Effect of spinning parameters on mechanical strength of polysulfone and polyvinylidene difluoride capillary ultrafiltration membrane

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ARTICLE INFO

Received: 25.06.2021
Revised: 20.07.2021
Accepted: 28.07.2021

Keywords: *Capillary membrane, Polysulfone, Polyvinylidene difluoride, Ultrafiltration, Ultimate Tensile strength (UTS)*

ABSTRACT

Fiber breakage often limits the life of the ultrafiltration capillary module and calls for the module replacement in an operating installation. Ultimate tensile strength (UTS) of the capillary fiber is thus an important qualifying parameter along with the separation performances and hence development of capillary membranes having enhanced mechanical strength is need of the time. In this paper, the effect of various governing parameters of wet spinning process for making UF capillary membrane fibers namely, polymer casting composition, bore fluid flow rate and drying conditions on ultimate tensile strength of polysulfone and polyvinylidene difluoride capillary membranes have been studied. It was observed that the fiber strength increases with increase in bore fluid flow rate and concentration of drying solution. Addition of surfactant in drying medium has shown decreased fiber strength.

1. Introduction

Advances in membranes have led to significant inroads in the separation technologies in many areas because of flexibility and performance reliability of membrane system, cost competitiveness, increasing demand and environmental awareness as reported by Eykamp W. Ultrafiltration (UF) is one of the pressure driven membrane process having wide industrial applications. One such application is pretreatment of seawater in place of conventional pretreatment system. UF process offers advantages such in being modular system, ease of operation and is a robust alternative to conventional treatments which is known to be complex, labour intensive and requiring larger footprint area as reported by Voutchkov N. UF membrane in capillary configuration has high packing density is amenable for easy backwash, causes lower pressure drop, etc. Polysulfone (PSf) and polyvinylidene difluoride (PVDF) are widely used polymer materials for preparation of capillary UF membrane as described by

Mamtani V. S. *et al.* The advantages of these polymers are that the PSf has high mechanical strength, good thermal and chemical resistance while PVDF is a specialty thermoplastic fluoropolymer having outstanding oxidative, thermal and hydrolytic stability.

During application of UF capillary membranes, fiber failure has been reported with an annual fiber failure rate in between 1 to 10 per million fibers as reported by Gijsbertsen-Abrahamse A. J. et al. Fiber failure causes leakage of feed water to the permeate side of the membrane resulting in deteriorating the quality of the permeate. Failure of fiber can be caused by chemical attack, faulty installation, presence of foreign bodies or poor membrane strength. Prediction of fiber failure due to chemical attack, faulty installation and due to presence of foreign body can be identified and corrective actions can be taken either by replacement of membrane material compatible to chemicals present in the feed, by proper installation of membrane & module, and by proper treatment of feed before sending it to UF membrane module. However, Childress Amy E. *et al.* reported the failure of fiber resulting from an external load applied under normal operating conditions, necessitates consideration of the development of the membrane fiber with sufficient strength to withstand the required operating pressure condition. Mechanical properties especially, tensile strength is very important parameter to be considered for capillary membrane fibers as they are self-supporting. When a high pressure is applied to a fiber with a low ultimate tensile strength (UTS), the fiber may break whereas a fiber with high UTS can easily withstand higher pressures.

Normally capillary membranes are prepared by wet spinning phase separation process wherein a polymer dissolved in appropriate solvent along with suitable additives is used. The final tensile strength of the fiber is affected by various capillary membrane preparation process parameters namely polymer concentration, type of polymer chosen, bore fluid flow rate, fiber spinning rate [Qin Jianjun *et al.*] drying methodology, etc. During the preparation membrane symmetry or asymmetry is decided which plays a significant role in the strength of the final fiber. Further, fiber diameter and wall thickness which contributes to strength of the fibers is also dependent to a lesser extent on the preparation process parameters. As very large formulation space exists for making capillary membrane using PSf or PVDF polymers, there is wide flexibility in preparing the membrane for a particular application with sufficient tensile strength. The objective of this work is to study systematically the effect of polymer concentration, type of polymer chosen, bore

fluid flow rate, drying methodology on the final strength of the PSf and PVDF capillary fiber. More details study is focused on PVDF polymer which has comparatively lower strength inherently than the PSf polymer.

