6	3	0	**6**	0
3 ¼	M	7.2	10.4	0	32.0	3.6	3.2	96.4	64.8	6	0	**8**	0
3 ¼	M	8.2	14.1	4.2	28.6	3.2	3.0	92.6	69.4	0	0	**-0**	0
2	F	4.5	9.1	0	26.0	4.2	2.8	95.8	71.2	2	0	**4**	0
17	M	7.8	10.1	11.0	62.7	2.0	1.1	87.0	36.2	0	0	**4**	0
14	F	7.2	9.5	-	34	2.6	2.4	97.4	-	0	0	**0**	0

Table 2. Mean and standard deviation of the selected parameters at the time of first diagnosis (for HbA, HbA2, HbF) and at the time of entry into the study (for Total Hb, size of the liver and spleen) and at end of the study for all parameters: There was no need of transfusion in this group of patients soon after starting the *Fagonia cretica* treatment.

Parameter	Mean		95% Confidence Interval	
	Before	After		
Hb (g/dL)	6.59	9.62	1.79-4.28	Improved significantly
HbA2 (%)	2.85	2.19	0.34-0.99	Reduced significantly
HbA (%)	5.18	29.44	13.35-35.19	Improved significantly
HbF (%)	91.9	61.30	8.69-52.51	Reduced significantly
Spleen, cm below coastal margin	5.25	0.50	2.07-7.42	Reduced significantly
Liver, cm below coastal margin	2.50	0.00	0.40-4.60	Reduced significantly

liver below costal margin were measured at the start of the study and again at the end of study (Table 3), whereas the initial reports for HbF, HbA2, HbA were recorded, when the patients were first diagnosed and again at the end of study.

The mean values of different parameters are listed in Table 4. The Hb significantly improved from 1.45 g/dL to 2.22 g/dL (P≤0.05). The mean values for hemoglobin before and after the treatment were 7.26 g/dL and 9.10 g/dL respectively. The HbA also significantly improved from 33% to 41% (P≤0.05). The respective values for HbA were 0.00% and 36.92% before and after the treatment. Whereas the mean values for HbF before and after treatment were 95.99% and 59.76%, respectively. The HbF was reduced from 32.36% to 40% (P≤0.05). The average values for HbA2 were 4.01% and 3.38% before and after treatment, respectively, and the reduction was from 0.32 to 0.94 (P≤0.05). The liver size reduced significantly in the range of 1.04 cm to 2.66 cm (P≤0.05). The mean values of the size of the liver before and after the start of treatment were

Table 3. Group-2 Patients of Thalassemia Major: The transfusion needs were significantly reduced (Electrophoresis after treatment done just before the next transfusion, i.e., 60±10 days after the last transfusion).

Age (Years)	Sex	Hb (g/dL)		% Hb A			% Hb A_2			% Hb F			Liver/Spleen palpable below costal margin (cm)	
		Before	After	Before		After	Before		After	Before		After	Before	After
				i	ii		i	ii		i	ii			
6	M	7.6	9.4	-		42	3.5		3.2	96.5		54.8	6—8	2--2
5	F	6.8	8.8	-		53	3.4		3.3	96.6		43.7	3—7	0---2
7	F	5.6	8.6	-		54	3.4		3.1	96.6		42.9	4—11	2--4
3	M	8.2	9.8	-		63	3.2		3.2	96.8		34.8	6—10	2--3
6	M	6.8	9.8	-		45	4.8		3.2	95.2		51.8	5—8	2--2
5	M	8.2	10.2	-		34	3.8		3.2	96.2		62.8	5—8	2--3
5	M	6.8	9.3	-		47	3.4		3.4	96.6		49.6	4—11	2--5
6	F	7.6	8.4	-		52	3.3		3.3	96.7		44.7	2—9	0--3
6	F	8.4	8.8	-		34	3.5		3.4	96.5		62.6	6—9	2--4
3	M	9.2	9.4	-		41	3.1		3.1	96.9		55.9	5—8	2--3
7	F	7.5	8.9	-		42	3.2		3.3	96.8		54.7	6—9	2--3
3	M	9.2	9.8	-		36	3.5		3.2	96.5		60.8	4—9	0--2
5	F	8.2	10.2	-		38	3.4		3.2	96.6		58.8	4—9	2--3
4	M	9	10.2	-		38.6	3.4		3.2	96.6		58.2	5---11	2---4
6	F	8.4	8.8	-		37	3.6		3.3	96.4		58.7	4—8	2--2
11	M	10.2	11.2	-		41	3.4		3.5	96.6		55.5	6---9	2---4
12	F	8.5	9.8	-		36	3.4		3.3	96.6		59.7	6--11	0--3
3	M	9.5	9.8	-		41	3.6		3.2	96.4		55.8	0----0	0---0
7	F	7.8	10.8	-		36	3.2		3.1	96.8		60.9	6—14	2--6
5	M	4.6	7.4	-		28	3.8		3.2	96.2		68.8	4---6	0--2
4	F	5.8	8.5	-		34	5.7		3.2	94.3		63.8	0—5	0--0
4	M	6.8	10.8	-		41	4.6		3.5	95.4		55.5	0—4	0--0
4	M	7.4	9.8	-		45	3.4		3.2	96.6		51.8	0—6	0--2
2	F	3.6	7.4	-		36	3.2		3.2	96.8		60.8	0--0	0--0
1	M	4.6	7.7	-		28	4.6		3.4	95.4		68.6	0—0	0--0
2	M	7.8	7.8	-		22	6.8		3.6	93.2		74.4	2—2	4--2
5	M	5.8	7.5	-		22	3.6		3.4	96.4		74.6	4—2	6--2
3	M	6.8	9.7	-		36	3.8		3.2	96.2		60.8	5—2	6---2
4	F	6.5	8.6	-		26	3.8		3.2	96.2		70.8	4--2	8--3
2	M	4.5	6.8	-		22	6.6		4.3	93.4		73.7	0—0	0--0
1	M	5.8	7.5	-		16	8.3		5.6	91.7		78.4	0—0	0--0
6	F	9.4	9.6	-		28	4.4		3.2	95.6		68.8	4—8	0--2
4	M	6.8	9.2	-		24	3.6		3.5	96.4		74.5	3—5	0---2

Table 4. Mean and standard deviation of the selected parameters at the time of first diagnosis (for HbA, HbA2, HbF) and at the time of entry into the study (for Total Hb, size of the liver and spleen) and at end of the study for all the parameters for the patients, where transfusion need was reduced significantly.

Parameter	Mean		95% Confidence Interval	
	Before	After		
Hb (g/dL)	7.26	9.10	1.45-2.22	Improved significantly
HbA2 (%)	4.01	3.38	0.32-0.94	Significantly Reduced
HbA (%)	0.00	36.93	33.23-40.62	Improved significantly
HbF (%)	95.99	59.76	32.36-40.11	Reduced significantly
Liver, cm below costal margin	3.42	1.58	1.04-2.66	Reduced significantly
Spleen, cm below costal margin	6.00	2.15	2.79-4.91	Reduced significantly

Table 5. Group-3 Patients in Thalassemia Major: Various parameters selected were the same as in the previous studies. This Group of patient did not respond to the treatment, i.e., transfusion needs remained almost the same. (Electrophoresis after the treatment done just before the next transfusion,i.e., 40±6 days after the last transfusion).

Age (Years)	Sex	Hb (g/dL)		% Hb A			% Hb A_2			% Hb F			Liver/Spleen palpable below costal margin (cm)	
		Before	After	Before		After	Before		After	Before		After	Before	After
				i	ii		i	ii		i	ii			
15	F	6.5	7.8	-	32	64	3.5		3.2	96.5		32.8	-/2	-/-
11	M	7.2	7.9	-	28	54	3.6		3.2	96.4		42.8	2/3	-/-
15	M	4.6	5.8	-	34	62	3.2		3.2	96.8		34.8	-/-	-/-
16	F	5.8	7.6	-	28	42	3.8		3.1	96.2		54.9	-/2	-/-
14	M	6.5	7.8	-	28	34	3.2		3.2	96.8		62.8	-/-	-/-
17	M	7.2	8.1	-	29	28	3.6		3.1	96.4		68.9	-/-	-/-
15	M	5.4	7.6	-	32	35	3.3		3.1	96.7		61.9	2/4	-/-
13	M	6.4	8.2	-	35	42	3.5		3.2	96.5		54.8	3/4	-/-
8	M	5.8	7.2	-	28	36	5.6		3.2	94.5		60.8	4/5	2/2
15	M	6.3	7.4	-	25	42	3.2		3.1	96.8		54.9	-/-	-/-
16	M	7.8	8.2	-	32	32	3.5		3.2	96.5		64.8	-/-	-/-
13	M	5.8	6.9	-	35	42	4.8		3.2	95.2		54.8	-/-	-/-

3.42 and 1.58 cm, respectively. The mean values for spleen before and after treatment were 6.00 and 2.15 cm and the reduction to size of spleen was 2.79 cm to 4.91 cm ($P \leq 0.05$) which was quite significant.

3.3. Thalassemia Major: Group-3

There were 12 patients in Group-3. In these Patients, no significant difference was observed in the interval between the two transfusions, even after eighteen months of treatment (Electrophoresis after treatment done just before the next transfusion, i.e., 40±6 days after the last transfusion)

Their total hemoglobin, the size of spleen and liver below costal margin were measured at the start of the study and again at the end of study (Table 5); whereas HbF, HbA2, HbA, HBS were also recorded, when the first diagnosis was made and then at the end of the study.

The mean values of different parameters are listed in Table 6. The Hb significantly improved from 0.95 g/dL to 1.58 g/dL ($P \leq .05$). The mean values for hemoglobin before and after the treatment were 6.27 g/dL and 7.54 g/dL, respectively. The HbA also significantly improved from 35% to 50% ($P \leq 0.05$).

Table 6. Mean and standard deviation of the selected parameters at the time of first diagnosis (for HbA, HbA2, HbF) and at the time of entry into the study (for Total Hb, size of the liver and spleen) and at end of the study for all the parameters for the patients not responding to the treatment.

Parameter	Mean		95% Confidence Interval	
	Before	After		
Hb (g/dL)	6.27	7.54	0.95-1.58	Improved significantly
HbA2 (%)	3.73	3.17	0.11-1.02	Reduced significantly
HbA (%)	0.00	42.75	35.41-50.09	Improved significantly
HbF (%)	96.28	54.08	34.75-49.63	Reduced significantly
Liver, cm below costal margin	0.92	0.17	0.03-1.47	Reduced significantly
Spleen, cm below costal margin	1.67	0.17	0.43-2.57	Reduced significantly

Table 7. Thalassemia Intermedia (no transfusion was required after the *Fagonia cretica* treatment was started. Follow up period varied from 10 to 18 months).

Age (Years)	Sex	Hb (g/dL)		% Hb A		% Hb A_2		% Hb F		Spleen palpable below costal margin (cm)	
		Before	After	Before	After	Before	After	Before	After	Before	After
14	M	9.4	12.5	34.0	43	6.5	3.6	59.5	53.4	6	2
19	F	8.7	12.8	44.0	58	5.8	3.4	59.2	38.6	4	-
18	F	8.8	13.2	41.0	64	3.4	3.2	55.6	32.8	5	2
16	M	7.8	11.8	38.0	78	3.2	3.1	58.8	18.9	-	-
21	F	8.2	12.4	28.0	67	3.4	3.3	68.6	29.7	5	2
14	M	9.2	13.4	31.0	66	5.4	3.2	63.6	30.8	5	-
16	M	10.4	14.2	44.0	67	4.3	3.1	51.7	29.9	4	1
24	M	11	13.0	34.0	45	3.6	3.3	62.4	51.7	-	-
16	F	7.8	11.4	32.0	54	3.2	3.2	64.8	42.8	3	-
14	M	8.4	11.6	41.0	68	3.2	3.1	55.8	28.9	5	2
12	M	9.5	12.6	38.6	64	3.8	3.1	57.6	32.9	4	1
14	F	11	12.8	36.4	71	6.2	3.1	57.4	25.9	3	2
23	F	7.9	11.8	42.5	82	4.5	3.2	53.0	18.8	5	-
22	F	8.4	13.2	28.5	78.2	3.6	3.1	67.9	18.6	4	2
16	M	9.4	12.8	32.8	82.5	3.6	3.4	63.6	14.1	6	2
15	F	8.8	10.8	41.2	78.4	3.8	3.2	55.0	18.4	-	-
11	F	9.2	12.6	38.4	78.8	3.5	2.8	58.1	18.4	4	2
14	M	11	12.6	42.0	81.4	3.2	3.2	54.8	15.4	2	-
11	F	8.0	11.0	26.0							

The respective these values for HbA were 0.00% and 42.75% before and after the treatment. Whereas the mean values for HbF before and after treatment were 96.28% and 54.08%, respectively. The HbF was reduced from 34.75% to 49.63% ($P \leq 0.05$). The average values for HbA2 were 3.73% and 3.17% before and after treatment, respectively and the reduction was from 0.11% to 1.02% ($P \leq 0.05$). The size of the liver reduced in the range of 0.03 cm to 1.47 cm ($P \leq 0.05$). The mean values of the size of liver before and after the start of medicine are 0.92 cm and 0.17 cm, respectively. The mean values for spleen before and after treatment were 1.67 cm and 0.17 cm and the reduction to size of spleen was 0.43 cm to 2.57 cm ($P \leq 0.05$) which was quite a significant reduction.

3.4. Thalassemia Intermedia

Thalassemia Intermedia is invariably recognized by infrequent blood transfusions with usually enlarged spleen.

Table 8. Mean and standard deviation of the selected parameters at the time of first diagnosis (for HbA, HbA2, HbF) and at the time of entry into the study (for Total Hb, size of the liver and spleen) and at end of the study, for the Intermedia Patients.

Parameter	Intermediate Mean		95% Confidence Interval	
	Before	After		
Hb (g/dL)	9.16	12.53	2.89-3.84	Improved significantly
HbA2 (%)	4.12	3.20	0.40-1.44	Reduced significantly
HbA (%)	37.08	68.13	24.94-37.17	Improved significantly
HbF (%)	59.30	28.89	24.46-36.36	Reduced significantly
Spleen, cm below costal margin	3.61	1.00	1.83-3.40	Reduced significantly

Table 9. Sickle Cell Thalassemia: No transfusion was required after the *Fagonia cretica* Treatment was started.) Follow up period varied from 7 to 18 months.

Age (Years)	Sex	Hb (g/dL)		% Hb A		% Hb A_2		% Hb F		Hb S		Spleen palpable below costal (cm)	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
11	M	7.8	8.8	-	24	3.6	3.3	34	21	62.4	51.7	6	4
27	M	5.6	9.4	-	32	3.4	3.4	28	8	68.6	57.6	9	5
22	F	8.2	11.6	-	28	3.4	3.3	38	17	58.6	51.7	7	5
15	M	7.2	13.1	12	38	3.5	3.4	42	21	57.5	37.6	3	2
16	M	9.2	12.8	22	52	3.6	3.4	24	12	50.4	32.6	9	2
17	M	8.7	12.3	12	45	3.7	3.2	35	18	49.3	33.8	6	-

The response to the treatment with *Fagonia cretica* in Thalassemia Intermedia patient in terms of their total hemoglobin, and the size of spleen palpable below costal margin were measured at the start of the study and again at the end of study (Table 7), whereas the values for HbF, HbA2, HbA, were had initially from the data, when first diagnosed and then at the end of study**.** No transfusion was needed after the treatment of *Fagonia cretica* started. This follow up period varied from 10 to 18 months.

The mean values of different parameters are given in Table 8. The Hb significantly improved from 2.89 g/dL to 3.84 g/dL ($P \leq 0.05$). The mean values for hemoglobin before and after the treatment were 9.16 g/dL and 12.53 g/dL, respectively. The HbA also significantly improved from 25% to 37% ($P \leq 0.05$). The respective these values for HbA were 37.08% and 68.13% before and after the treatment. Whereas the mean values for HbF before and after treatment were 59.30% and 28.89% respectively. The HbF was reduced from 24.46% to 36.36% ($P \leq 0.05$). The average values for HbA2 were 4.12% and 3.20% before and after treatment, respectively, and the reduction was from 0.40% to 1.44% ($P<0.05$). The mean values for spleen before and after treatment were 3.61 cm and 1.00 cm and the reduction to size of spleen is 1.83 cm to 3.40 cm ($P \leq 0.05$) which was quite significant.

3.5. Sickle Cell Thalassemia

The total hemoglobin, and the size of spleen below costal margin were measured at the start of the study and again at the end of study, whereas the values for HbF, HbS, HbA2, HbA, were had initially from the data, when first diagnosed and then at the end of study (Table 9). The HB significantly improved from 1.92 g/dL to 5.18 g/dL ($P \leq .0.05$). The mean values of different parameters are listed in Table 10. The mean values for hemoglobin before and after the treatment were 7.78 g/dL and 11.33 g/dL respectively. The HbA also significantly improved from 25% to 33% ($P \leq 0.05$). The respective these values for HbA were 7.66% and 36.50% before and after the treatment. Whereas the mean values for HbF before and after treatment were 33.50 and 16.16,

Table 10. Mean and standard deviation of different parameters, in Sickle Cell Thalassemia, selected at the time of first diagnosis (for HbA, HbA2, HbF) and at the time of entry into the study (for Total Hb, size of the liver and spleen) and at end of the study.

Parameter	Mean		95% Confidence Interval	
	Before	After		
Hb (g/dL)	7.78	11.33	1.92-5.18	Improved significantly
HbS (%)	57.80	44.17	8.47-18.80	Reduced significantly
HbA2 (%)	3.53	3.33	0.01-0.39	Reduced significantly
HbA (%)	7.66	36.50	25.17-32.49	Improved significantly
HbF (%)	33.50	16.16	13.10-21.57	Reduced significantly
Spleen, cm below costal margin	6.66	3.00	1.12-6.20	Reduced significantly

respectively. The HbF was reduced from 13.10 to 21.57% (P≤0.05). The average values for HbA2 were 3.53% and 3.33% before and after treatment, respectively and the reduction was from 0.01% to 0.39% (P≤0.05). The HbS significantly, reduced in the range of 8.47% to 18.80% (P≤0.05). The mean values of HBS before and after the start of medicine were 57.80% and 44.17%, respectively. The mean values for spleen before and after treatment were 6.66 cm and 3.00 cm and the reduction to size of spleen is 1.12 cm to 6.20 cm (P≤0.05) which was quite a significant reduction in the size of spleen.

Response to the treatment in all these patients for Total Hemoglobin; Hb A; Hb A; Hb F is given in Fig. 1, 2, 3, 4. Where the response to the treatment in Sickle Cell thalassemia for Total Hb; Hb S; Hb A2 ; Hb A and Hb F are given in Fig. 5.

The usual clinical symptoms observed in Thalssemia Major, Intermedia and Sickle Cell Thalassemia patients:

- Arthralgia with and without swelling of joints (Fig. 6)
- Easy fatigability, shortness of breath (Fig. 7)
- Fever and chest infection (Fig. 8)
- Sleep disturbances and frequent headaches (Fig. 9)
- Bleeding gums or epistaxis (Fig. 10)
- Maxillary Hyperplasia and Frontal bossing (Fig. 11)

These symptoms alleviated in majority of the patients, who received *Fagonia cretica* medication and they were behaving like normal subjects, except for the need of transfusion at usual frequent intervals in Group-3 of Thalassemia Major patients (Table 11).