2. Experimental

2.1 Materials

Polysulfone (Av. molecular weight: ~50-60KDa, Solvey India make), polyvinylidene difluoride (Av. molecular weight: ~300KDa, Solvay Solef make), N-methyl pyrrolidone (NMP; 99.0% pure, Sigma-Aldrich make), polyvinylpyrrolidone (PVP, Molecular Weight: 40KDa, Sisco Research Laboratory make), glycerol (Sisco Research Laboratory make) and surfactant 'sodium lauryl ether sulfate (Sisco Research Laboratory make)' were used as such without further purification.

Table 1. Compositions of PVDF/PSf dope solution

Components of dope solution	Compositions (wt. %)			
Polymer	17.86 (PSf)	15.7 (PVDF)	17.2 (PVDF)	17.86 (PVDF)
PVP	12.6	12.6	13.8	12.6
NMP	69.54	71.7	69.0	69.54

2.2 Process Set-up and Membrane Preparation

The dope solutions were prepared using either PSf or PVDF homopolymer along with PVP and NMP at different compositions given in Table 1. The dope solution was pressurized under nitrogen pressure of 1 bar above atmospheric pressure along the annular gap in the spinneret. The bore fluid (UF treated service water) was pumped through the axial bore of spinneret using peristaltic metering pump. The polymer dope solution and the non-solvent 'water' are contacted at the outlet of spinneret (O.D = 2 mm and I.D = 1 mm; Fig. 2) causing phase inversion and capillary membrane formation. As depicted in Fig.1, the nascent capillary fiber coming out from the spinneret is passed into water bath (external coagulant) where solvent is replaced by water. During the membrane preparation, dope solution is kept at ambient temperature in the range of 24-26°C; humidity was in the range 45-51%; bore fluid and external coagulant were deionized water. The air gap between spinneret and external coagulant bath was maintained in the range of 100-120 mms. Further, the water bath (about 200 litres) was drained and replenished each day for 4 days after which capillary membrane fibers were dried with glycerol at different compositions (2-10 vol. %). In few experiments, washing with surfactant sodium lauryl sulfate (0.2 %) was used before drying with glycerol (10 vol. %).

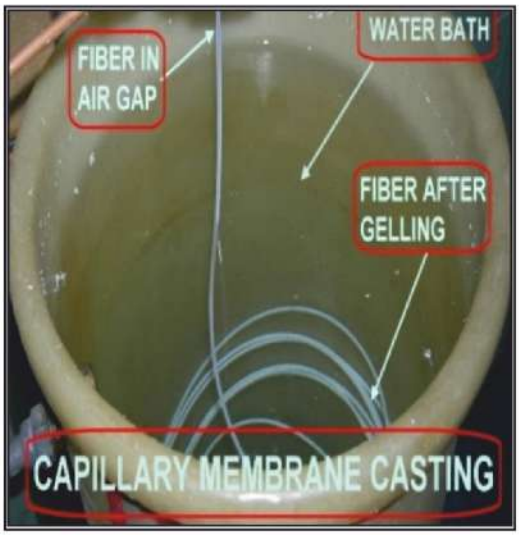

Fig. 1 Spinning of capillary membrane

Fig. 2 Dismantled spinneret

Fig. 3 Universal Testing Machine (UTM)