The most important observation made in the

Table 11. Symptoms recorded in three treatment groups and the control group on day zero and after 10 to 18 months (Test & Control) treatment.

Symptoms	Total Patients	Test Group										Control (50)	
		1 (9)		2 (33)		3 (12)		Intermedia		SC Th*:			
		B	A	B	A	B	A	B	A	B	A	Before	After
Arthralgia / swelling of joints	45	8	1	27	1	10	1	4	0	2	0	24	31
Fatigability; Shortness of breath	44	9	1	23	1	12	3	3	0	2	0	34	40
Fever and chest infection	35	5	0	21	1	9	1	1	0	1	0	41	46
Sleeplessness	32	6	2	16	1	10	2	0	0	0	0	25	31
Bleeding Gums	32	5	0	19	2	8	1	0	0	0	0	36	46
Maxillary Hyperplasia/ Frontal bossing	24	4	0	13	1	-	-	0	0	0	0	32	37

B: Before Treatment; A: After Treatment; *SC Th: Sickle Cell Thalassemia

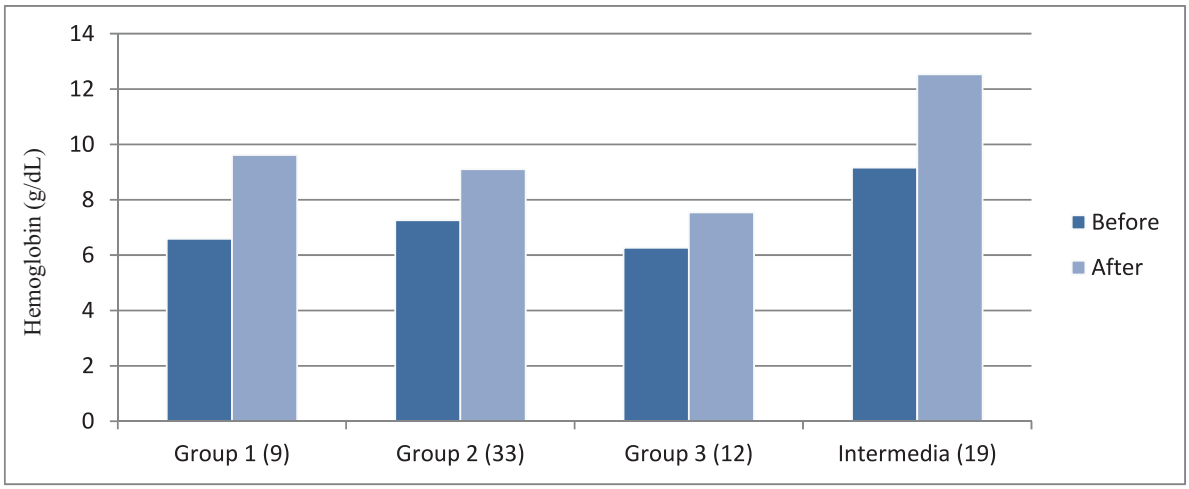

Fig. 1. Total hemoglobin response to *Fagonia cretica* treatment in different groups of patients Number of patients for each group given in brackets

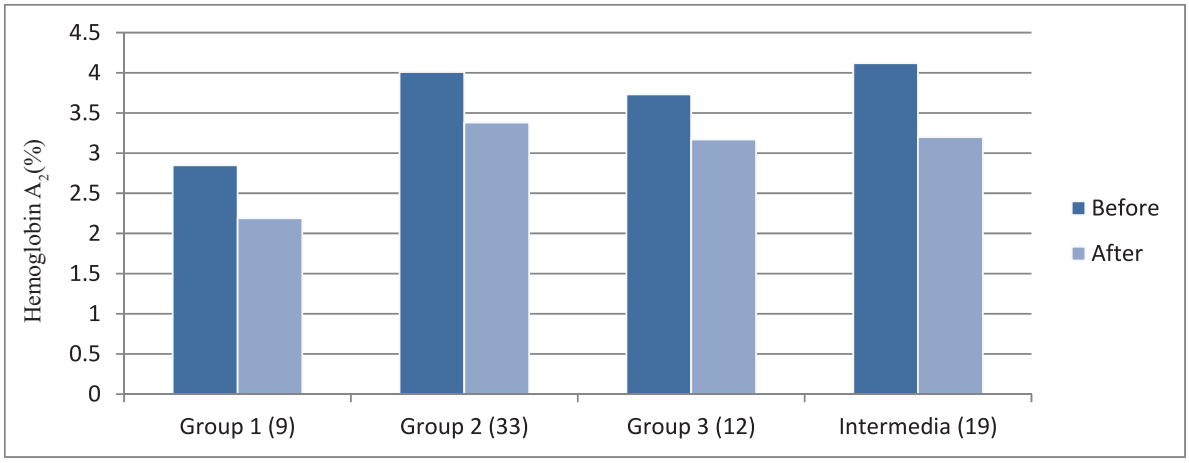

Fig. 2. HbA2 in response to *Fagonia cretica* treatment in different groups of patients. The number of patients for each group is given in brackets.

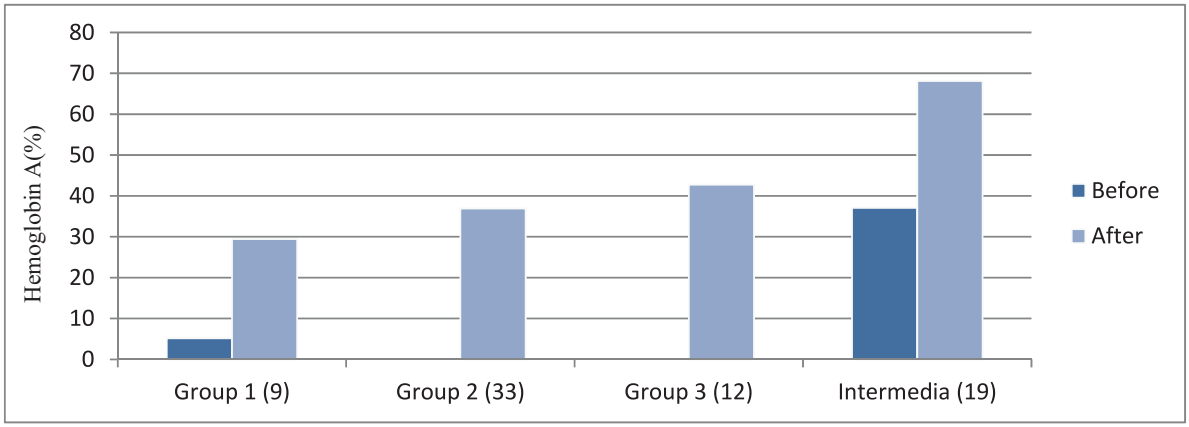

Fig. 3. HbA in response to *Fagonia cretica* treatment in different groups of patients. The number of patients for each group given in brackets.

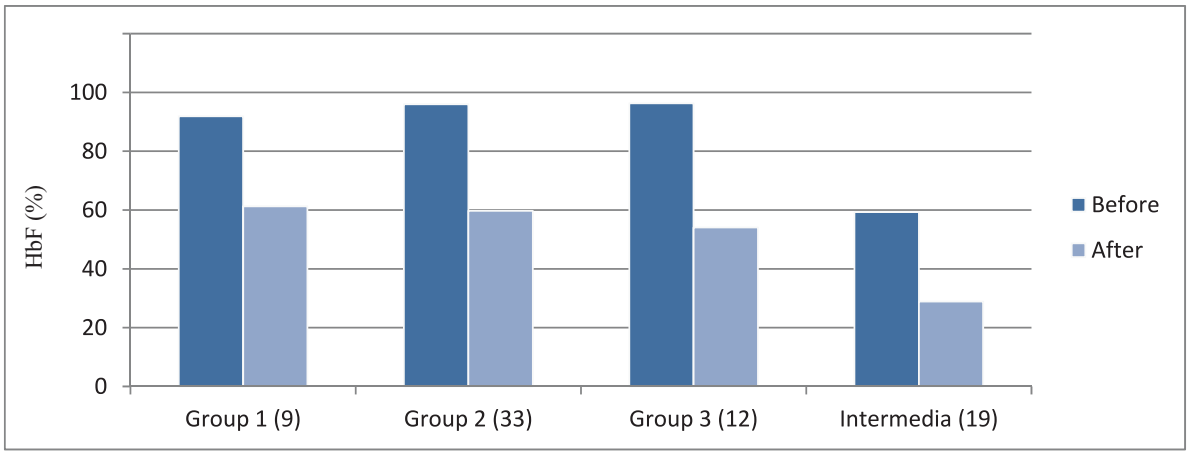

Fig. 4. HbF in response to *Fagonia cretica* treatment in different groups of patients. The number of patients for each group given in brackets.

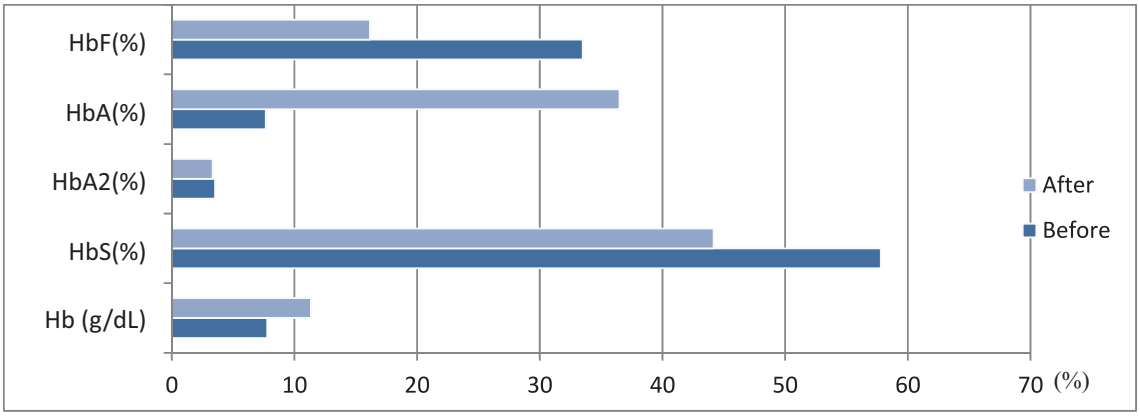

Fig. 5. Sickle Cell Thalassemia: Response of different parameters: Total Hb, HbS, HbA2, HbA, HbF to *Fagonia cretica* treatment: There was no need of Transfusion soon after the treatment in this group.

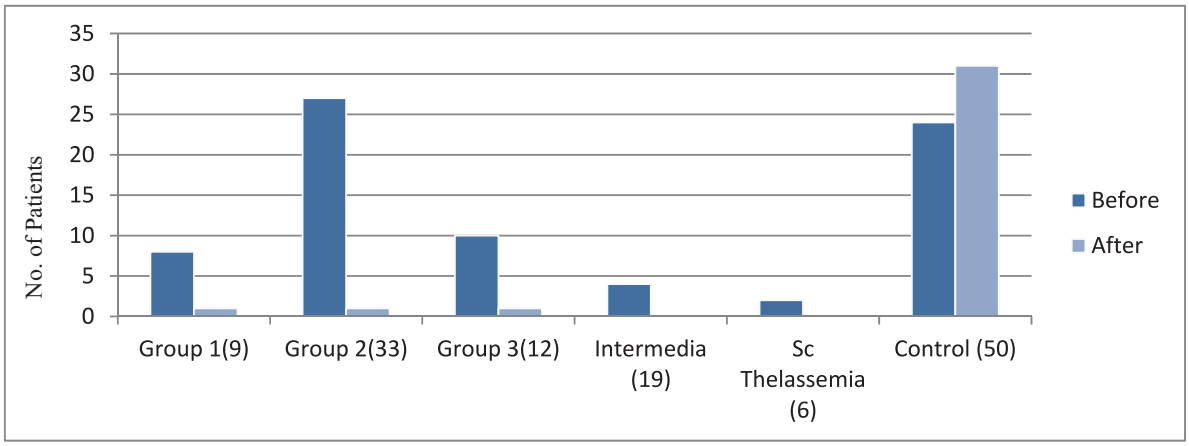

Fig. 6. Response to the Treatment for Arthralgia with swelling of joints in different Groups of Patients. Total number of patients in each group given in brackets.

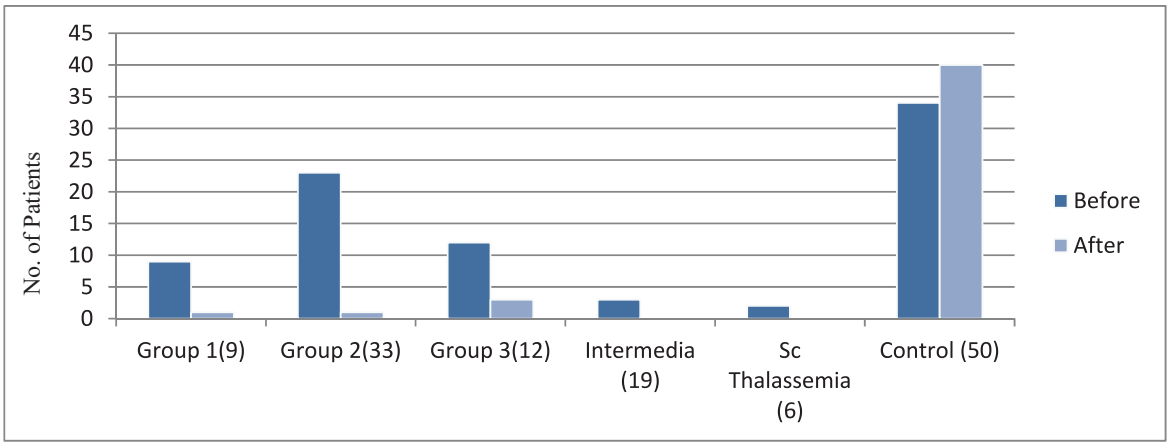

Fig.7. Response to the Treatment for: Fatigability: Shortness of breath in different Groups of Patients. Total number of patients in each group given in brackets.

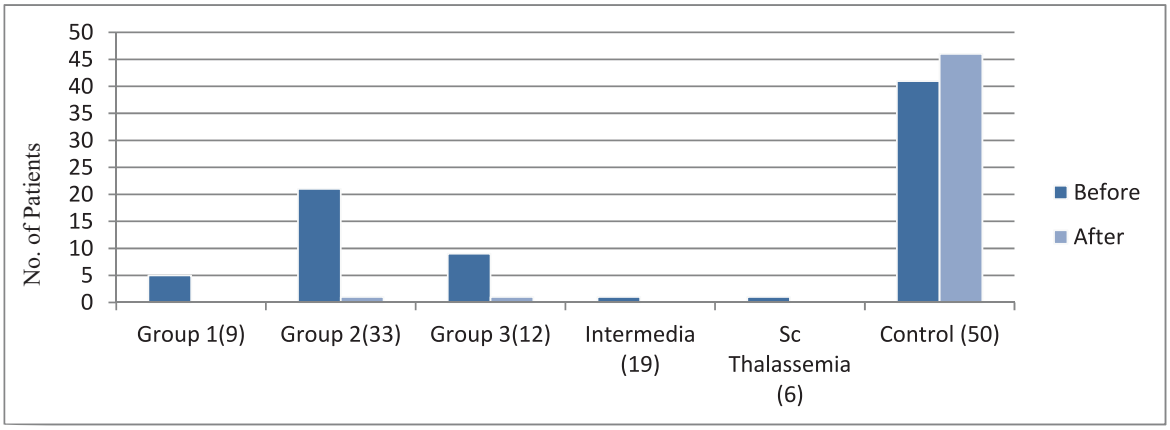

Fig. 8. Response to the Treatment for Fever and chest infection in different Group of Patients. Total number of patients in each group given in brackets.

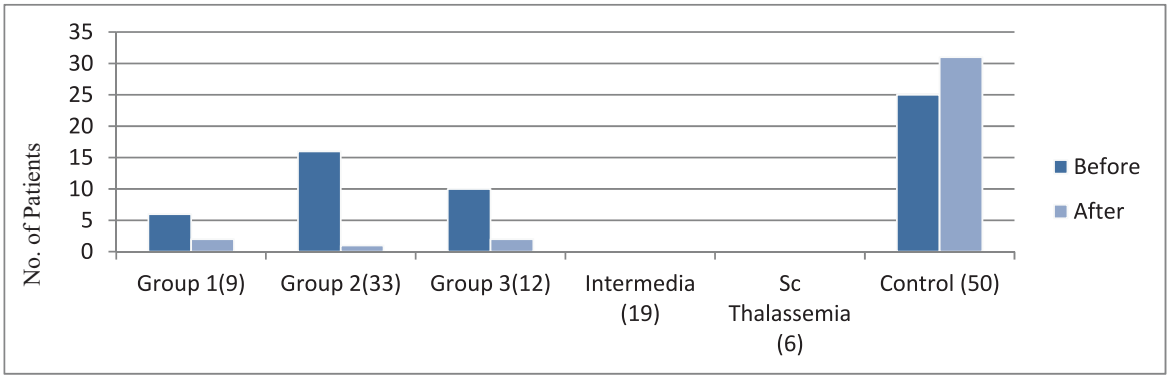

Fig. 9. Response to the Treatment for Sleeplessness in different Group of Patients. Total number of patients in each group given in brackets.

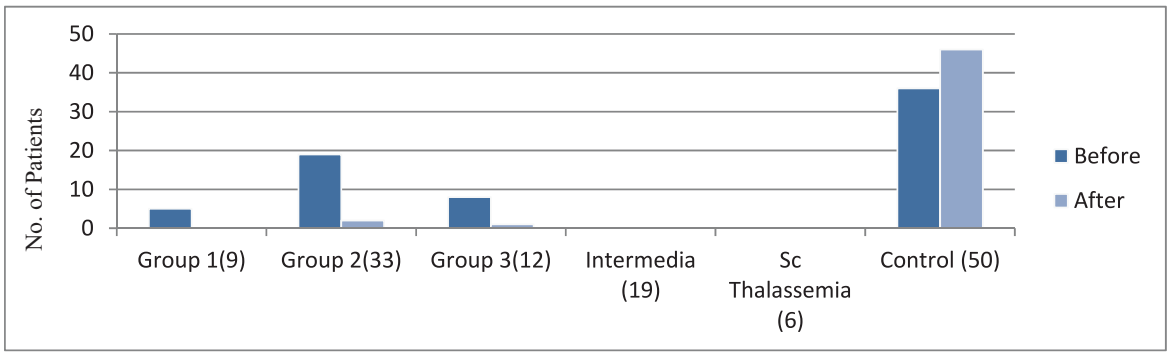

Fig. 10. Response to the Treatment for Bleeding gums in different Group of Patients. Total number of patients in each group given in brackets.