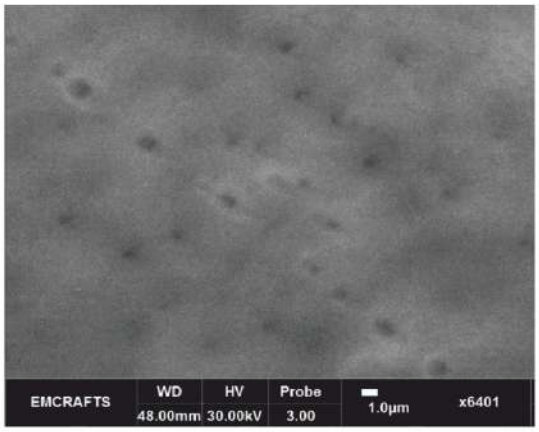

Fig. 4 SEM image of outer surface of PSf capillary membrane

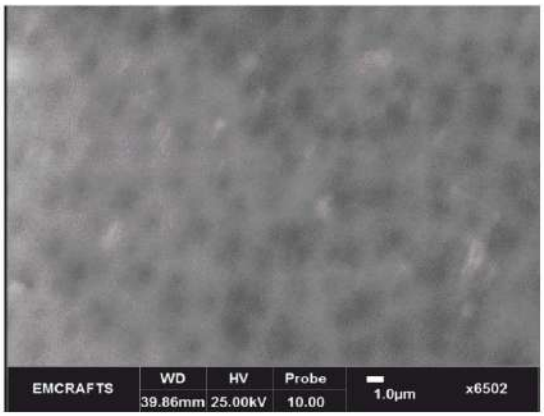

Fig. 5 SEM image of outer surface of PVDF capillary membrane

2.3 Capillary Membrane fiber testing

Fibers of capillary membrane were tested with Universal Testing Machine (UTM) [make: HEMETEK LRX plus, Llyod Instruments] for its Tensile strength as shown in Fig. 3. The testing was done by keeping clamp distance of 120mm & extension rate of 50mm/min at 22-25°C. The outer surfaces of capillary membrane were inspected using Scanning Electronic Microscopy (SEM) [make: EMCRAFTS, Cube 100]. SEM samples of the membranes were prepared by coating the membrane with Gold using Ion Coater (EMCRAFTS).

3. Results and Discussion

The outer surfaces of PSf and PVDF capillary membranes were inspected using SEM as shown in Fig. 4 & Fig. 5. The images shows that both the membranes do not have any surface defects and PSf capillary membrane has more porosity than PVDF membrane. The approximate pore diameter for both the membranes is in the range of 0.5 to 2 µm.

The fibers were further tested with UTM. The effects of nature of the polymer, composition of polymer in dope solution, bore fluid flow rate and different drying conditions on the strength of the fiber were studied by obtaining the final UTS values of the fiber and the Stress vs. Strain curve for each fiber. The results are described and discussed in the following sections.

3.1. Effect of polymer (PSf and PVDF) in dope on UTS of the final capillary membrane fiber

The PSf and PVDF capillary membrane fibers prepared under the same polymer dope composition pressurized by Nitrogen gas at 2 bar and under same bore fluid rate (8ml/min) with drying condition of 10 % glycerol without washing with surfactant were tested for finding UTS. As can be seen from Table 2, the UTS of PSf (2.7 MPa) is more compared to UTS of PVDF (1.25 MPA). The typical Stress vs. Strain relationship obtained for PSf and PVDF fibers are shown in Fig.6 and Fig.7 respectively. The stress and the strain that PSf capillary membrane fiber can sustain are much higher than that from PVDF. The curves obtained are in relation to the strength of pure polymer [higher for PSf (69 MPa) than that of PVDF (43 MPa)] as reported by Mamtani V. S. *et al*.

Table 2. Ultimate Tensile Strength for capillary fibers membrane made from different polymers (PSf and PVDF)

Sl. No.	Name of the parameter	PSf	PVDF
1	PVDF, (wt. %)	17.86	17.86
2	PVP, (wt. %)	12.6	12.6
3	NMP, (wt. %)	69.54	69.54
4	Drying (Glycerol vol.% for 24 hours)	10	10
5	Bore fluid flow rate, (ml/min)	8	8
6	Tensile Strength, (MPa)	2.7	1.25

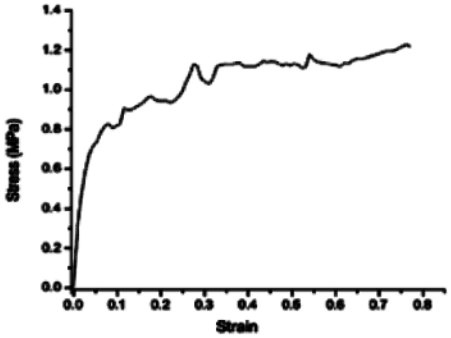

Fig.6. Stress vs. Strain for PSf capillary membrane fiber (17.86 wt. % of PSf in dope)

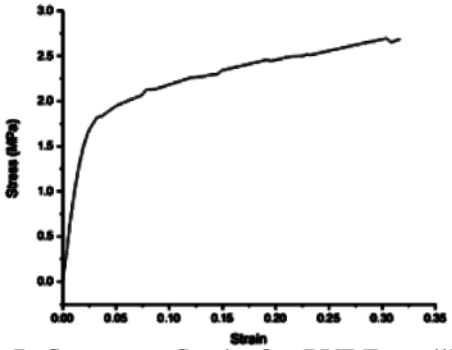

Fig.7. Stress vs. Strain for PVDF capillary membrane fiber (17.86 wt. % of PVDF in dope)