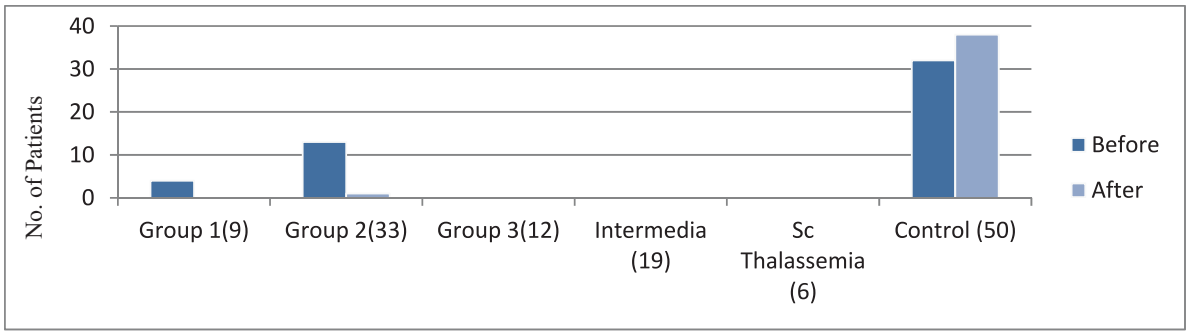

Fig. 11. Response to the Treatment for Maxillary Hyperplasia- Frontal bossing in different Group of Patients. Total number of patients in each group given in brackets.

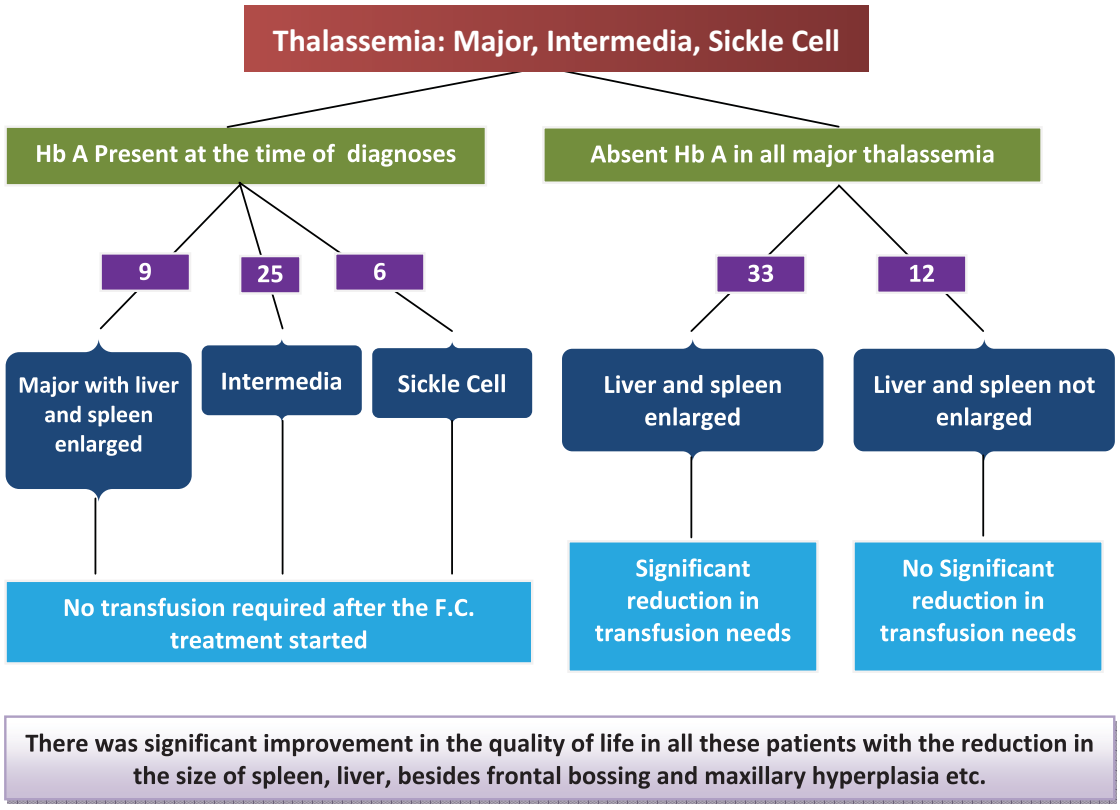

Fig. 12. All patients of Thalassemia.

Group 111 Thalassemia Major patients was quite striking, they were all looking normal and there was no:

- Frontal bossing or maxillary hyperplasia
- Over and above the spleen and liver were hardly palpable below costal margins
- The bone marrow biopsy in these patients revealed severe himosidrosis (Table 15).

4. DISCUSSION

The inconsistent response to the treatment in different patients, particularly those suffering Thalassemia Major, was quite evident from the short case study mentioned in this report. Some of the causes that we could appreciate:

This study revealed interesting results. In Group-1 patients belonging to Thalassemia major, Intermedia and Sickle Cell Thalassemia, there was no need of transfusion soon after these patients started using *Fagonia cretica*. HbA on electrophoresis was present in almost all these patients, when they were first diagnosed.

Thalassemia major (Group-2) and (Group-3), there was no HbA initially present. Patients belonging to Group-2 did show significant reduction in transfusion, as the interval between two transfusions increased significantly from 10±3 to 60±10 days. But Group-3 patients hardly showed any change in the need of transfusions. The Group-3 patients belonged to a typical class. They were looked normal and there was hardly any enlargement of liver or spleen; and there were no bony abnormalities, like frontal bossing and maxillary hyperplasia.

We observed a significant relief of disease related symptoms like arthralgia, myalgia, swelling of joints, frequent fevers and chest infection, bleeding gums, sleep disturbances and easy fatigability with shortness of breath. We also noted that in patients, "Group-1 and 2" of Major and patients belonging to Intermedia and Sickle Cell Thalassemia, the size of the spleen had significantly reduced. *Fagonia cretica* also reduces the serum ferritin level in a good number of patients, even in the absence of other chelating agents. The poor response in Group-3 of Thalassemia Major could possibly be attributed to the collection of iron in the bone marrow.

This study gives convincing results in Thalassemia Syndrome, as there was significant ($P \leq .0.05$) decrease in the Hb F and Hb A2 and Hb S, whereas it showed significant increase ($P \leq .0.05$) in Hb A in all the patients. This report indicates that the transfusion requirements had almost seized in a group of patients belonging to Thalassemia Major and all patients belonging to Intermedia and Sickle cell anemia.

We tried to compare the results of different parameter including total Hb, HbA, HbF, HbA2 besides the size of spleen, liver, frontal bossing, maxillary hyperplasia, bleeding gums or epistaxis, arthralgia or swelling of the joints and shortness of breath or easy fatigability, in the two groups treatment and control Groups. Group-2 and the Group-3 in the treatment category of Thalassemia Major, the possibility of error due to the overlap of the donor's blood could not be avoided.

This study yielded persuasive results of *Fagonia cretica* in Thalassemia syndrome and sickle cell thalassemia, as there was significant decrease in the Hb F and Hb A2 and Hb S, whereas it showed significant increase in Hb A, over and above there was quite significant improvement in the quality of life of all these patients. A diagrammatic illustration of the results is given in Fig. 1-11 with a flow diagram elucidating the response to the treatment. (Fig. 12).

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Research Article

Characterization of Hospital Wastewater, Risk Waste Generation and Management Practices in Lahore

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Abstract: Hospitals generate both, liquid and solid waste. High public health risks are involved in managing these wastes. Objectives of this study were: (i) to determine the characteristics of hospital wastewater; (ii) analysis of current risk waste management practices and compliance level with hospital waste management rules-2005 (HWMR-2005); and (iii) analyse the risk waste generation rates. Three main hospitals of Lahore, i.e., Services Hospital, General Hospital and Gulab Devi Chest Hospital, were selected for this study. Wastewater characteristics were determined by taking samples from each hospital. Results were compared with National Environmental Quality Standards (NEQS). Survey of hospitals was conducted, using a questionnaire, to determine the compliance status with HWMR-2005. Risk waste generation data for the year 2012 was collected and analysed. Wastewater analysis revealed that BOD, COD and Cadmium concentrations were more than the permissible limits prescribed in NEQS. Compliance with HWMR-2005 was found better. Mean risk waste generation rates in Services Hospital, General Hospital, and Gulab Devi Chest Hospital were 0.22, 0.2 and 0.02 kg/bed/day. No significant variations were observed in risk waste generation rates on daily, weekly, monthly and seasonal basis.

Keywords: Hospital, wastewater, risk waste, generation rates, Lahore

1. INTRODUCTION

Disinfectants, pharmaceuticals, radionuclides and solvents are widely used in hospitals for medical purposes and research. After application, these reach the municipal sewer network [1]. If left untreated, these could lead to outbreak of communicable diseases, water contamination, and radioactive pollution [2]. Study conducted on bacteriological and physiochemical qualities of hospital wastewater revealed that there was contamination of the receiving environment (water, soil and air) due to the discharge of hospital wastewater. It could also be hazardous to human health [3]. Hospitals generate significant volumes of wastewater on daily basis [4]. Average wastewater production from hospitals is estimated to be 362 to 745 litters per occupied bed per day [4, 5-7]. This huge volume of hazardous wastewater needs special attention.

Outside Pakistan, many studies have been conducted on hospital wastewater in different countries such as France, India, Nigeria, Ethiopia, Iran, Morocco, Indonesia and Korea. These studies showed that BOD values varied from 242 mg/L to 632 mg/L and COD values varied from 616 mg/L to 1388.75 mg/L. Heavy metals such as Cadmium, Chromium, Copper, Lead, Mercury, Nickel and Zinc were also found in hospital wastewater [1-17, 36]. However, there is no known study on hospital wastewater in Pakistan and little data exist on its characteristics.

In addition to wastewater, hospital also generate risk waste like infectious waste, pathological

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waste, sharps, pharmaceutical waste, genotoxic waste, chemical waste, and radioactive waste. Studies have been conducted in different countries like Iran, South Africa, China, Germany, Korea, Egypt, UK, Turkey, Bangladesh, India and Congo on the generation and management of risk waste [18-27, 39]. Rules and regulations relating to the definition and disposal of hospital waste vary widely in different countries. In European countries classification and disposal of hospital waste is regulated by ordinances [21].

In Pakistan, Ministry of environment issued hospital waste management rules (HWMR) in 2005 [11]. According to the rules, waste originating from healthcare facilities like clinic, laboratory, dispensary, pharmacy, nursing home, health unit, maternity centre, blood bank, autopsy centre, mortuary, research institute and veterinary institutions is termed as hospital waste. It includes both, risk waste and non-risk waste. Non-risk waste includes paper and cardboard, packaging, food waste and aerosols and the like. Risk waste is described in the above para. According to World Health Organization, normally, 15 to 20% of waste originating from a healthcare facility is risk waste; and it needs special handling and treatment. For different types of risk waste, HWMR-2005 specify colour coding for its proper segregation at source of generation. It suggests to use while colour bags for non-risk waste. While for risk waste yellow colour bag should be used. For sharps, yellow colour, leak proof and penetration resistant, container should be used [11].

In Pakistan, little attention is so far paid to risk waste management. Study conducted in eight hospitals of Faisalabad city shows that 90% of the hospital staff was not trained in hospital risk waste management. 80% of the hospitals did not ever received any notice from Pakistan Environmental Protection Agency. There was no segregation of risk and non-risk waste in 76.7 % of the hospitals. Sanitary workers transport the waste without any personal protective equipment (gloves, boots etc.). The research indicates that doctors and hospital management were totally unaware of basic methods of risk waste disposal [27].

Study conducted in ten large public and private hospitals of Rawalpindi and Islamabad shows that segregation practices (for risk and non-risk waste) at the point of generation were not followed. Waste segregation issues were due to lack of training of medical and other staff including sweepers and ward servants. There were no waste bins. Waste was collected without using standard operating procedures for final disposal and treatment. The study suggests that training of hospital staff can lead to improved hospital risk waste management practices [28].

Another study was conducted in eight teaching hospitals of Karachi. It revealed that out of eight hospitals visited, 2 (25%) were segregating the risk waste at source. Only one (12.5%) hospital arranged training sessions for its waste handling staff regularly. Five (62.5%) hospitals had storage area for risk waste but mostly it was not protected from access of scavengers. Five (62.5%) hospitals disposed their risk waste by burning in incinerators, two (25%) disposed it in municipal landfills and one (12.5%) was burning waste in open air without any specific treatment. No record of risk waste was generally maintained. Only two (25%) hospitals had well documented guidelines for risk waste management and a proper waste management team. Study concluded that HWMR-2005 should be followed and implemented by law enforcement agencies [29]. In order to improve risk waste management and develop a management strategy, it is important to understand and evaluate current practices [20]. Information about hospital waste management in Pakistan is currently inadequate. Compliance rating of hospitals with HWMR-2005 is non-existent.

Different factors affect the hospital risk waste generation rates. Tabasi and Marthandan [30] reviewed 20 research papers that reported relevant associated factors in hospital risk waste production. Out of 20 studies, 13 studies (65%) reported that the type of healthcare establishment has significant

effect on risk waste generation. Other factors include the number of patients, number of beds and the percentage of bed occupancy. Hospital risk waste generation rate were determined in some of the developing countries like India, Bangladesh, China, Taiwan lie in a range of 0.14 to 0.88 kg/bed/day [20, 22, 32, 34, 35, 40]. In 2010, study conducted on quantification, classification and management of hospital waste in Lahore city showed that 785 million ton of risk waste was produced and incinerated in Lahore per annum [33].

Evaluation of waste generation rates and quantities is essential for the establishment of a waste management system for hospitals [31]. The objectives of the present study were to; (1) characterize hospital wastewater; (2) evaluate compliance with HWMR-2005 and (3) evaluate the risk waste generation rates and its variations. In Pakistan, previous studies on hospital risk waste generation rates are not rigorous, since these were based on the data of only one to three weeks [38, 39]. However, this study is based on risk waste data of 52 weeks (one year). Thus all possible variations like weekly, monthly and seasonal were accounted for. In addition, no previous work exists on hospital wastewater characteristics which is pre-requisite for the selection of an appropriate treatment technology.

2. MATERIALS AND METHODS

2.1 Hospitals Selected for the Study

In Lahore, there are 232 hospitals. Out of these 47 are public and the rest are private. To study wastewater characteristics and risk waste generation, it was necessary to select major hospitals with plenty of instrumentation, a range of medical services and large outrun of patients. For this study, hospitals having 200 or more beds were considered as major. Thirteen public hospitals in Lahore meet this criteria. Out of these, 3 hospitals were selected randomly making a sample size of 23%. Ten percent or more sample is considered to be a good sample size for small populations [41]. The selected hospitals included: Services hospital (SH) having 1196 beds, General hospital (GH) with 1048 beds and Gulab Devi (GD) chest hospital (1500 beds).

2.2 Sampling and Analysis of Wastewater

There were several wards in the selected hospitals. Each ward generated wastewater having different characteristics. All these wastewaters join at the tank of disposal station and are homogenized. To take a representative sample, it was decided to collect wastewater from the disposal tank. The parameters tested and the testing procedures are mentioned in Table 1. The heavy metals in the wastewater were analysed by using atomic absorption spectrophotometer (PerkinElmer Analyst 800).

Table 1. Parameters tested and the testing procedures.*

Parameter	Testing Method
pH	pH paper
Five-day biochemical oxygen demand (BOD)	5210 (B)
Chemical oxygen demand (COD)	5220 (B)
Total dissolved solids (TDS)	2540 (C)
Chlorides	4500 Cl^- (C)
Alkalinity	2320 (B)
Total nitrogen	4500 N_{org} (B)
Ammonia nitrogen	4500 NH_3 (B&C)
Iron	3111 Fe
Manganese	3111 Mn
Cadmium	3111 Cd
Copper	3111 Cu
Nickel	3111 Ni
Lead	3111 Pb
Zinc	3111 Zn
Chromium	3111 Cr

*All the testing methods are based on Standard Methods for the Examination of Water and Wastewater, 20th edition (1998), www.standardmethods.org.

2.3 Methodology for Analysis of Hospital Waste Management Practices

For analysis of current hospital waste management practices, a survey questionnaire based on HWMR-2005 was developed. Questionnaire was filled through visits of the selected hospitals. It contained 25 questions about different aspects of hospital waste management.

2.4 Methodology for Determination of Generation Rates

Proper record of risk waste generated, in the selected hospitals, was maintained on daily basis. For this study risk waste generation data from 1st January to 31st December 2012 (365 days) were collected from the available record of selected hospitals. Statistical analysis was performed on the yearly data including mean, minimum, maximum, standard deviation and coefficient of variation.

Separate analysis was performed to find out weekly, monthly and seasonal variations in risk waste generation. There are 52 weeks in a year, therefore each day approximately occurs 52 times in a year. For weekly variation, data for a specific day of the week, for the entire year, was added and mean and standard deviation (SD) was calculated. Mean of different days were compared by calculating standard error of mean (SEM) and lastly values for 95% confidence interval were found out.

Similarly, for monthly variations, the mean of each month was calculated from the daily risk waste generation data, along with SD for each month. Mean of different days were compared by calculating standard error of mean (SEM) and lastly values for 95% confidence interval were found out. For seasonal variations, period from May to September was taken as summer, from October to November as autumn, from December to February as winter and March to April as spring. Mean of each season was calculated from the daily data for that season with SD for each season. SD for weekly, monthly and seasonal variations are shown as error bar on the relevant figures in section 3.4. Standard error of mean could not be calculated for seasonal variation due to difference is sample size.

In order to compare the amount of risk waste generated from each unit of a hospital, one week risk waste generation data was taken for two hospitals i.e. GH and GD. The week was randomly selected. However, the same week for the two hospitals was taken. Mean of the entire week, for each unit, was then plotted for the sake of comparison.

3. RESULTS AND DISCUSSION

3.1 Characteristics of Hospital Wastewater

Hospitals investigated had no wastewater treatment plant. The results of physico- chemical parameters are presented and compared with NEQS in Table 2. Values of pH varied from 6.8 to 7.5. These values were within the permissible limits of NEQS. Similar results were obtained in other studies. Beyene and Redaie [7] determined pH value in hospital wastewater to be 7.4. Study on hospital wastewater in India showed pH value of 7.36 [2].

BOD and COD values varied from 112 mg/L to 750 mg/L and 251 mg/L to 1400 mg/L respectively. These concentrations were more than the permissible limits of NEQS. Highest concentrations of BOD and COD were in General hospital and Services hospital. TDS and Chlorides concentrations were in a range of 620 mg/L to 1400 mg/L and 70 mg/L to 200 mg/L, respectively. These values were within

Table 2. Physicochemical charcterisation of hospital wastewater.

Parameters	NEQs	General Hospital	Services Hospital	GulabDevi Chest Hospital
pH	6 – 9	6.8	7.2	7.5
BOD	80 mg/L	120	750	300
COD	150 mg/L	280	1480	680
TDS	3500 mg/L	900	800	1400
Chlorides (Cl^-)	1000 mg/L	110	110	70
Alkalinity	*	480	600	670
Total Nitrogen	*	27.6	45.2	18.6
Ammonia Nitrogen	40 mg/L	16.7	24.2	17.6

* No NEQs for this parameter.

Table 3. Concentrations of heavy metals in hospital wastewater.

Heavy metals	NEQS	General Hospital	Services Hospital	Gulab Devi Chest Hospital
Cadmium (mg/L)	0.1	0.032	0.045	0.676
Chromium (mg/L)	1.0	0.042	0.107	0.088
Lead (mg/L)	0.5	0.012	0.104	0.229
Nickel (mg/L)	1.0	0.593	0.631	0.634
Zinc (mg/L)	5.0	0.077	0.174	0.150
Copper (mg/L)	1.0	BDL*	BDL*	BDL*
Manganese (mg/L)	1.5	0.027	0.057	0.027
Iron (mg/L)	8.0	0.339	0.447	0.445

*BDL=Below Detection Limit

the permissible limits of NEQS. Alkalinity was in a range of 480 mg/L to 670 mg/L as $CaCO_3$. Total nitrogen and ammonia nitrogen were in a range of 18.6 mg/L to 45.2 mg/L and 16.7 mg/L to 24.2 mg/L respectively. These values were within the permissible limits of NEQS.

The results of heavy metal concentrations in hospital wastewater samples are presented and compared with NEQS in Table 3. It can be seen that concentration of all heavy metals were within permissible range except Cadmium in Gulab Devi hospital. Possible reasons of high Cadmuim contents are old and discarded nickel-cadmium batteries, pigments, coatings and plating, used in the hospitals. High concentration of cadmium may cause kidneys, lungs, and bones effects.

3.2 Analysis of Hospital Waste Management Practices

The findings of the questionnaire filled, during field visits, are discussed below.

3.2.1 Waste Management Team

HWMR-2005 specify that each hospital must have a notified waste management team; duties of team must be defined and hospital administration must make waste management plans. It was observed that waste management teams were notified under rule (u/r) 4(1). Waste management officer was nominated u/r 4(4). Duties and responsibilities of waste management team were notified u/r 4 (3) & 5. Meetings of waste management team u/r 6 were conducted twice a month.

3.2.2 Segregation of Waste

HWMR-2005 prescribe that risk waste should be segregated, on site, inside the hospital. After segregation, it should be weighed and packed in color coded bags as described in Section 1. It was observed that risk waste was separated from non-risk waste at source u/r 16(1). Syringe needle cutting u/r 16(2) was practiced. Plastic bags, infusion bags, drip bags were being cut down u/r 16(2). Broken syringes and needles were placed in yellow boxes u/r 16(4). Sharp containers were yellow in color u/r 16(4). Sharp containers were marked “Danger! Contaminated Sharps” u/r 16(4). The sharp container was closed and sealed when 03 quarters u/r 16(4). Non risk waste containers were

Table 4. Results of statistical analysis of risk waste generation data for year 2012.

Hospital Name	Average (kg/day)	Minimum (kg/day)	Maximum (kg/day)	Standard Deviation	Coefficient of variation	Total Annual (kg/day)	Average (kg/bed/day)
Services Hospital	234	127	326	40	17	73,118	0.22
General Hospital	204	115	324	46	22	63,863	0.20
Gulab Devi Hospital	28	12	64	11	39	8,906	0.02

lined with white waste bags u/r 16(8).

3.2.3 On-Site Collection and Transportation of Waste

Directions of on-site collection and transportation of waste were followed as per HWMR-2005. Waste was collected once daily u/r 17(3) a. All waste bags were labelled indicating point of production and contents u/r 17(3) b. The transportation of waste was properly documented u/r 18(5) g. Risk waste was transported by trollies to the central storage facility. Before transferring the waste was again weighed and proper record of waste generation was maintained. There was violation of rule 17(2) in all the studied hospitals as sanitary staff and sweeper did not wear personal protective equipment (gloves, boots, and clothes).

3.2.4 Waste Storage

HWMR-2005 direct to store risk waste in a separate room inside hospital for temporary storage, at suitable temperature. It was observed that the above facility u/r 19(1) was provided in all the studied hospitals. These storage facilities were away from the public approach. Proper cooling was provided in the storage rooms to maintain temperature between 3^0C to 8^0C.

3.2.5 Treatment/Disposal of Risk Waste

It was told by the concerned persons in the hospitals that risk waste is sent to the incinerator installed in the Children hospital Lahore. Before transportation, it is again weighed and proper record was maintained by both the authorities operating incinerator and the hospital. There was a small scale incinerator available in the Services hospital Lahore. It is based on old technology and thus causes air pollution. Concerned staff at incineration plant told that it is utilized only in case of emergency such as shutdown of the incinerator at Children hospital.

3.3 Generation Rates of Risk Waste

Statistical analysis of the risk waste data is presented in Table 4. It shows that average daily risk waste generation rates were high in SH (234 kg/day; 0.22 kg/bed/day) and GH (204 kg/day; 0.20 Kg/bed/day) as compared to GD (28 kg/day; 0.02 kg/bed/day). Risk waste generation in GD is much less than other hospitals. The major reason is that type of healthcare facilities provided in GD hospital are different from other two hospitals. It is discussed in more detail in section 3.5.

The range in which risk waste generation per day varied in SH, GH and GD were 127 to 326, 115 to 325 and 12 to 64 kg/day, respectively. The respective standard deviation for the yearly data, for the above hospitals, was 40, 46 and 11, whereas the coefficient of variation were 17, 22 and 39. This shows that variations/scatter in the risk waste generation in GD is more than the other two.

An important parameter, for reporting and designing systems for risk waste management, is risk waste generated per bed per day. These figures for SH, GH and GD hospital were evaluated to be 0.22, 0.2 and 0.02, respectively. The figure for GD does not lie in the reported range for other developing countries (0.14 to 0.88 kg/bed/day). The reason are discussed in detail at the end of this section.

3.4 Variations in Risk Waste Generation

In addition to daily variation of risk waste discussed in section 3.3, seasonal, monthly and weekly variation are also important to study. As these are taken into account while designing waste management system. The results for weekly variation are presented in Fig. 1, which shows average value for each day of the week and standard deviation as error bar. It can be seen that there is no significant variation between different days of the week for all the hospitals. Table 5 shows the confidence interval for 95% confidence level, for all the hospitals, for weekly variation. The margins of error shows the standard error of the mean. It can be seen that margin of error for GH and SH are very close to each other. This may be due to the similar nature of treatment facilities provided. For 95% of the time, the risk waste on a specific day of the week lied in a range of 190 to 218, 25 to 32 and 220

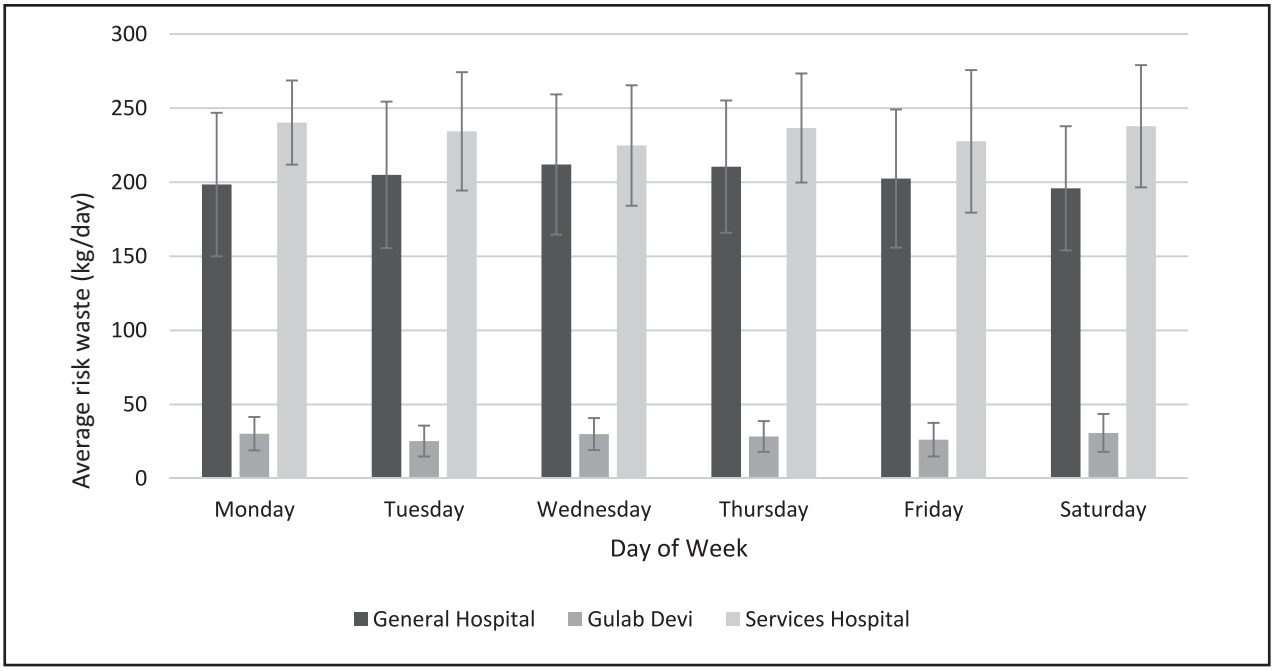

Fig. 1. Average week days risk waste generation rates.

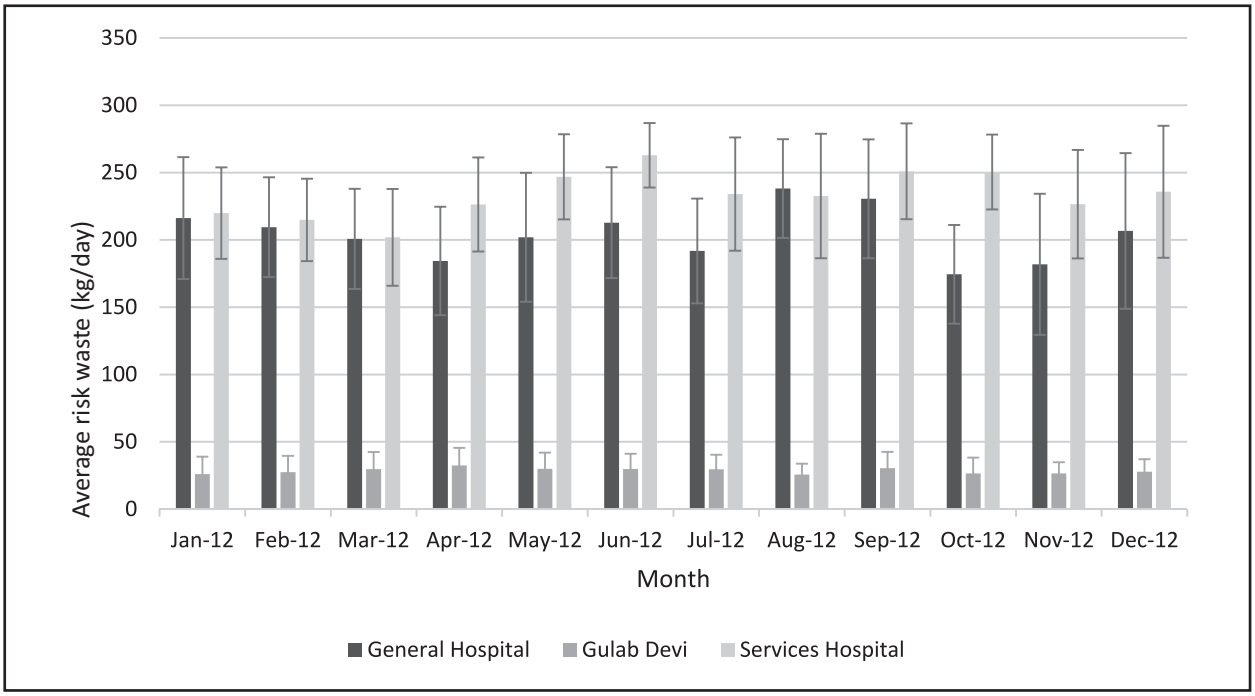

Fig. 2. Average monthly risk waste generation rates.

Table 5. Standard error and confidence intervals for weekly variation.

Hospital Name	Confidence level	Confidence coefficient	Margin of error	Confidence interval	
				Upper bound (kg/day)	Lower bound (kg/day)
General Hospital	95%	1.96	14	218	190
Gulab Devi Hospital			3	32	25
Services Hospital			13	247	220

Table 6. Standard error and confidence intervals for monthly variation.

Hospital Name	Confidence level	Confidence coefficient	Margin of error	Confidence interval	
				Upper bound (kg/day)	Lower bound (kg/day)
General Hospital	95%	1.96	19	223	185
Gulab Devi Hospital			5	33	24
Services Hospital			18	251	216

to 247 kg/day for GH, GD and SH, respectively.

The monthly variation of risk waste is shown in Fig. 2. It can be seen that risk waste generation is maximum in the month of August and June for GH and SH, respectively; probably it may be due to Dengue fever. While it is minimum during the month of October and March for GH and SH, respectively. This finding is helpful while finding storage capacity for risk waste. More storage is required in the month of August and June. It can be observed that for GD, there is no significant monthly variations; it may due to the fact that GD is only for tuberculosis patients. No other patients are entertained here. Table 6 shows the confidence interval for 95% confidence level, for all the hospitals, for monthly variation. Again the margin of error for GH and SH are very close; the reason being the same as stated for weekly data. For 95% of the time, the risk waste in a month lied in a range of 185 to 223, 24 to 33 and 216 to 251 kg/day for GH, GD and SH, respectively.

The seasonal variation is shown in Fig. 3. This figure shows mean risk waste generated during each season and standard deviation as error bars. It can be concluded from this figure that there is no significant seasonal variation in GH and GD. However, in the case of SH slight seasonal variation has been observed. More risk waste is generated during autumn and summer. This finding may help to design waste management system. More storage is required during the season when more risk waste is generated.

3.5 Type of Healthcare Facility and Risk Waste

The risk waste generation from each unit of GH and GD are shown in Fig. 4 and 5. It is evident from a comparison of risk waste generated in a unit, within the same hospital, that type of unit has a significant impact on the amount of risk waste generation. For example, in the case of GH (Fig. 4), Homio Dialysis unit generates the maximum amount of risk waste (48 kg/day) and is followed by Surgical Operation Theatre (17 kg/day) and Medical Emergency (17 kg/day). It can also be observed that minimum risk waste is generated from eye wards (< 1 kg/day) while no risk waste is generated from Angiography unit.

From Fig. 5, it is evident that in GD maximum amount of risk waste is generated in Micro Lab (4.7 kg/day), which is followed by Cardiac OT (4.5 kg/day). Earlier studies conducted on this hospital did not collect the risk waste generation data from all the wards/ units. e.g. the data for GD did not include Micro Lab and Cardiac OT which produces highest risk waste in this hospital [38].

It is also evident from Fig. 4 and 5 that the type of healthcare facility has a significant impact on the

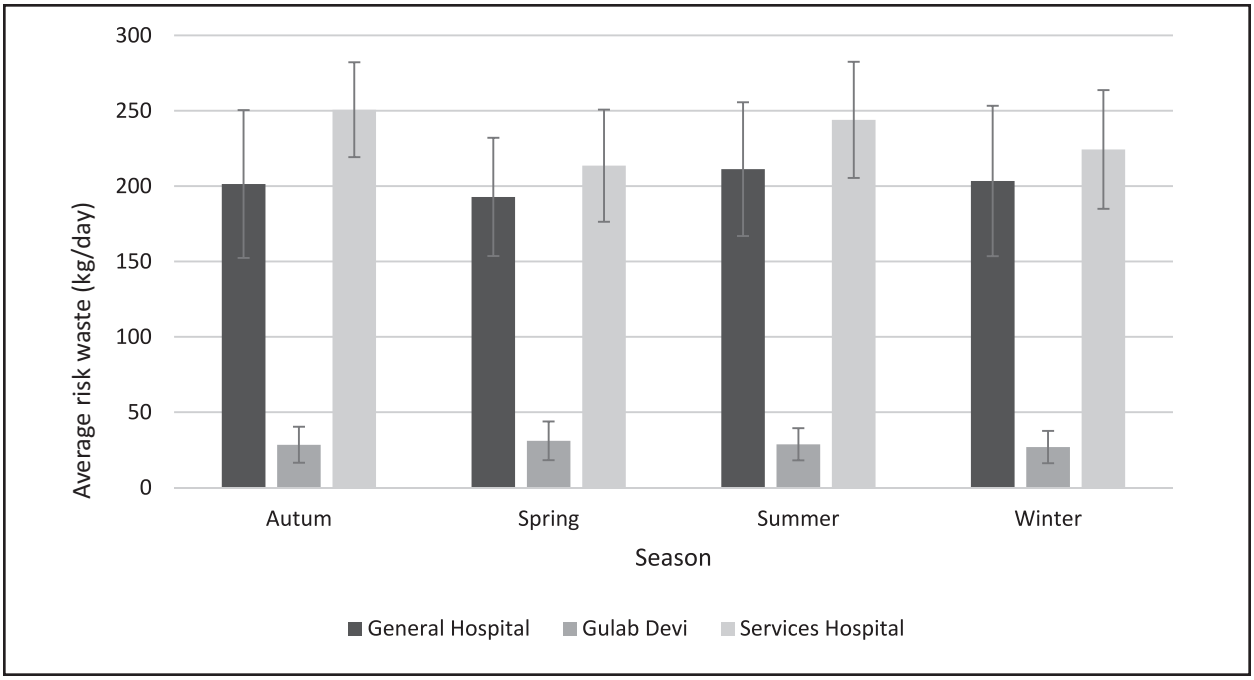

Fig. 3. Average seasonal risk waste generation rates.

amount of risk waste. GD is solely for chest while GH deals with all types of patients. This can also be seen from Table 4 that mean (yearly mean) daily risk waste from GD is 28 kg/day while the same for GH is 204 kg/day. This finding is endorsed by other studies in Lahore [39].

4. CONCLUSIONS AND RECOMMENDATIONS

Following conclusions can be drawn from the current study with a few recommendations:

Wastewater treatment is not on the priority list of the management of selected hospitals. Since none of the hospitals have wastewater treatment plant. BOD and COD of hospital wastewater are above the limits prescribed in NEQS, while rests of the parameters tested were within the limits. Except Cadmium, all heavy metals analysed were within the permisible limit of NEQS. Cadmium was high in the wastewater of Gulab Devi Chest hospital due to its specialzied nature. The high amounts of BOD, COD and Cadmium may harm the aquatic life and even human health, since the wastewater in Lahore is finally disposed in river Ravi and agriculturel fields. This situation calls for immediate attention.

Compliance level of HWMR-2005 in the selected hospitals was better. Risk waste was disposed through incineration. Average daily risk waste generation rates in the hospitals varied from 28 to 234 kg/day (yearly mean). When related to number of beds, it varied from 0.02 to 0.2 kg/bed/day. The unit producing maximum amount of risk waste is Homio-dialysis. Thus, it can safely be concluded that the type of healthcare facilities significantly affect the amount of risk waste generation. There was no significant variations in mean weekly and monthly risk waste generation. However, season may affect the generation.