3.2. Effect of polymer composition (PVDF) in dope solution on UTS of the final capillary membrane fiber

PVDF capillary membrane fibers prepared under different polymer dope composition pressurized by Nitrogen gas at 2 bar and under same bore fluid rate (8ml/min) with drying condition of 10 % glycerol without washing with surfactant were tested for finding UTS. As can be seen from Table 3 that the UTS of fiber increases with increase in polymer concentration in the dope solution. The typical Stress vs. Strain relationship obtained for PVDF fibers with different composition of PVDF in dope viz. 15.7 wt. %, 17.2 wt. % and 17.86 wt. % are shown in Fig.8, Fig.9 and Fig.7 respectively. The reason for increased strength in fiber can be attributed to increased packing of the polymers and reduction of the porosity. It can also be noted that the strength increases with polymer composition significantly initially however further increase in polymer composition increases strength slightly. Also increase in PVDF composition more than 18 wt. % in NMP dope solution results in polymer chunk formation which cannot be used for spinning capillary membrane. Hence, to obtain higher strength, the polymer composition for PVDF in the NMP dope can be restricted in the range of 17-18 wt.%.

Table 3. Ultimate Tensile Strength for PVDF

capillary membrane fibers made at different polymer composition

Sl. No.	Name of the parameter	Batch 1	Batch 2	Batch 3
1	PVDF, (wt. %)	15.7	17.2	17.86
2	PVP, (wt. %)	12.6	13.8	12.6
3	NMP, (wt. %)	71.7	69.0	69.54
4	Tensile Strength, (MPa)	0.85	1.20	1.25

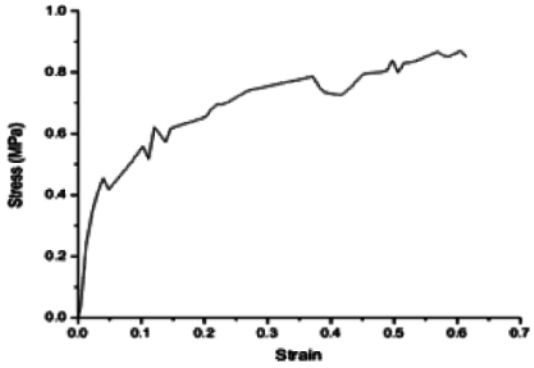

Fig.8. Stress vs. Strain for PVDF capillary membrane fiber with 15.7 wt. % of PVDF in dope and bore fluid flow rate of 8 ml/min

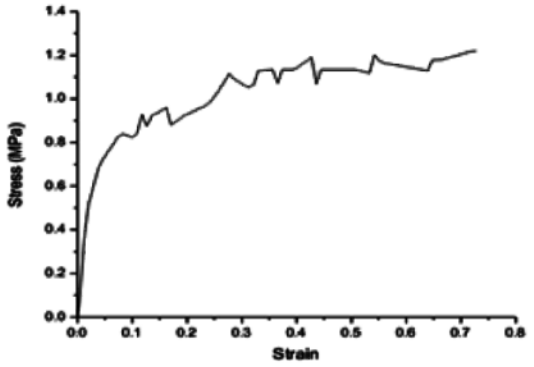

Fig.9. Stress vs. Strain for PVDF capillary membrane fiber with 17.2 wt. % of PSf in dope and bore fluid flow rate of 8 ml/min

Table 4. Ultimate Tensile Strength for PVDF capillary membrane fibers made at different bore fluid flow rate

Sl. No.	Name of the parameter	Batch 1	Batch 2	Batch 3
1	PVDF, (wt. %)	17.2	17.2	17.2
2	PVP, (wt. %)	13.8	13.8	13.8
3	NMP, (wt. %)	69.0	69.0	69.0
4	Drying (Glycerol vol.% for 24 hours)	10	10	10
4	Bore fluid flow rate, (ml/min)	6	8	10
5	Tensile Strength, (MPa)	1.10	1.20	1.23