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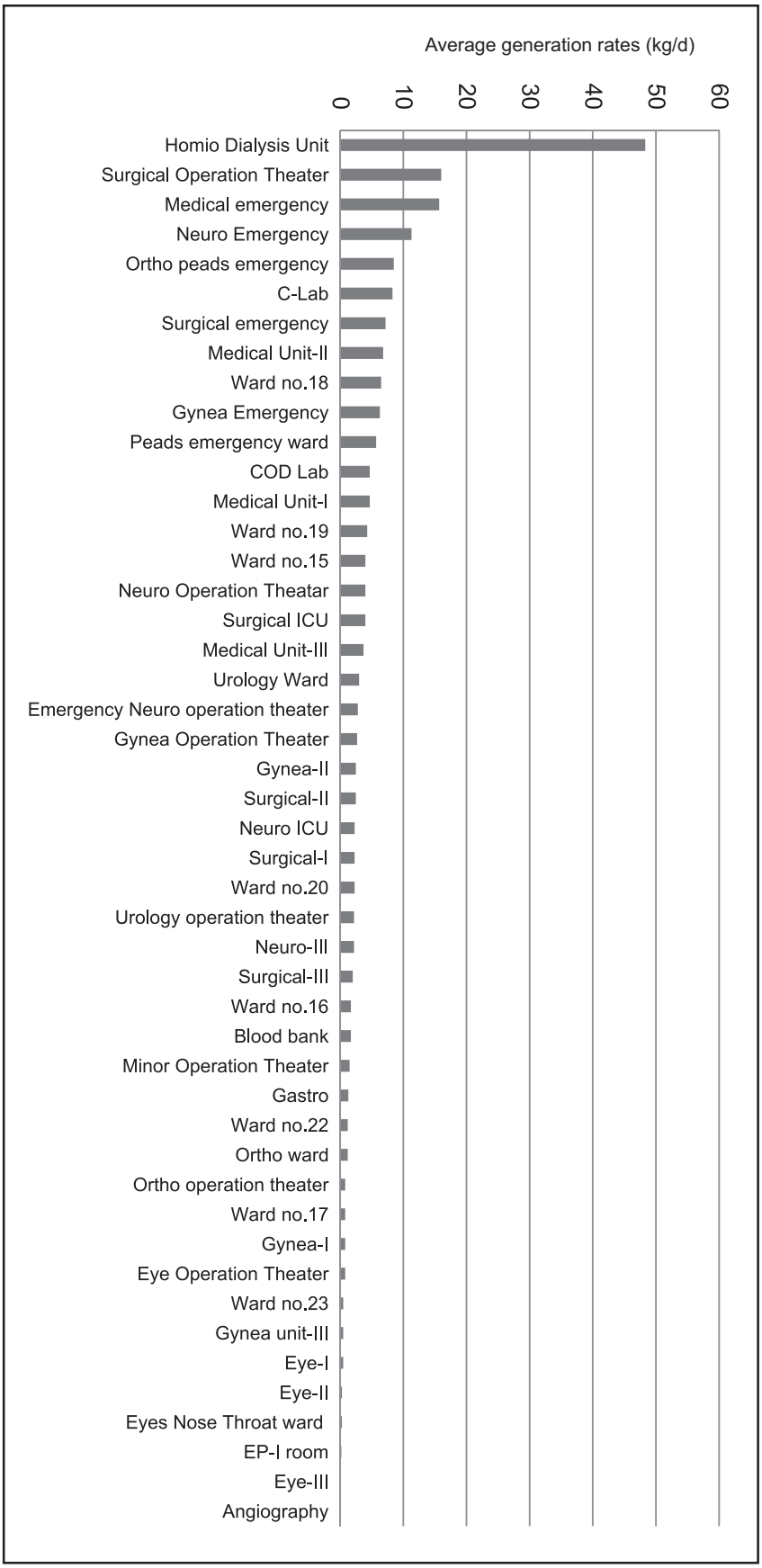

Fig. 4. Average risk waste of different wards/units of General Hospital (weekly average).

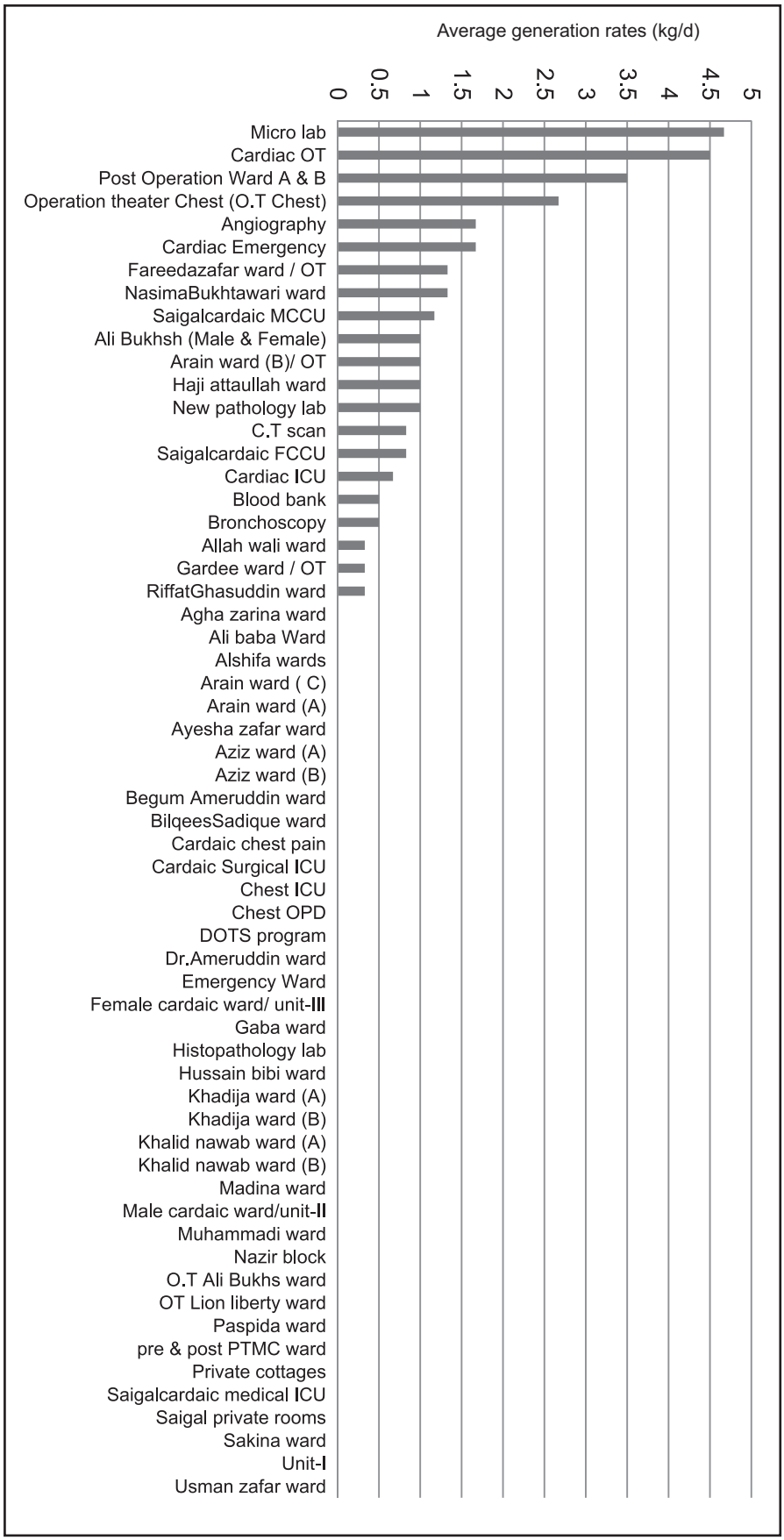

Fig. 5. Average risk waste of different wards/units of Gulab Devi Chest Hospital (weekly average).

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Research Article

A Study of the Internal Defects of Terrazzo and Engraved Construction Materials using Direct Film Neutron Radiography Technique

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Abstract: Neutron radiography technique has been utilized in the work for studying internal defects of various types of construction materials through optical density measurements of the samples. Two kinds of locally developed construction materials have been used as samples in the experiment. They are Terrazzo and Engraved construction materials. Tangential Neutron Radiography Facility of 3 MW TRIGA Mark-II research reactor is used here to find out the internal defects of the samples. From the observation of neutron radiographic images of the samples and variation of optical density at different positions, it revealed that the associated composites of Terrazzo construction material are uniformly distributed. No voids or any inclusions in the materials have been observed in the radiograph. The neutron radiographic image of Engraved construction material shows that the optical density values at different reference positions are different. The density at the central position of the image is different from its neighboring reference positions. Moreover, some voids are observed in the neutron radiograph of the material. This confirms that in this material the associated composites are not uniformly mixed and distributed during its fabrication. So, the fabrication of this construction material is relatively faulty. This faulty material may have several bad impacts while it is used as a construction material. It can absorb rainwater and thus may be damaged. Due to its structural disorder, its strength is deteriorated and may be damaged easily from even a very minor environmental turmoil.

Keywords: Neutron radiography, non-destructive testing, optical density

1. INTRODUCTION

Neutron radiography (NR) is an imaging technique, which provides images similar to X-ray and Gamma-ray radiography. Interactions of neutron with matter can be divided into scattering and absorption. Neutrons can detect light elements, which have large neutron absorption cross-sections like hydrogen and boron. The information provided by spatial and temporal beam attenuation is recorded on magnetic media via analogy or digital signals.

All radiographic methods, whether making use of X-rays, γ-rays or neutron beams are based on the same general principle that, radiation is attenuated on passing through an object (sample). The object under examination is placed in the incident radiation beam. The beam, which remains after passing through, enters a detector that registers the fraction of the initial radiation intensity that has been transmitted through each point of the object. Any inhomogeneity in the object or an internal defect (such as voids, cracks, porosity, inclusion, corrosion, etc.) will show up as change in radiation intensity reaching the detector.

Neutron radiography is a non-destructive

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testing (NDT) technique of testing the nuclear and non-nuclear materials as well as industrial products [1]. It concerns neutrons and radiography using neutron beam. Recently, NR method has been applied to detect faults and to study water absorption properties of building materials [2]. A neutron radiography standard testing method for the moisture analysis was introduced by Peterka et al [3] to the building industries in order to evaluate the properties, functions and the efficiency of their water protective agents against the penetration of water, water solution etc. In another study [4], quality of leather and ceramics has been studied. Study of corrosion in aluminum has been reported by Islam et al [5]. In the present study, the NR set up at the tangential beam port of the 3.0 MW TRIGA Mark-II research reactor of AERE, Savar, Dhaka, Bangladesh has been used. Details of the NR facility of AERE, Savar, Dhaka can be found in reference [6]. Details of the parameters of the facility have been given by Ahsan et al [7]. A study of defects and water absorption behavior of jute products was reported by Rahman et al [8].

The following experiments were carried out using direct film neutron radiography technique:

A) Determination of optimum irradiation time for the present sample.

B) Determination of defects in the samples through optical density variation measurements.

Any inhomogeneity in the object or an internal defect, e.g., void, crack, porosity or inclusion will show up as a change in radiation intensity reaching the detector, irradiation intensity varies after passing through an object under examination. This intensity variation obeys the general attenuation law [9] applicable for X-rays, gamma rays or neutrons

$$I = I_0 e^{-\mu x} \quad (1)$$

where,

I_0 = initial intensity of the incident beam,
I = intensity of the emergent beam from the object,
μ = attenuation coefficient,
x = thickness of the object.

When the radiation beam is neutron, the above equation can be written as

$$I = I_0 e^{-\mu x} \quad (2)$$

where,

ϕ_s = number of neutrons transmitted through the sample, n cm^{-2} sec^{-1},
ϕ_0 = number of neutrons incident upon the sample cm^{-2} sec^{-1},
N = number of nuclei per cm^3,
σ = microscopic cross-section, cm^2,
x = thickness of the sample, cm.

The attenuated neutron beam enters a detector that registers the fraction of the initial radiation intensity reaching the detector and is then recorded in the X-ray film. This is the principle of NR. The rate of depletion of the control rod material can also be detected by taking regular neutron radiographs. Irradiated TRIGA fuel elements could be used as object for all these experiments.

2. EXPERIMENTAL PROCEDURE

2.1 Pre-Irradiation Procedure

1. Sample collection/preparation
2. Loading the film and converter foil in the NR cassette
3. Setting the sample in the neutron beam

2.2 Sample Preparation/Collection

Some locally developed construction materials have been collected from the Concord Ready-Mix and Concrete Products Ltd, Gulshan, Dhaka. The names of these products are terrazzo construction product and Engraved construction product. Terrazzo building product is made from marble chips, marble dust and white cement. Engraved product is made from cement, sand and pigment.

2.3 Loading the Film and Foil in the NR Cassette

Gadolinium (Gd) metal foil of 25 μm thickness

was used as converter in the NR cassette and Agfa structruix D4pDW industrial X-ray films were used as detector in our experiment. The films have emulsions in single side only. The sample and the NR camera were placed on their respective tables across the neutron beam. In this position the camera was placed just after the sample. The sample holder table was set at the optimum sample position from the reactor biological shielding assembly.

2.4 Irradiation of the Sample

To find out the optimum irradiation time of the sample a series of experiments were performed with different exposure time. To do these experiments the reactor was operated at 250 kW power level. Finally, we found the optimum irradiation/exposure time for the sample. From the observation of the final radiograph we found out the internal details such as cracks, voids, homogeneity of their compositions etc. of the sample.

2.5 Post-Irradiation

After irradiation of the sample, the irradiated film was separated from the NR camera in the dark room and then following procedures have been carried out to make the radiographic image of the irradiated samples:

2.6 Developing

Developing ensures latent image, which was produced during irradiation to visual one. The film was then immersed into the developer for some time and was then agitated into the developer horizontally without touching the beaker (which contains the developer chemicals).

2.7 Washing

For cleaning the developing chemicals, the film was washed in cool water for a few minutes.

2.8 Fixing

The developed film was immersed in the fixture chemicals to obtain the clear image.

2.9 Final washing

The silver compounds, which were formed during the fixing stage have to be removed, since they can affect the silver image at the later stage. For this reason the film was washed thoroughly in running water.

2.10 Drying

After the final washing, the films were dried by clipping it in a hanger and simultaneously flowing fresh air from the air cooler.

The neutron radiographic images of the sample show that the region in which the sample was at close contact on the neutron radiography cassette were light whereas, the backgrounds were comparatively dark. This is because more neutrons were attenuated by the test sample and allowing more neutrons to pass freely through the rest.

3. BASIC PRINCIPLE OF THE STUDY

The quality/homogeneity of an object depends on the proper distribution of the composite materials. In the present work we have studied the quality of the test samples by the densitometric measurements of the neutron radiographic images of the sample.

When neutron beams hits an absorber, some of them are absorbed and scattered while the rest pass through it. Attenuation of radiation in the object is the difference between the radiation intensity before and after passing through this object. It has been expressed mathematically in eqn. (1).

$$I = I_0 e^{-\mu x} \tag{3}$$

where e=base of natural logarithms, x = thickness of the test object, μ = linear neutron attenuation coefficient, which depends on the atomic number and the density of the material. I and I_0 is the neutron intensity after passing the object and the neutron intensity incident on the object.

In this work, the term homogeneity means the uniformity in the distribution of the composite materials. The homogeneity of a material depends on the proper distribution of the composite materials. Measuring the optical density of the radiographic film background (without image), the optical density of the center point of the

sample image, and at different reference levels of the radiographic image of the sample, one can comment about its homogeneity/inhomogeneity. The best homogeneity is ensured would constant optical density values at all places/levels.

The mathematical expression [10] for the optical density D, at a point of the film/image is given by:

$$D = \ln\left(\frac{A_0}{A}\right) \quad (4)$$

where, A_0 = response of densitometer without the image and A = response of densitometer with the image.

Fractional change in the image density of the neutron radiograph can be represented by ΔD and the expression can be written as,

$$\Delta D = \left(\frac{D_c - D_n}{D_c}\right) \quad (5)$$

where D_c = Average optical density of the total radiographic image and D_n = Optical density at different positions of the radiographic image.

The optical density of the neutron radiographic images of the sample have been measured by a digital densitometer (Model – 07 - 424, S - 23285 Victorian, USA). Densitometric data of the optical density of the radiographic image of the sample tiles are given in Table 2.

4. RESULTS AND DISCUSSION

To calculate the optimum irradiation time for the two samples, the samples were irradiated for different time intervals at reactor power 250 kW. The optimum irradiation time was found out to be 45.0 ±3.7 minutes for both the samples. The results are shown in Table 1. The radiographs of Terrazzo and Engraved tile samples are shown in Fig. 1(a) and Fig. 1(b), respectively. The optical density of the neutron radiographic images of the samples were measured by a digital densitometer (Model – 07-424, S-23285 Victorian Inc., USA). Densitometric data of optical density of the radiographic image of the sample is shown in Table 2.

Table 1. Optimum irradiation/exposure time of the objects.

Construction material	Irradiation time (Minute)	Optimum irradiation time (Minute)
Terrazzo	60.0 50.0 40.0 45.0	45.0 ±3.7
Engraved	60.0 50.0 40.0 45.0	45.0±3.7

Table 2 Densitometric data for Terrazzo and Engraved building materials.

Construction material	Optical density at the centre	Average density (D_c)	Optical density at different positions (D_n)	Fractional change in image density $\Delta D = (D_c - D_n) / D_c$
Terrazzo	2.54 2.54 2.54 2.53 2.53 2.54	2.537±0.002	2.54 2.54 2.54 2.55 2.54 2.54 2.54 2.55 2.54	0.001 0.001 0.001 0.005 0.001 0.001 0.001 0.005 0.001
Engraved	2.48 2.48 2.48 2.48 2.48 2.47	2.478±0.001	2.48 2.50 2.47 2.52 2.49 2.44 2.50 2.44	0.000 0.009 0.003 0.017 0.005 0.015 0.009 0.015

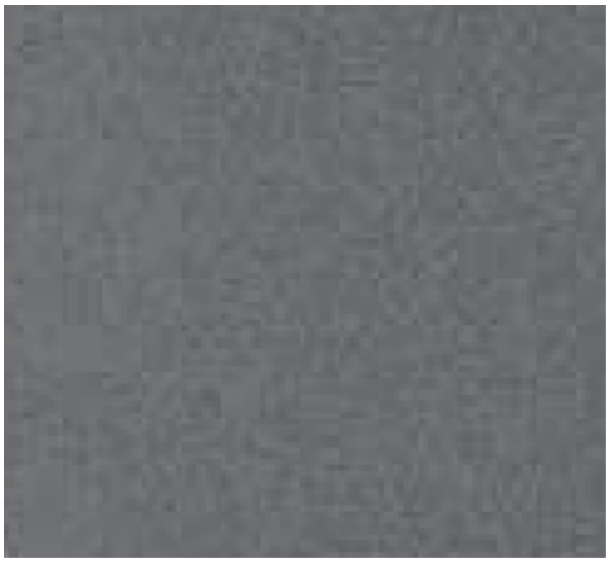

Fig. 1(a). Neutron radiographic image of a Terrazzo tile.

Fig. 1(b). Neutron radiographic image of an Engraved tile.

The Terrazzo tiles are usually used in the floor of buildings. The radiograph of Terrazzo is almost clear which shows that the mixture of the constituent elements of the sample is quite uniform. The densitometric data also proves the uniformity in mixing the constituent elements in the Terrazzo material. From experience in handling the radiographic films, we can conclude that the quality of Terrazzo tiles is good. From the radiograph of the Engraved construction materials it seems that the sample was not perfectly homogeneous. From the densitometric data it may be concluded that mixing of the constituent elements in the Engraved tiles is not uniform. The quality of the Engraved tiles should be improved further by mixing the constituent elements in a much better and improved way. The samples were dipped into water for 24 hours and then radiographs were taken but no absorption of water was seen in the radiographs. The reason was that the tiles were coated by water resistant porcelain type materials.

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Research Article

Preparation of Double Action Surfactant using Protein Hydrolyzate from Fleshing Waste and Its Utilization as a Lubricant with Retanning Property in Leather Making

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Abstract: Leather fleshing are generated during beam house leather processes when lower layer of skin is scrapped off through sharp knife. Generally these are thrown out in land area as waste material without any proper treatment. Collagen protein hydrolyzate was recovered by alkaline hydrolysis and further reacted with fatty acid ester product from glycerol. The reaction was promoted by direct addition of alkaline catalyst. The yield of fatty acyl amido carboxylic acid based surfactant was found excellent calculated based on raw extracted collagen protein hydrolyzate. In this study, prepared product was applied in wet blue split from cow hide as a fatliquoring agent with additional retanning property. Investigations were also focused on the characteristics of prepared leather to evaluate the effect of product on resulted leather. The results of standard physical characteristics such as tensile strength, tear strength, % age Elongation at break, softness, etc. showed significant improvement with lubricating effect on leather through smoothness of surface. In this way, a waste is converted into a useful surfactant product.