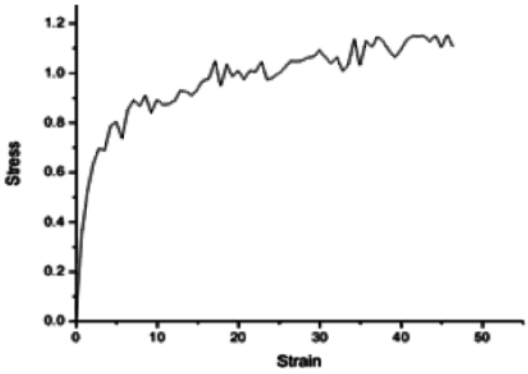

Fig.10. Stress vs. Strain for PVDF capillary membrane fiber with 17.2 wt. % of PVDF in dope and bore fluid flow rate of 6 ml/min

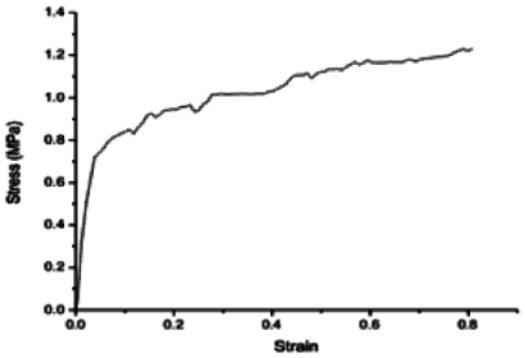

Fig.11. Stress vs. Strain for PVDF capillary membrane fiber with 17.2 wt. % of PVDF in dope and bore fluid flow rate of 10 ml/min

3.3. Effect of bore fluid flow rate on UTS of the final capillary membrane fiber

The PVDF capillary membrane fibers prepared under same polymer dope composition pressurized by Nitrogen gas at 2 bar with different bore fluid rate (6ml/min, 8ml/min and 10 ml/min) under same drying condition of 10 % glycerol without washing with surfactant were tested for finding UTS. The UTS data for PVDF capillary membrane fibers made at different bore fluid flow rate is given in Table 4. It can be seen that the UTS of fiber increases with increase in bore fluid flow rate. The typical Stress vs. Strain relationship obtained for PVDF fibers with different bore fluid flow rate viz. 6ml/min, 8 ml/min and 10 ml/min respectively are given in Fig.10, Fig. 9 and Fig.11 respectively. The reason for increased strength in fiber can be attributed to increased solvent removal rate by bore liquid at higher bore fluid flow rate and hence reducing the pore size of the capillary membrane fiber. It is also be noted further that similar to polymer composition, the increase in bore fluid flow rate from 6ml/min. to 8ml/min. increases the strength significantly but from 8ml/min to 10 ml/min., strength of the fiber increases slightly. The higher bore fluid flow rate is desirable for obtaining higher fiber strength, however the performance of the membrane is also to be considered while fixing the bore fluid flow rate as it can significant effect the pore size of the membrane and thus change the separation characteristics.

Table 5. Ultimate Tensile Strength for PVDF capillary membrane fibers made with different drying conditions

Sl. no.	Name of the parameter	Batch 1	Batch 2	Batch 3	Batch 4
1	PVDF, (wt. %)	17.2	17.2	17.2	17.2
2	PVP, (wt. %)	13.8	13.8	13.8	13.8
3	NMP, (wt. %)	69.0	69.0	69.0	69.0
4	Bore fluid flow rate, (ml/min)	8	8	8	8
5	Surfactant wash vol.% + Drying (Glycerol vol.% + water for 24 hours)	Yes (0.2% surfactant washing); 10% glycerol+ 90% water	No; 2% glycerol+ 98% water	No; 6% glycerol+ 94% water	No; 10% glycerol+ 90% water
6	Tensile Strength, (MPa)	0.93	1.05	1.1	1.20