Keywords: Leather fleshings, protein hydrolyzate¸ protein based surfactants, cow split leather

1. INTRODUCTION

Solid waste management in most of the developing countries is a major environmental challenge as reported by Daskalopolous [1], because leather processing commonly has been associated with high pollution due to the generation of different organic wastes during traditional manufacturing processes in tanneries as reported by Taylor et al [2]. It is generally known that approximately 200 kg leather product is produced from one ton of wet salted hides. Therefore, a huge amount of solid wastes is generated as previously described [3-5].

This solid waste creates a major problem for tanneries in terms of both their variety and quantity. However a huge amount of proteineous waste is reusable in various useful products after necessary modification as earlier reported [6-7]. But the ways of the leather waste processing are associated to the innovative separation and processing procedures for fats, protein and chromium [2, 6]. Leather fleshing mainly consists of protein, fat and water with dirt as earlier reported [7-8].

Skin fleshings are one of the major solid wastes generated during pretanning operations of leather processing. These skin fleshings contain high protein content (50–60%) and collagen (3–5%) content which are currently being wasted, thus creating the solid waste disposal problem in tanneries as reported in previous research [9-10]. In the previous studies, skin fleshings have been anaerobically digested for the production of methane gas using biomethanation process as reported earlier [11]. These skin fleshings have been used for glue manufacture, enzyme production and animal feed production as reported earlier [12]. Base-catalyzed transesterification

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reaction of raw fleshing oil for the production of fuel has been investigated by preparation of ester product as an alternative fuel as well as a feed stock in lubricant production or cosmetic industry [13]. Limed skin fleshings have been co-digested with biodegradable fraction of municipal solids waste and optimized for biogas production as reported earlier [14]. Transesterification reactions of raw fleshings oil using methanol as the alcohol and alkali catalyst was reported to be preferred in the case of low acid value, sodium hydroxide was used as a catalyst in the reaction have been reported by several authors[15-19]. Enzymatic hydrolysates of waste collagen proteins obtained from leather, edible meat product casings, etc. of mean molecular mass 20–30 kDa have been reacted with dialdehyde starch (DAS) to produce biodegradable hydrogels used for packaging materials such as food, cosmetic and pharmaceutical products [3] and thermoreversible hydrogels as reported in previous study [20]. Leather fleshings have been used for the production of protein based feed for fish cultures. Growth profile was study on the Labeo rohita for duration of eight weeks by supplements with prepared feed. This feed was prepared by raw animal fleshings chemically treated with hydrogen peroxide (approximately 3%), sunflower oil (nearly 10%) and groundnut oil was used in the feed as a lipid source while wheat flour and rice bran (approximately 14%) were used as a carbohydrate source for fermentation as reported earlier [21].

Protein-based surfactants have got special attention due to their hydrophilic emulsifying group with no toxicity on biological systems and strong antimicrobial activity as reported earlier [6]. Consequently protein-based surfactants have been studied for important applications in various areas, such as foods, cosmetics and pharmaceutical formulations, etc as reported earlier [22]. However, high tech purification is needed for such sophisticated product that may be cost effective. In this work, we have investigated on simple and safe side application of this extracted collagen hydrolyzate protein after modification with fatty acid ester into surfactant product for cow split leather.

2. MATERIALS AND METHODS

Tannery Chemicals for processing were purchased from local market and used without further purification. Centrifugation of product was done by using Variable Speed Centrifuge Model 2010H from Scientific LTD, UK. Samples were conditioned prior to physical testing in a standard atmosphere of temperature 20±2 ºC and Relative humidity 65±2 %. The samples were tested according to standard methods [23-29] using a Universal Testing Machine (H5KS) from Tinius Olsen Ltd, UK, having a uniform speed of separation of jaws of 100± 20 /mm for tensile strength and % elongation, tear strength and tearing load. Leather softness tester (ST300) from SATRA was used for softness. Calibrated Equipments were used for testing of leather.

2.1 Leather Fleshing Treatment for Recovery of Protein Hydrolyzate

Leather fleshing wastes (grayish brown colored obtained after fleshing of skins through knife) were received from the tanneries of SITE Area, Karachi stored in refrigerator till further use. After deliming fleshing (1 kg) were hydrolyzed in autoclave at 110 ^{o}C for two hours with the addition of water, 4% magnesium oxide and 2% sodium hydroxide. After two hours fleshings were completely liquefied and resulted a dark colored viscous mass having some insoluble particles. These particles were re-filtered and the solution was freezed. Three fractions were isolated after hydrolysis process using separating funnel as briefly described in Scheme 1. Second fraction was used in this experimental work while other two fractions consisting fats and sludge respectively would be used in other research experiments as per their possible applications. Total solids of all three fractions were determined using the official method as well as delimed leather fleshings as in Table 2.

2.2 Preparation of Amino Acid based Surfactant

In the first step, 250 g protein was dissolved in 200 mL water by heating and stirring to prepare

homogenized solution .Then pH of the protein solution was checked (9.0) and it was used for further reaction. Long chain fatty acid (Lauric acid) 0.1 mole was reacted with 110 g glycerol by adding 1 mL of concentrated high strength Sulfuric acid as a catalyst. This prepared fatty acid ester was added into above protein solution flask. Then, the mixture was heated and stirred for one hour at 60-65^{0}C. During the reaction pH of mixture was maintained at 11-13 by adding drop by drop freshly prepared sodium hydroxide solution. Product was cooled at room temperature and washed twice with water. Transfer the contents in Centrifuge tubes and white Waxy lump product was obtained in the thick paste settled at the bottom of tubes. Prepared surfactant was collected stored in a cool place till further used. The confirmation of product was done through FTIR spectras. The pH of the product was 11.0 and adjusted at 6.5-7.0 with diluted acetic acid solution to apply in leather processing at fatliquoring step.

2.3 Application of the Product

A cow hide was processed up to wet blue stage by conventional chrome tanning process. After preparation wet blue was kept for ageing for 3-4 days at room temperature. A split was achieved using the splitting machine. Then it was shaved at 1.2 mm thickness by shaving process and cut into two equal halves parallel to backbone. Condition of wet blue pieces was normal without any defects. First, both wet blues were washed with 200% water and added 0.2%Acetic Acid, second wash was carried out with 15% water for 15 min each. Wet blues were then basified with 2% Sodium Formate and 0.2% Sodium Bicarbonate for 90 min. Drain the float and Wash. Then 3% dye and 3% (Edaplin MK) was given with 100% water for 15 min drumming 4% (Tanigan O.S) and 12% Quebracho for 30 min drumming. Left Overnight in the drum for thorough dyeing of leather. Next day drum was run for 5 min. Then three times washing was carried with 250% water each for 15 min. Then solution of prepared surfactant 10% in 100% warm water (60^0 C) was applied by continuous drumming for 60 min. After that, 0.3% Formic Acid was given by drumming for 15 min and then 2.5% Chromium Sulfate by drumming for 40 min. Drain the float and Leather was washed two times with water. Finally float was drained. Leathers were horse-up in tannery area then dried at room temperature. The other wet blue was processed with the same procedure and chemical only surfactant dose was applied with 10% commercial synthetic (Derminol ASN) fatliquor. All chemicals were added on shaved weight of wet blue.

3. RESULTS AND DISCUSSION

We have worked on very simple but most important surfactant from the protein hydrolyzates recovered by the hydrolysis of leather fleshing wastes under alkaline conditions as shown in Scheme 1. These solid wastes can be converted into useful by products in many ways such as filler, lubricant, glue, surfactants, etc. as earlier [31]. Before synthesis of product all three fractions were characterized for their contents, physical appearance and % age yield on dry basis (Table 1). Total solids were also determined to calculate the active matters in the material (Table 2). Second fraction showed amino acid composition similar to collagen as previously described [31]. There are two types of methods for the production of surfactants from

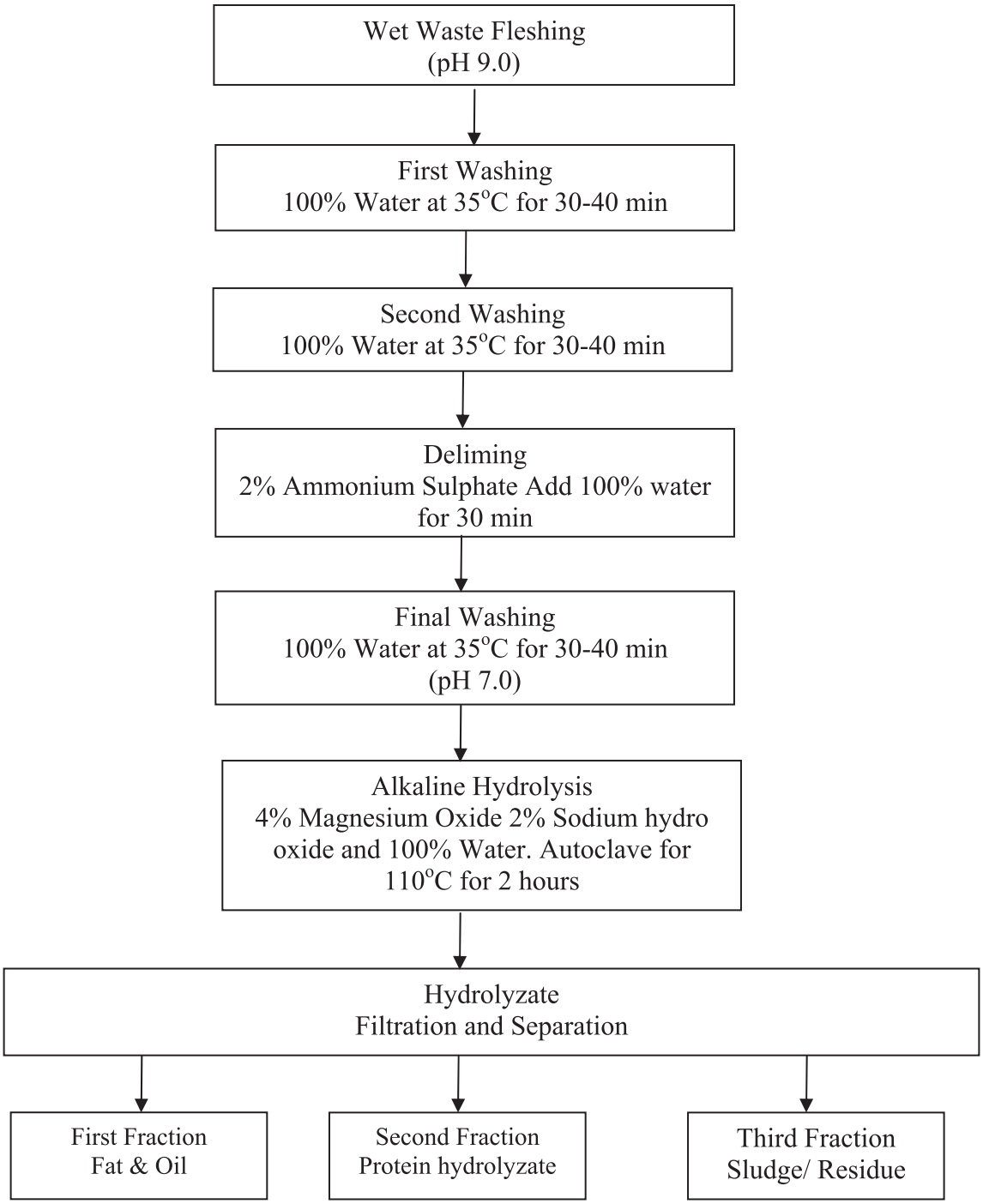
Table 1. Characteristics of recovered fractions**.**

Recovered fractions*	% Yield	Appearance	pH
Fat	20.63	Light Creamy	4.0
Protein Hydrolyzate	65.14	Light Brown	9.0
Sludge /Residue	14.23	Blackish Grey	9.0

*Yield was calculated on moisture-free basis

Table 2. Determination of total solids.

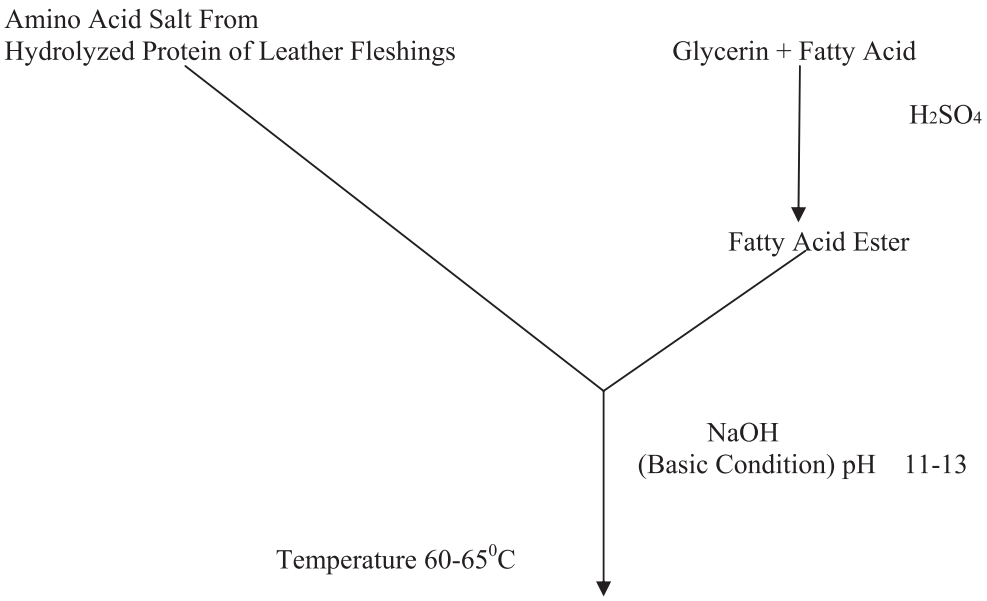
Sample Material	Total Solids (%)
De-limed Fleshings	33.40
Recover Fat	48.74
Protein Hydrolyzate	19.99
Sludge/Residue	23.62



Scheme 1. Separation of fractions from wet raw fleshing waste material.

hydrolyzed protein: 1) biotechnological and 2) chemical methods [32-35]. The well known is the condensation with long-chain carboxylic acids where the acid may be used in the form of chloride, anhydride or various esters as reported earlier [36]. The extracted protein from leather fleshings contain 20 different amino acids in different proportions, these all amino acids reacts with the fatty acid ester of the glycerol in the basic condition as described in the material and methods, thus finally formed a fatty acyl amido carboxylic acid based surfactant as shown in Scheme 2.

This product has the special characteristics due to the two structural features: 1) they contain a long



Scheme 2. Fatty acyl amido carboxylic acid based surfactant.

chain alkyl group for hydrophobic moiety and they contain amido and carboxylic groups acids group for hydrophilic moiety [37]. This product has been applied in the solution form with warmed water in selected wet blue. The prepared surfactant has free base form sites that will react with the acidic sites of leather. In this way the product gives the retanning effect in addition to lubrication. Good result was observed when leather was soaked in the solution and constant drumming for specific period usually about 30 min to 90 min [38]. To study the comparison of standard results of leather, another wet blue was cut in the same size and processed in the similar conditions. The product showed uniform distribution in the leather due to the good interaction of charges. The prepared leathers were evaluated for physical tests. Three samples from each leather were tested and the results are given in (Table 3). All results were improved as compare to reference leather. The difference in shrinkage temperature of surfactant applied leather and the reference was 2 ˚C which is common due to the structural difference in natural skin. The product has increased the strength and the suppleness of leather with lubrication of fibers as shown in the (Table 3). The improvement in all physical properties is due the retanning effect of amino acid. Further research on double action of surfactant will clear the work mechanism.

Table 3. Physical characteristics of the resulted leathers.

Physical Test	Surfactant Applied leather	Reference leather applied Commercial Product
Thickness (mm)	3.46±0.14*	3.23±0.25
Tensile Force at break (N)	297.82±12.73	257.55±6.87
Tensile Strength (N/mm^2)	8.39±0.69	7.811±0.67
Tearing Load (N)	191.00±9.75	152.71±3.69
Tear Strength (N/mm)	78.18+3.98	49.194±2.02
Softness (mm)	3.96±0.37	4.1±0.26

*Standard deviation, calculated from 3 observations of each test.

4. CONCLUSIONS

Protein hydrolyzate from leather fleshing has been separated from other fractions and further converted in to a double action surfactant using fatty acid ester. Application of product has resulted normal soft leather with improved physical characteristics and smooth surface. Therefore, it is concluded that this surfactant from waste protein can be utilized for lubrication with additional retanning benefits.

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Research Article

Solar Flares Data Analysis on application of Probability Distributions and Fractal Dimensions and a comparative analysis of North-South Hemispheric Solar Flares Data Behavior

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Abstract: Since Solar Flares are associated with the sunspots. Solar flares have different length, duration and peakness in different intervals as sunspots have. The Solar Flares index data starting from 1966 to 2008 (Monthly Flare Index Cycles "20, 21, 22, 23") in both North and South hemisphere separately along with total Solar Flares data. This paper compared the North, South and total hemispheric Solar Flares data in perspective of probability distributions such as Gamma, Log-Gamma and Chi-square which tested by Kolmogrove-Smirnov D-test and then their persistency by using fractal dimension. Further we estimate the Hurst exponent with the help of fractal dimension. Fractal dimension shows complex of the data while Hurst exponent represents smoothness of the data. All the tested probability distributions in this paper show persistency and positively correlated. The use of probability distributions allows to represents the uncertainties in the solar flares data. This study will be helpful to verify the long term smoothness of data trends and conclude the results will be present so forecast values will be more accurate.

Keywords: Solar Flares, Gamma Distribution, Log-Gamma Distribution, Chi-Squared Distribution, Fractal Dimension , Hurst Exponent

1. INTRODUCTION

The sun is releasing energy continuously the major types of it are namely solar flares (SF) and coronal mass ejection (CMEs) [15-6]. The explosive event that produces a sudden brightness on the sun is known as SF these flares produces a burst of energetic particles the energy released by the SF is equal to 10^{32} erg [14, 10]. The SF are normally classified by their brightness for example the X-class, M-class and C-class which are the basic types of SF. The X-class SF can produces the major storms on the earth, M-class SF can cause brief radio blackouts at polar latitudes and the C-class SF have no significant consequences [14, 7]. SF occurs near or inside the sunspots but it is not necessary that all the sunspots have the SF so there is a interaction between SF and the sunspots [11, 7].

In this paper we use FD method to observe the complexity of the SF data. Higher the complexity of the data lower will be the smoothness or wise versa. Fractal dimension expresses the space filling property of the data whereas, Hurst exponent represents the smoothness of the data.

The FD is defined by the following expression.

$$\mathrm{FD} = \frac{\text{no of small pieces}}{\text{magnification}} \tag{1.1}$$

FD and H are related as follows

$$F = 2 - H \tag{1.2}$$

FD and H represent the dynamical behavior of the time series data. Particularly, Hurst exponent compares the persistency, and anti persistency and Brownian nature of the time series data.

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Relation (1.2) in this study will be used to analyze the persistency of SF data.

2. MATERIAL AND METHODS

We collected the SF data from WDC (1966 to 2008). We applied most suitable probability distributions on each cycle of SF data as well as for total data. For this kind of estimations we used statistical software Easy Fit (EF).

The second part of this paper is based on the estimations of FD of each SF cycle with total SF data. We have used Fractalyse 2.4.1 software to obtain the FD. We also obtained the parameter H (Hurst exponent) with the help of FD and analyze the persistency of each cycle for North, South and total SF data.

2.1 Probability Distribution Approach

In most of the experimental analysis of any branch of science one often encounters the problems where the probability distributions are applicable. These probability distributions can be helpful in generating the random numbers [2, 5].

In the term of probability distribution the comparative study of SF is very helpful to observe the change and variation on the earth climate. There is a marked correlation between sunspots cycles and earth climate (rainfall and temperature) [4]. The study of probability distributions of random numbers for climatic parameters generally gives the basic knowledge about the physical processes governing in it.

The KST [Kolmogorov-Smirnov *D*-test] for the check of deviation of normality to assessing the nature of the distribution with the real time data. This tests that whether the statistic

$$D = \max |F(x) - G(x)| \qquad (2.1)$$

exceed a critical value in the K-S table or not. Where G(x) is sample cumulative distribution and F(x) is the predetermined cumulative distribution corresponding to a given sample of size n [9]. The normal distribution is well define distribution for continuous variables applied to symmetrically distributed data. Mathematically it can be define as follows:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-1}{2}(\frac{x-\mu}{\sigma})^2}, \quad (-\infty < x < +\infty) \qquad (2.2)$$

Where, σ and μ are the standard deviation and mean respectively of the sample.

The Log-Gamma Distribution (LGD) is define as it is the natural log of variable x which is Gamma distributed, the Log-Gamma Distribution has minimum value 1 when Gamma variable = 0. The LGD is given by

$$f(x) = \frac{[\ln(x-y+1)]^{\alpha-1}(x-y+1)^{-(\frac{1+\beta}{\beta})}}{\beta^{\alpha}\Gamma(\alpha)} \qquad (2.3\ a)$$

where $(1-\beta)^{-\alpha} + \gamma - 1$ is the mean of LGD and $x \geq \gamma$, $\alpha > 0$, $\beta > 0$ [9,2]. Γ and β are the parameters of the distribution. Similarly if the random variable Y follows gamma distribution with parameters α and β , then the likelihood of Y is expressed as,

$$g(y) = \frac{\beta^{\alpha}}{\Gamma(\alpha)} Y^{\alpha-1} e^{-\beta y}, \quad (y \geq 0, \alpha > 0, \beta > 0) \qquad (2.3\ b)$$

Where, α and β are the shape parameter and scale parameter respectively

$$\Gamma(\alpha) = \int_0^{\infty} t^{\alpha-1} e^{-t} dt \qquad (2.3\ c)$$

$$E[Y] = \frac{\alpha}{\beta}, \quad \text{Var (Y)} = \frac{\alpha}{\beta^2} \qquad (2.3\ d)$$

zThe Chi-square Distribution (CSD) which can be approximated by the Normal Distribution (ND) and is given by the following.

$$f(x;n) = \frac{\left(\frac{x}{2}\right)^{\frac{n}{2}-1} e^{-\frac{x}{2}}}{2\Gamma\left(\frac{n}{2}\right)} \qquad (2.4)$$

Where the variable $x \geq 0$ in CSD and the positive integer represents the number of degrees of freedom [2]

2.2 Fractal Dimension

Box-counting or box dimension is one of the most widely used dimensions [3]. Its popularity is largely due to its relative ease of mathematical calculation and empirical estimation. The definition goes back at least to the 1930s and it has been variously termed Kolmogorov entropy, entropy dimension,

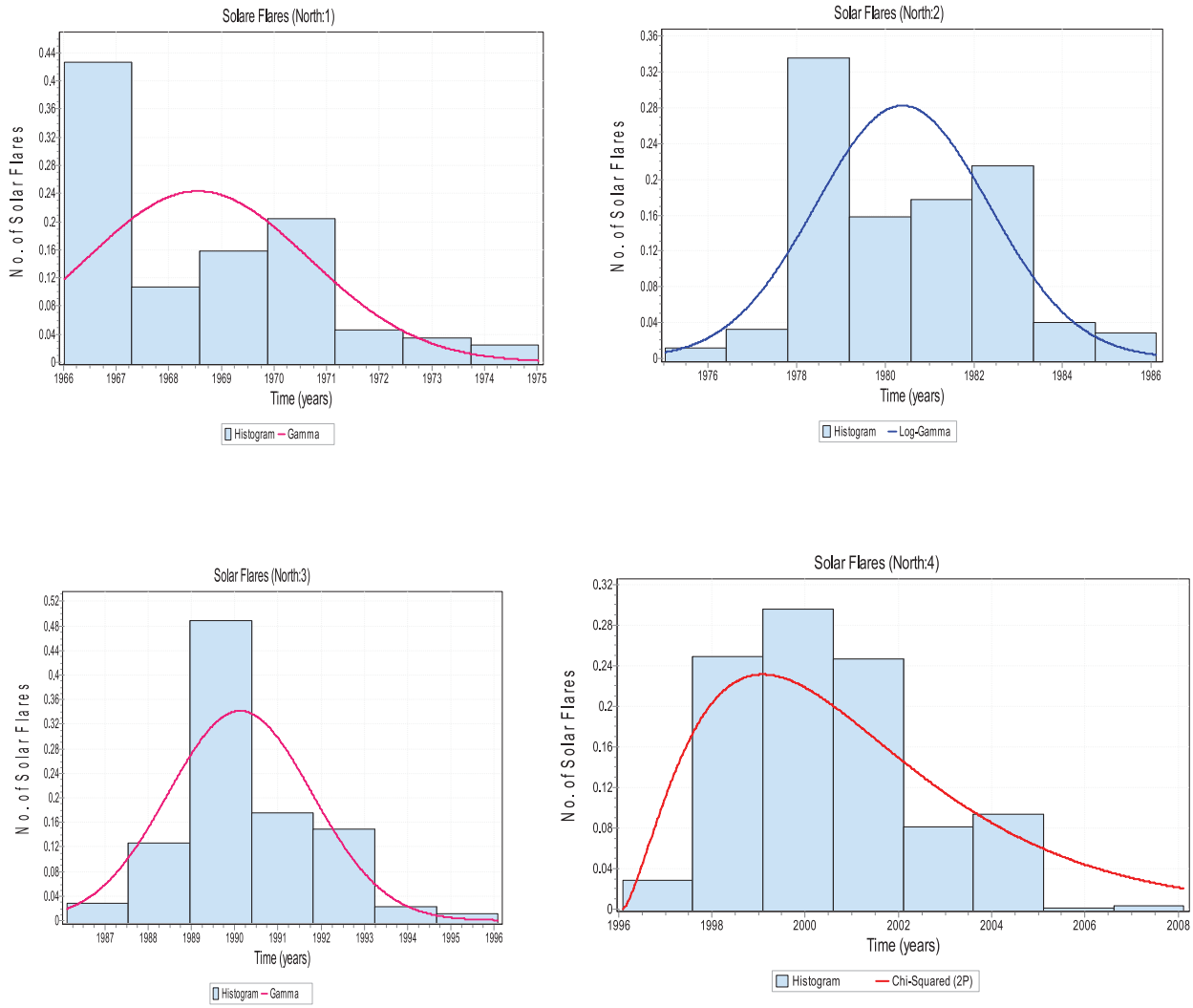

Fig. 1. Probability distributions of 4 cycles of the solar flares North-Hemisphere data.

capacity dimension (a term best avoided in view of potential theoretic associations), metric dimension, logarithmic density and information dimension. We shall always refer to box or box-counting dimension to avoid confusion [3]. This kind of analysis includes more insight into complexity and structure of the system [1]. The FD of self similar object with self similar pieces **r** scaled down by a factor **s** can be express mathematically as.

$$D = \frac{lnr}{lns} \tag{2.5}$$

Fractal dimensions are generally comparison of different numbers that are associated with fractals [8]. The importance of Fractal dimensions is because they can be defined in connection with real world data which can be measured approximately by experiments.

The clouds, trees, feathers, coastlines, neurons networks in the body, dust in the air, the clothes, and the distribution of frequencies, the colors emitted by the sun, and the wrinkled surface of the sea during a storm are attached with the fractal dimensions [1].

Consider the relationship between fractal dimension and Hurst exponent

H	FD	Correlation	Nature of Process
0.5	<1.5	Positive	Persistent
=0.5	=1.5	Zero	Brownian
<0.5	>1.5	Negative	Anti-Persistent

This relationship is summarized by [14] which

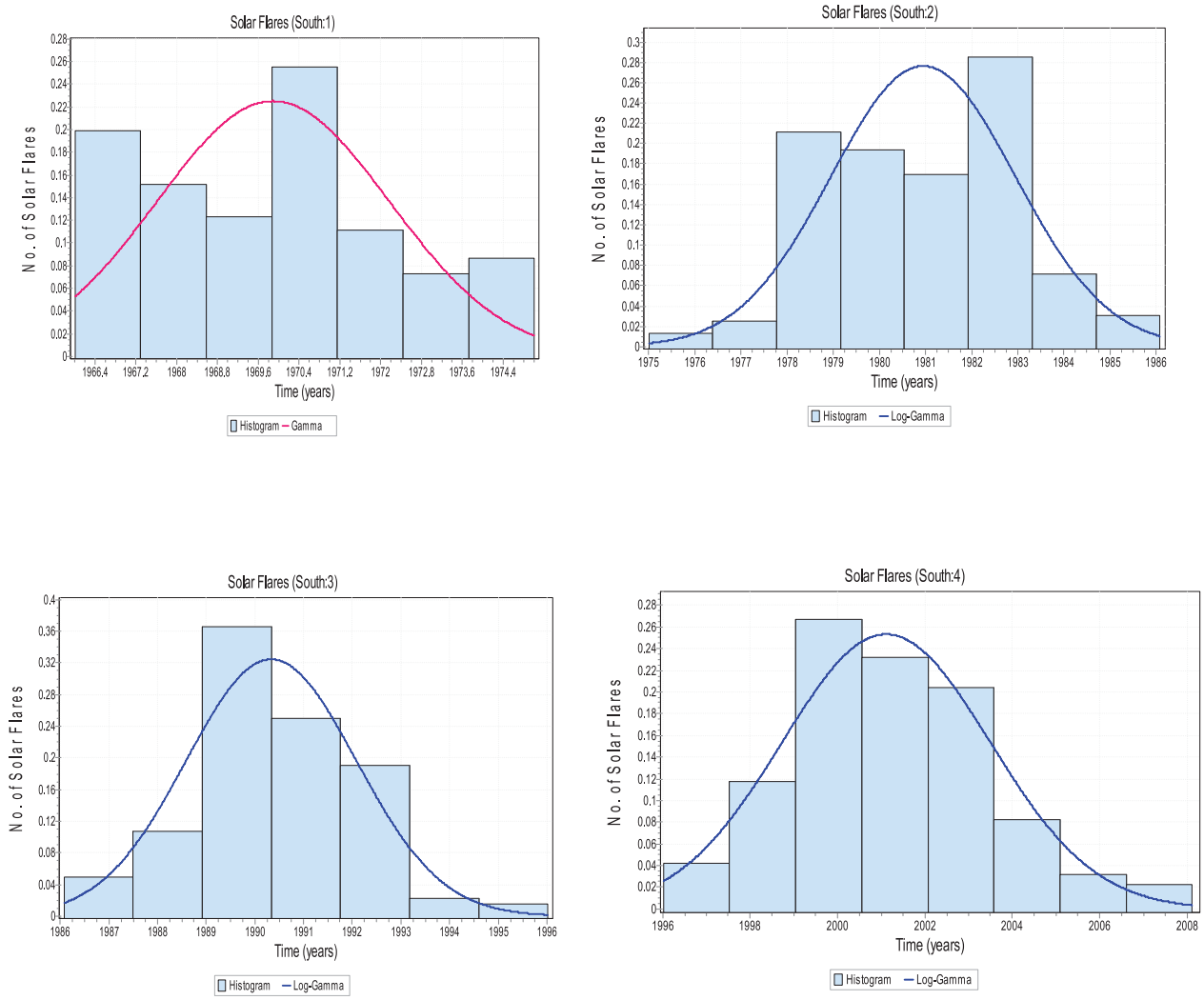

Fig. 2. Probability distributions of 4 cycles of the solar flares South-Hemispheric data.

represent correlation as well as nature of the data.

3. RESULTS AND DISCUSSION

In the first section we have obtained the most suitable probability distributions for SF North-South hemispheric data sets along with the total SF data from 1966 to 2008. The SF data of all kind is maintained by the world data center. The results show that the probability distribution for solar flares cycles 1 and 2 of North-South hemispheric SF data follows the GD and LGD respectively (see figure:1 and 2). This is also same for the total solar flares cycles 1 and 2 (depicted by Tables: 1 and 2). The results represent that the fluctuation of solar flares activity are almost similar for N-S hemispheric and total SF data particularly for Cycle 1 and 2. The variations of probability distributions in solar flares cycles 3 and 4 represent that there is fluctuation in solar activity cycles for North and South hemisphere (depicted by Tables: 1 and 2 also figures:1, 2 and 3). This variation of solar flares activity cycles corresponds to the change in climate in two hemispheres. Solar flares cycles 3 and 4 follows the GD and CSD respectively of North hemisphere (see figures:1 and Table:1). While for South hemisphere the results show GD for cycles 3 and 4 as depicted by the table: 2 (see also figure:2). This represents no change in the probability distribution for South hemisphere SF data. But the change in North hemisphere shows a prolonged

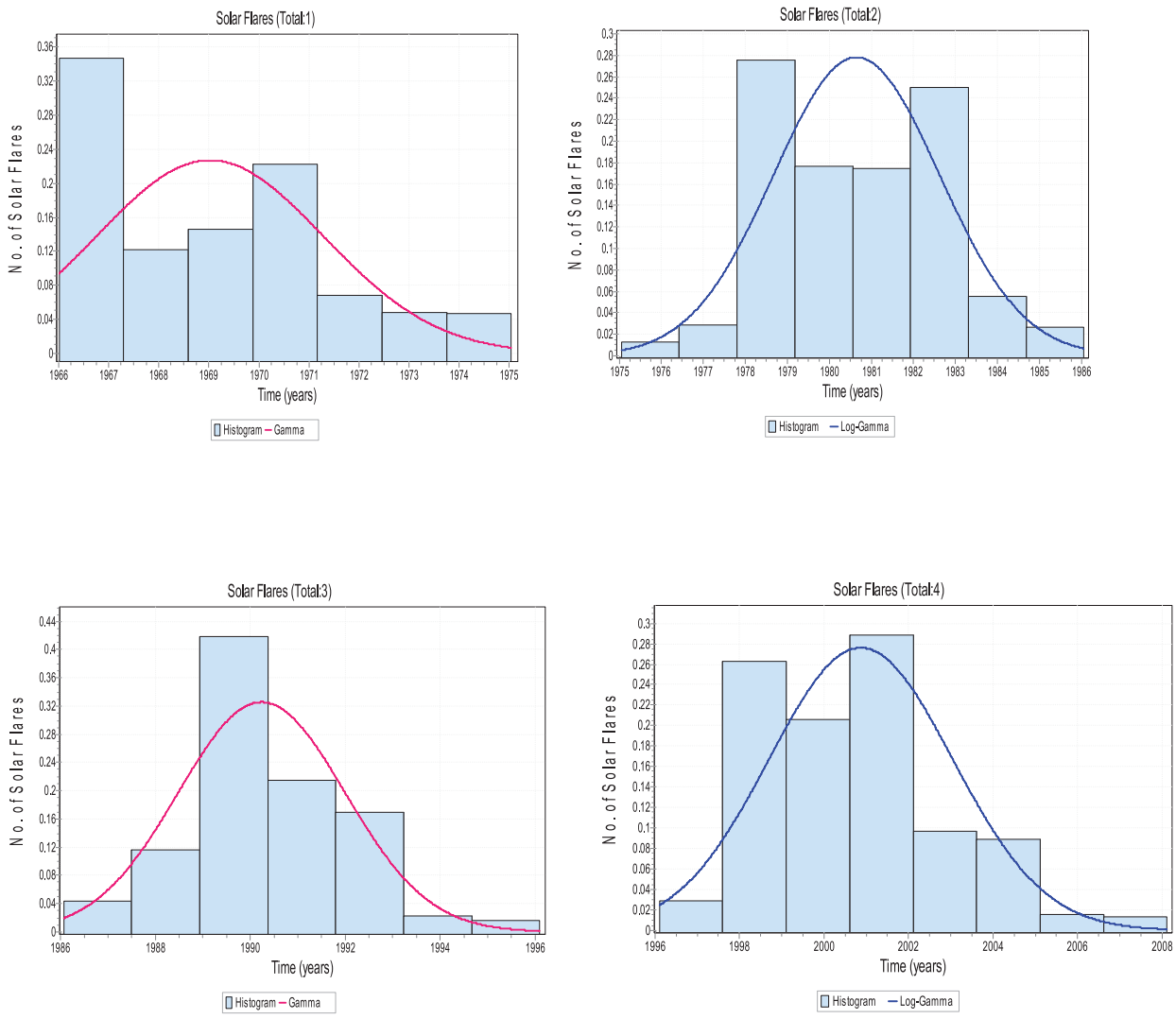

Fig. 3. Probability distributions of 4 cycles of the solar flares Total-Hemispheric data.

more smooth solar flares activity. The total SF data cycle 3 and 4 shows GD and LGD respectively (Table: 3 and figure: 3 depicts the distributions). This kind of variation in total solar activity indicates increase in cycle length but not as much as in North hemisphere. The probability distributions for complete SF data of both hemispheres represents CSD while for complete total SF data it is GD (as depicted by Tables: 1, 2 & 3). Same probability distribution on N-S hemisphere shows that total solar flares activity on two hemispheres have same ascending and descending phase and time while positive tail means prolonged length of cycles. These variation can also be observed with the help of mean and standard deviation of each cycle which shows a fluctuation between the hemispheres as depicted by tables:1, 2 and 3.

In the second section we estimated the FD for each solar flares cycle and observe complexity for both the N-S hemispheres along the total SF data. We also compare the complexity for each hemisphere as depicted in table: 4 depicts the FD of each cycle. The FD for cycle wise data indicates that solar flares data on North hemisphere is more or less complex (smoother) as compared to the South hemisphere. This means that SF data on North hemisphere is more persistent than the South hemispheric SF data and also than the total SF data sets. Table: 4 depicts the basic information and comparison between the fractal behavior of each hemispheric data.