3.4. Effect of drying methodology and surfactant washing on UTS of the final capillary membrane fiber

The PVDF capillary membrane fibers prepared under same polymer dope composition pressurized by Nitrogen gas at 2 bar with same bore fluid rate (8ml/min) under different drying conditions of (2%, 4%, 10%) with or without washing with surfactant which were further tested for finding UTS. As can be seen from Table 5 that the UTS of fiber increases with increase in glycerol concentration in drying solution. Hence, a higher glycerol content (10 vol.% or more for PVDF membrane prepared using NMP) in drying solution is desirable so that water inside fibers is replaced by glycerol and pores do not collapse when the fiber is dried. It was also be noted that the washing of fiber with surfactant (sodium lauryl sulfate at 0.2 vol. %) decreased the strength of the fiber significantly as further drying with 10% glycerol solution also could not improve the strength. However, visible smoothness was seen in the fibers having surfactant wash before drying while those fibers without surfactant washing were little rough by visual observation. The typical Stress vs. Strain relationship obtained for PVDF fibers with different drying conditions are shown in Fig.12, Fig.13, Fig.14 and Fig.15 respectively. The reason for increased strength in fiber with increase in glycerol composition in drying solution can be attributed to higher deposition of glycerol in the pores and thus preventing the pore collapse and hence increasing the strength of the fiber.

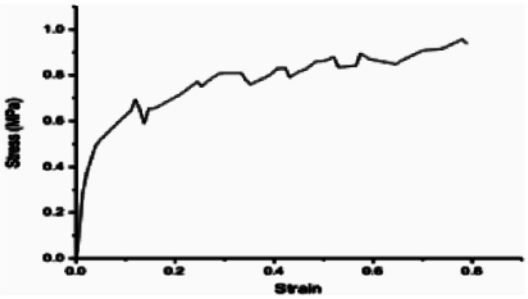

Fig.12. Stress vs. Strain for PVDF capillary membrane fiber with drying condition of 0.2 vol. % sodium lauryl sulfate surfactant washing followed by glycerol (10 vol. %) solution drying

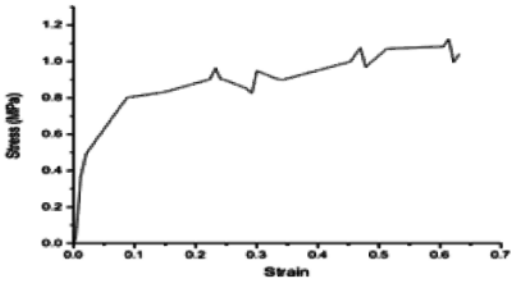

Fig.13. Stress vs. Strain for PVDF capillary membrane fiber with drying condition of no surfactant washing and by glycerol (2 vol. %) solution drying

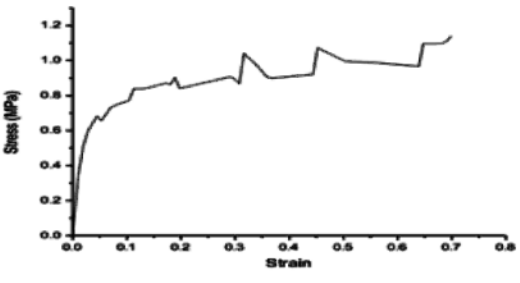

Fig.14. Stress vs. Strain for PVDF capillary membrane fiber with drying condition of no

surfactant washing and by glycerol (6 vol. %) solution drying

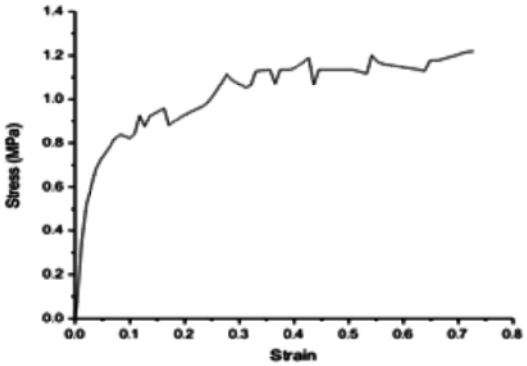

Fig.15. Stress vs. Strain for PVDF capillary membrane fiber with drying condition of no surfactant washing and by glycerol (10 vol. %) solution drying

4. Conclusions

The strength of capillary membrane fiber is strongly related to the polymer used, the composition of the polymer in dope solution, bore fluid flow rate and drying condition among other parameters. The UTS for PSf capillary membrane fiber is higher as compared to PVDF. The increase in concentration of the polymer in casting dope solution increases the strength significantly initially and then later slightly in lower rate. The PVDF polymer concentration in the NMP dope should be in the range of 17-18 wt.% for having good mechanical strength of the fiber without sacrificing separation performance properties. Increase in bore fluid flow rate increases strength of the fiber significantly initially and later slightly but it can also change the separation characteristics of the membrane significantly. Optimum bore fluid flow rate should be decided based on the desired strength of the fiber and its desired separation characteristics. The increase in glycerol concentration in drying solution increases the strength of the fiber and accordingly higher glycerol content in drying solution is desirable but it should be related to overall pore volume of capillary membrane fibers. The surfactant washing before drying significantly decreases the strength.