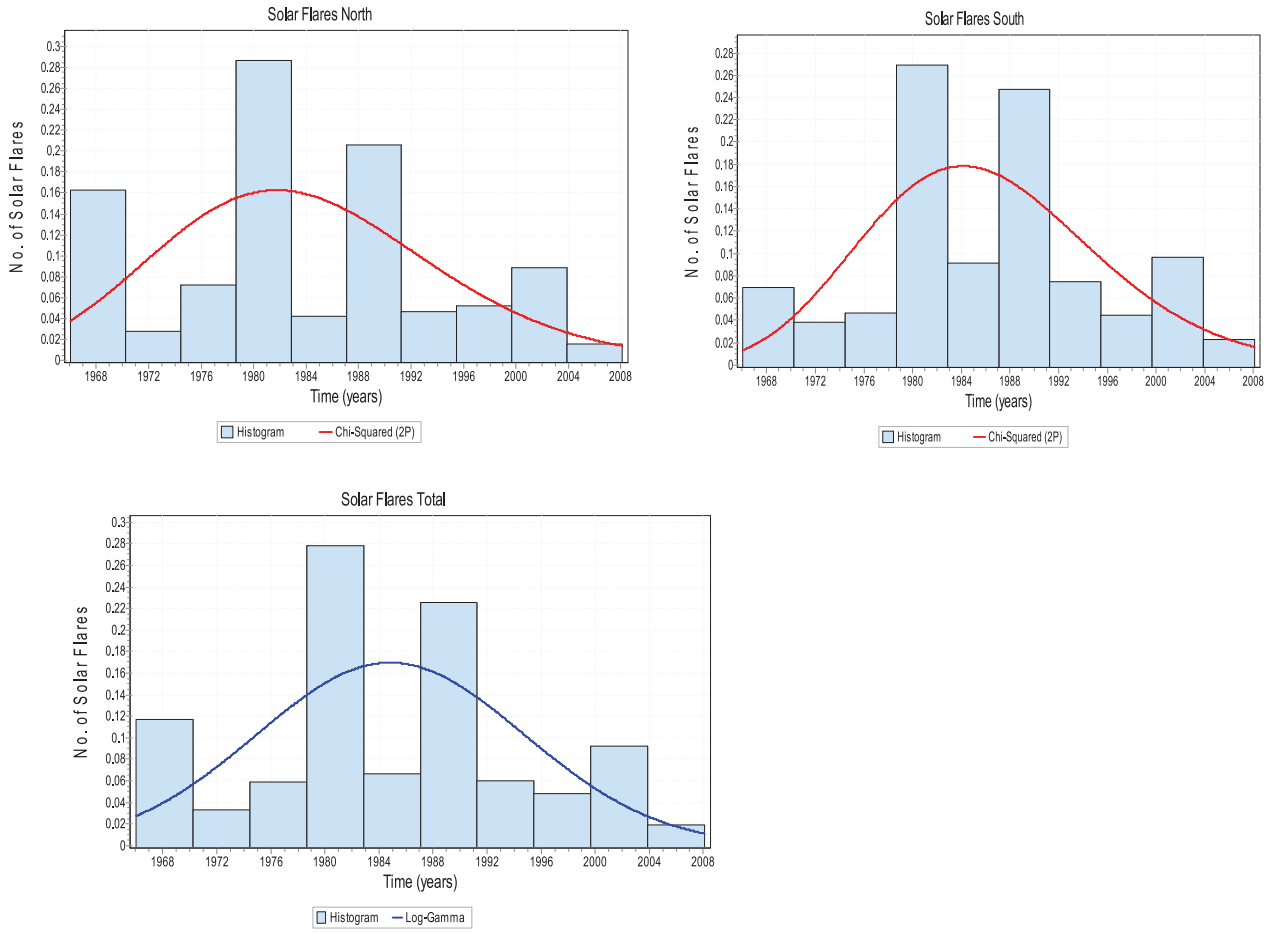

Fig. 4. Probability distributions of North, South and Total solar flares data.

We have also analyzed the probability distributions of the SF data as used above. This also shows persistency in the perspective of FD as depicted by table: 4. It also confirms that persistency of any data points can be analyzed in the perspective of probability distribution. So the technique will be helpful to study the physical nature of any data. The results of FD for North, South and Total SF data indicates that North hemisphere and total SF data are more persistent than South hemisphere SF data. This is because of the shifting of solar flares from North to South and South to North. The North and total SF data have fluctuation in probability distribution in different cycles although South hemisphere SF data have the same probability distribution for cycles 2, 3 and 4 (table: 5). The Hurst exponent for each cycle are also obtained with the help of FD to observe the smoothness and persistency more accurately. Table: 4 depicts H for smoothness.

4. CONCLUSIONS AND OUTLOOK

The first section of this paper contains the probability distributions of 4 cycles of solar flares data. We have compared the probability distributions of North and South hemisphere along with the total solar flares data starting from 1966 to 2008. Tables: 1 and 2 depict the related distribution. The SF cycles have different durations in accordance with sunspots data. The shortest duration of SF data is 9 years and longest is 12 years approximately. Tables: 1 and 2 depict the length of the two durations for N and S hemispheres. The length of cycles 1, 2, 3 and 4 for North, South and Total SF data are same. Cycles 1,

Table 1. North-solar flares.

Cycle	Duration	years	mean	St.dev	Distribution	Statistic(KST)	Parameters
1	1966.01-1975.04	9	2.5659	2.289	Gamma	0.17799	$\alpha=8.6763E^{+5}$ $\beta=0.00227$
2	1975.05-1986.06	11	4.1737	4.7093	Log-Gamma	0.12278	$\alpha=5.9028E^{+7}$ $\beta=1.2860E^{-7}$
3	1986.07-1996.1	9	3.2277	3.6984	Gamma	0.16789	$\alpha=1.4309E^{+6}$ $\beta=0.00139$
4	1996.11-2008.12	12	1.5692	2.3141	Chi-Squared (2P)	0.15405	$\nu=5$ $\gamma=1996.1$
1- 4	1966.01-2008.12	42	2.8887	3.5753	Chi-Squared (2P)	0.09801	$\nu=55$ $\gamma=1928.8$

Table 2. South-solar flares.

Cycle	Duration	years	mean	St.dev	Distribution	Statistic (KST)	Parameters
1	1966.01-1975.01	9	1.3986	1.1179	Gamma	0.13215	$\alpha=7.4762E^{+5}$ $\beta=0.00263$
2	1975.02-1986.08	11	4.1737	4.7093	Log-Gamma	0.10387	$\alpha=5.6661E^{+7}$ $\beta=1.3398E^{-7}$
3	1986.09-1996.02	9	3.2277	3.6984	Log-Gamma	0.13005	$\alpha=7.5050E^{+7}$ $\beta=1.0121E^{-7}$
4	1996.03-2008.12	12	1.5692	2.3141	Log-Gamma	0.1094	$\alpha=4.0743E^{+7}$ $\beta=1.8657E^{-7}$
1- 4	1966.01-2008.12	42	2.7348	3.2472	Chi-Squared (2P)	0.0829	$\nu=46$ $\gamma=1940.1$

Table 3. Total-solar flares.

Cycle	Duration	years	mean	St.dev	Distribution	Statistic (KST)	Parameters
1	1966.01-1975.01	9	3.9061	2.6764	Gamma	0.14652	$\alpha=7.5396E^{+5}$ $\beta=0.00261$
2	1975.02-1986.08	11	4.1737	4.7093	Log-Gamma	0.09863	$\alpha=5.7980E^{+7}$ $\beta=1.3093E^{-7}$
3	1986.09-1996.02	9	3.2277	3.6984	Gamma	0.14317	$\alpha=1.2847E^{+6}$ $\beta=0.00155$
4	1996.03-2008.12	12	1.5692	2.3141	Log-Gamma	0.13373	$\alpha=4.9148E^{+7}$ $\beta=1.5466E^{-7}$
1- 4	1966.01-2008.12	42	5.6232	5.904	Log-Gamma	0.09822	$\alpha=2.3181E^{+6}$ $\beta=3.2757E^{-6}$

2, 3 and 4 have the respective length as 9, 11, 9 and 12 years. Cycle 1 and 2 for North, South and Total SF data follows GD and LGD respectively while cycle 3 of North and Total SF data follows the GD and for South hemisphere SF data shows LGD. The results for cycle 4 show LGD for South and Total SF data and for North hemisphere it follow CSD. Tables: 1 and 2 depict the related information. The probability distribution for complete cycle of North, South hemispheric SF data is CSD and for Total SF data it follows LGD. All the results of probability distribution are tested with the help of Kolmogorov-Smirnov *D*-test. Tables: 1, 2 and 3 depict the statistical analysis. In the second section of this paper we analyzed the complexity of each cycle of SF data and then compared them by

Table 4. FD and H of solar flares data.

Cycle	FD (North)	H (North)	FD (South)	H (South)	FD (Total)	H (Total)
1	1.271	0.729	1.463	0.537	1.394	0.606
2	1.072	0.928	1.282	0.718	1.047	0.953
3	1.121	0.879	1.154	0.846	1.048	0.952
4	1.122	0.878	1.176	0.824	1.112	0.888
1- 4	1.134	0.866	1.241	0.759	1.15	0.85

Table -5. Comparison of distribution and FD.

Cycle	FD (North)	FD (South)	FD (Total)	Distribution North	Distribution South	Distribution Total
1	1.271	1.463	1.394	Gamma	Gamma	Gamma
2	1.072	1.282	1.047	Log-Gamma	Log-Gamma	Log-Gamma
3	1.121	1.154	1.048	Gamma	Log-Gamma	Gamma
4	1.122	1.176	1.112	Chi-Squared (2P)	Log-Gamma	Log-Gamma
1- 4	1.134	1.241	1.15	Chi-Squared (2P)	Chi-Squared (2P)	Log-Gamma

estimating FD using FRACTALYSE 2.4 software. Hurst exponents also computed through FD which is depicted by table: 4. Results show that FD for North and Total SF data are less than those for South hemisphere SF data. This means that SF data for North and Total hemisphere are more persistent than South hemispheric SF data as depicted by table: 4 for FD and H. It also confirms the fact that if FD increases then H decreases. This kind of variation appears because of the difference in the ascending and descending phases of SF activity of each cycle. The cycle will prolong if the difference between the ascending and descending phase of SF activity has greater means and the tail prolongs.

In the end a relation between probability distribution and persistency is provided in table: 5 depicts. All probability distributions show persistency. The mean-tail analysis confirmed the FD-H analysis.

5. ACKNOWLEDGEMENTS

We are very thankful to the World Data Center (WDC) for providing the North, South and Total Solar Flares data. The contents of this paper form part of the first author's doctoral thesis.

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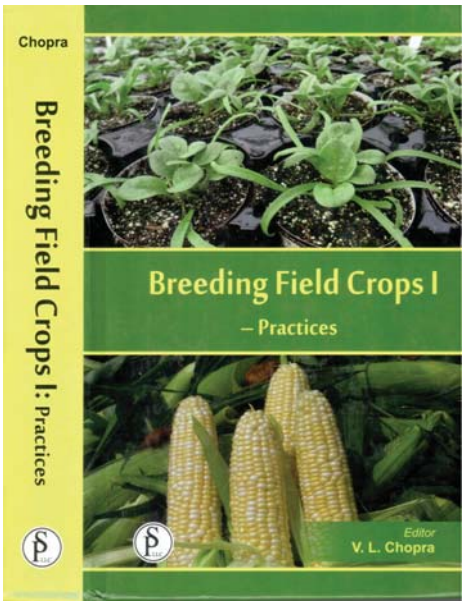
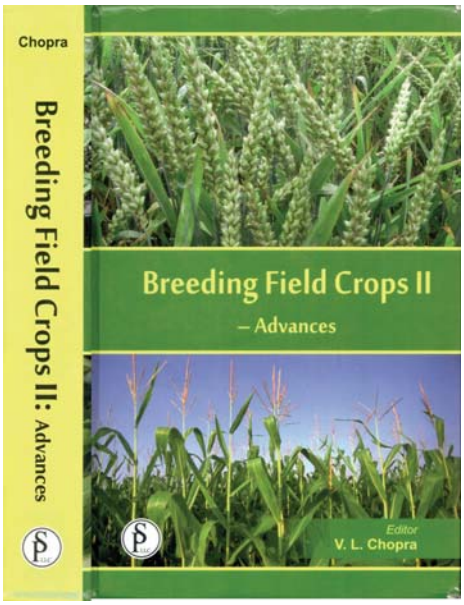
Book Review

Chopra, V.L. (Ed.), Breeding Field Crops (Volume I & II).

Studium Press LLC, Houston, Texas, USA, 650 pp. (2014).





This book is organized into 29 chapters, in two volumes. First volume of the book "Breeding Field Crops I: Practices" has addressed the practices related to improvement of various crops grown in India. In this volume, the authors have discussed the architecture of the crop plants and their breeding methods. The book Volume-I covers most of the major and minor crops, including cereals (i.e., Wheat, Maize, Rice, Sorghum, Pearl Millet), Cotton, Sugarcane, Pulse Crops (i.e., Pigeonpea, Chickpea and Mungbeans), and Oilseeds, (Brassicas, Soybean, Sunflower and Groundnut). There is good uniformity in description of the problems in breeding and use of germplasm and methodology for improvement of various crops.

The volume "Breeding Field Crops II: Advances" introduces modern and upto date breeding approaches, including biotechnology tools, related to crop improvement and developments in field crops. Topics such as breeders rights, patents, biosafety issues and other emerging concepts have been included. This book volume also includes expanded coverage on the role the genetics plays in development of new crop cultivars, and fully explores the exciting new developments in molecular biology. All of the chapters concerning breeding of specific crops, from major to minor crops, contain some new information, largely based on molecular genetics and conventional breeding.

Volume-II of the book also addresses global and local climate change in the contest of limited and reducing natural resources. It covers the area of development of varieties resilient to biotic and abiotic stresses, and nutritionally enriched and nutrient efficient crop varieties. The Volume II mainly counts for the achievements of the last decade and gives insight of the current breeding objectives.

As is well known, seed is one of the most important agricultural input for maximizing yield. The last section of this book deals extensively with maintenance and seed production of improved

cultivars and the practical problems associated with seed production of different crops. Along with relevant examples, this section depends heavily on the author's extensive field experience.

Combining of theory and practice in modern plant breeding has made this book more understandable to the reader. On the whole, this text nicely covers the basics of plant breeding and is suitable for an introduction to the field. It is highly readable, with many good examples and illustrations. Formatting has been streamlined with the incorporation of some good photographs and tables.

The authors have tried to make this book a state of the art publication in the field of plant breeding with a vast coverage of all the fields by providing updated and detailed information regarding crop areas, production, productivity trends, origin, evolution, taxonomy, breeding systems and release of crop varieties of 14 major crops of the Indo-Pak Subcontinent.

For examining the breeding methods and the methodology for improving various crop plants, this book is an important contribution to understanding the breeding approaches which can be used particularly in the Subcontinent. The chapters in the book carry much work done by the breeders in the Subcontinent which is more useful for the breeders from this area and such information is not much reflected in the international books with other origins. Thus this book is a useful source of fundamental information for graduate students and professional plant breeders.

On the whole, the book is a good effort but it lacks some important crops and aspects. For example, forage crops are missing and only sugarcane is considered amongst the vegetative propagated crops. Similarly, potato is also missing from field crops though it should not have been ignored because of its importance in the Subcontinent. Though book is expected to cover all plant breeding and research activities in the Subcontinent, but the chapters have relied upon and emphasized mostly the achievements within India and the relevant breakthroughs and achievements in other parts of the Subcontinent are ignored or not entertained.

This book, on the whole, is highly informative and well written in simple English, which should make it a ready reference tool on plant breeding for students and the applied scientists related to crop improvement.

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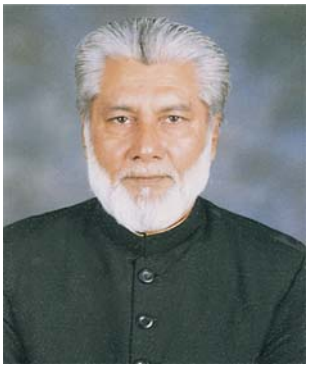
Pakistan Academy of Sciences

Obituary



Prof. Dr. Muhammad Zafar Iqbal (1942-2014)

Prof. Dr. Muhammad Zafar Iqbal was born in Lahore (British India) on 20th July, 1942. He was elected Fellow of the *Pakistan Academy of Sciences* in 1990. Lately, he was working as Rector, Superior University, Raiwind Road, Lahore. He obtained his B.Sc. and M.Sc. degrees in 1960 and 1962, respectively, from University of the Punjab, Lahore. In 1970, he got his Ph.D. from University of Bristol, UK. He worked as a Postdoctoral Fellow in University of Georgia, Athens, USA from 1976 to 1978.

During his illustrious career, Dr Zafar Iqbal held various positions, including Registrar, University of Health Sciences, Lahore; Acting Vice Chancellor, Pro-Vice Chancellor, Registrar, Director of Institute of Chemistry, and Dean of Faculty of Science, at University of the Punjab; Demonstrator, Chemistry Department, University of Bristol, UK; and Acting Chairman, Board of Intermediate and Secondary Education, Lahore.

In view of his significant contributions in the field of higher education, Prof. Zafar Iqbal earned many honors and awards which included *Sitara-i-Imtiaz*; *Tamgha-i-Imtiaz*; Albert Einstein World Award of Science, Germany; Best Teacher Award by University Grants Commission; Open Gold Medal by *Pakistan Academy of Sciences;* Prize by National Book Council, Govt. of Pakistan; First Prize by Pakistan Writers Guild; Medal by Science Forum, Lahore; World Cultural Council Award for contributions in Science; and Cash award as Distinguished Scientist by Ministry of Education, Govt. of Pakistan.

Prof. Zafar Iqbal was Life Member of Pakistan Institute of Chemists, Chemical Society of Pakistan, and Pakistan Association for Advancement of Science; and Member of Scientific Society of Pakistan, and Energy Society of Pakistan. He was Fellow of Pakistan Institute of Chemists, Royal Society of Chemists, UK, and Pakistan Chemical Society. Currently, he was President of Pakistan Institute of Chemists.

Prof. Zafar Iqbal's areas of research included Inorganic and Analytical Chemistry with special interest in Organometallic and Coordination Compounds, and Environmental Pollution of Air, Water and Food. He published a number of research papers both in national and international journals of repute. Also, he authored a number of books in the area of Chemistry. As evidenced by his book "*Quran-e-Hakeem aur Science*" (in Urdu) published in 2010, Prof. Zafar Iqbal also had a good knowledge and commitment towards the region.

I had known Prof. Zafar Iqbal personally since 1960 when he was a student of M.Sc. at Institute of Chemistry, University of the Punjab. After his appointment as Lecturer at the same Institute, I had the chance to interact with him quite frequently as my junior colleague in the University. He rose gradually from the rank of Lecturer to Assistant Professor, Associate Professor, Professor, and Dean of Faculty of Sciences by his shear devotion and hard work. He was a good teacher and was quite popular among students, particularly those who worked under him as researchers.

Temperamentally, Prof. Zafar Iqbal was a social

person, who could pull on nicely with his juniors and seniors. He possessed cool temperament, smiling face, and decent manners. He will never like to offend anybody.

In the death of Prof. Zafar Iqbal, Pakistan in general and *Pakistan Academy of Sciences* in particular have lost an eminent chemist. May the Almighty Allah rest his soul in eternal peace and give fortitude to his family to bear this irreparable loss! Amin.

In the end, I wish to place on record my sincere appreciation to Dr. Abdul Rashid, FPAS, and Editor-in-Chief of the PAS Journal, in the preparation of this script.

Prof. Dr. M. D. Shami, *SI*
Vice President,
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ACKNOWLEDGEMENTS (font size 10)**:** In a brief statement, acknowledge financial support and other assistance.

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3. Kay, R.R. & C.R.L. Thompson. Forming patterns in development without morphogen gradients: differentiation and sorting. *Cold Spring Harbor Perspectives in Biology* 1: doi: 10.1101/cshperspect.a001503 (2009).

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4. Luellen, W.R. *Fine-Tuning Your Writing*. Wise Owl Publishing Company, Madison, WI, USA (2001).
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