References

- Childress Amy E., Le-Clech Pierre, Daugherty Joanne L., Chen Caifeng, Leslie Greg L. Mechanical analysis of hollow fiber membrane integrity in water reuse applications. *Desalination,* 2005, 80: 5-14.
- Eykamp W., "*Microfiltration and ultrafiltration*", in 'Membrane Separation Technology: Principles and Applications', R.D. Noble, S.A. Stern (Eds.), Elsevier Science, Amsterdam, 1995,1-40.
- Gijsbertsen-Abrahamse A. J., Cornelissen E. R., Hofman J. A. M. H., Kiwa N.V. Fiber failure frequency and causes of hollow fiber integrity loss. *Desalination,* 2006,194: 251–258.
- Mamtani V. S., Bhattacharyya K. P., Prabhakar S. and Tewari P.K. Fouling Studies of Capillary Ultrafiltration

Membrane. *Desalination and Water Treatment,* 2014, 52: 542-551.

- Mamtani V.S., Bhattacharyya K.P., Ghosh A.K., Bindal R.C. and Tewari P.K., Screening of Polymers (PSf, PES, PE, PVC, PVDF, PP, PAN) for preparation of Capillary Membranes - A Critical Review, Proceedings of The Indian Chemical Engineering Congress (CHEMCON)-2012, Jalandhar, Punjab, India, 2012, Indian Institute of Chemical Engineers.
- Qin Jianjun, Chung Tai-Shung. Effect of dope Flow rate on the morphology, separation performance, thermal and mechanical properties of ultrafilltration hollow fibre membrane. *Journal of Membrane Science*, 1999, 157: 35-51.
- Voutchkov N., Pretreatment Technologies for Membrane Seawater Desalination, Australian Water Association, 2008, 1-132.

Energy consumption of reactive and proactive protocols in mobile ad hoc network in fuzzy inference system

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ARTICLE INFO
Received: 02.06.2021
Revised: 30.07.2021
Accepted: 06.08.2021

Keywords:

MANET, AODV, DSR, DSDV, MATLAB, ENERGY EFFICIENCY

ABSTRACT

The designing of an efficient routing protocol is a fundamental problem in a Mobile Ad-Hoc Network (MANET). This paper evaluates three ad-hoc network protocols (AODV, DSR & DSDV) in different network scales taking into consideration the mobility factor. It evaluates the performance of various ad hoc routing protocols such as DSR, DSDV and AODV in terms of energy efficiency by varying pause time, node velocity and packet sending rate. It has been verified through extensive simulations using MATLAB, which represent a wide spectrum of network conditions. AODV, DSR delivers the better performance as that of the state-of the art algorithms DSDV. Here, we present a performance comparison of the DSR, AODV and DSDV routing protocols with respect to energy consumption, evaluating how the different approaches. This paper identifies the security issues; challenges to security design and review the state-of-the-art security proposals that protect the MANET link and network-layer operations of delivering packets over the multihop wireless channel.

I. INTRODUCTION

Recently there has been a lot of interest in building and deploying sensor networks – dense wireless networks of heterogeneous nodes collecting and disseminating environmental data.Routing is one of the key issues in MANETs due to their highly dynamic and distributed nature also because the use of mobile networks is growing very fast. In particular, a very large number of recent studies focused on Mobile Ad-hoc Networks (MANETs) [1,2]. The performance of a mobile ad-hoc network depends on the routing scheme employed, and the traditional routing protocols

do not work efficiently in a MANET. Various protocols have been developed for ad hoc networks such as DSDV (Destination-Sequenced Distance Vector) ,DSR(Dynamic Source Routing) and AODV(Ad-Hoc On Demand Routing). These protocols offer varying degrees of efficiency [3]. In this paper, we'll concentrate on the routing problem. Current research has focused on protocols that are low power [4][5][6], scalable with the number of nodes [7] and fault tolerant (to nodes that go up or down, or move in and out of range) [8]. In Goswami et al. [9], determined a difference between routing protocols performance when operating in large area MANET with high speed mobile nodes. In Goswami et al. [10], evaluates the performance of various ad hoc routing protocols such as DSDV and AODV in terms of energy efficiency by varying pause time, node velocity and packet sending rate. Some of the previous work regarding energy efficient routing in *mobile ad-hoc networks* (MANETs) focused on performance comparison of existing ad hoc routing protocols (such as DSR, AODV and DSDV [11]) with respect to energy consumption (e.g. [12]). Recently, new power aware routing protocols for MANETs have been proposed. In Gomez et al. [13] a new technique has been introduced as a power aware enhancement for MANET routing protocols.

However, we think that a more useful metric for routing protocol performance is *network survivability*. By this we mean that the protocol should ensure that connectivity in a network is maintained for as long as possible, and that the energy health of the entire network should be of the same order. This is in contrast to energy optimizing protocols that find optimal paths and then burn the energy of the nodesalong those paths, leaving the network with a wide disparity in the energy levels of the nodes, and eventually disconnected subnets. Energy Aware Routing, the protocol that we have developed tries to ensure the survivability of low-energy networks. It is also a reactive protocol such as AODV and directed diffusion; however, the protocol does not find a *single* optimal path and use it for communication. Rather it keeps a set of *good* paths and chooses one based on a probabilistic fashion. As we will show later, this means that instead of a single path, a communication would use different paths at different times, thus any single path does not get energy depleted. It is also quick to respond to nodes moving in and out of the network, and has minimal routing overhead.

II. FUZZY SYSTEM:

Fuzzy logic is an approach to computing based on "degrees of truth" rather than the usual "true or false" (1 or 0) logic on which the modern computer is based. Fuzzy logic includes 0 and

1 as extreme cases but also includes the various states of truth in between. There are two types of Fuzzy logic inference system (FIS). One is Mamdani type and the other is Sugeno type FIS. Mamdani type system is very popular and is commonly used. In this paper, Mamdani type FIS has been used because it gives non-linear and variable fuzzy outputs.

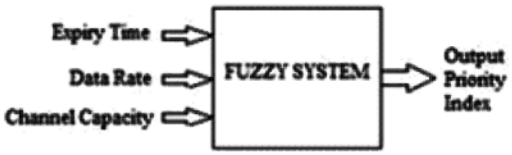

Fig.1. Fuzzy Seheduler

Thus, the following procedure is considered to define expert fuzzy system:

- Defining input-output sets which accept nor-malized input-output pairs.
- Generating if-else fuzzy rules based on input-output pairs.
- Creating fuzzy rule base.
- Implementing fuzzy system based on fuzzy rules.

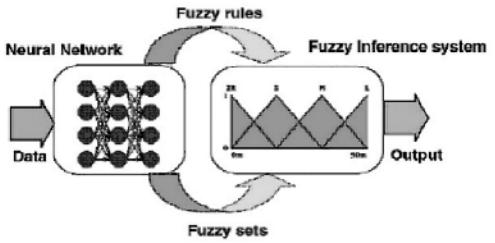

Fig.2. Fuzzy Inference System model

Fuzzy if–then rules

We write if-them rules as follows:

For Energy Consuption

1) If (Node is min) then (AODV-Energy is Normal) (DSDV- Energy is Min) (DSR-Energy is normal).
2) If (Node is normal) then (AODV- Energy is Max) (DSDV- Energy is Normal) (DSR-Energy is Normal).
3) If (Node is max) then (AODV- Energy is normal)) (DSDV- Energy is min) (DSR-Energy is normal).

III. RESULTS

The aim of these simulations is to analyze the DSDV protocol by comparing it with other protocols (AODV & DSR) for its efficiency in terms of power. This has been made by measuring the energy with respect to different network size and taking into consideration the remaining battery power. The simulation tool that has been used in this study is matlab [12]. So matlab is selected for evaluating these protocols. We simulate performance with different node such as 10, 15, 20, 30, 40 .

A Mamdani neuro-fuzzy system uses a supervised learning technique (back propagation learning) to learn the parameters of the membership functions.

In Fuzzy system, 1 factor of the number of nodes has been used in this system for evalu-ation of three AODV, DSDV and DSR routing protocols as input parameter and based on this input factor, effect of the factor on three

AODV, DSDV and DSR rout-ing . In this paper, Fuzzy system tools are used in Matlab software to determine efficiency of the test technique .

This system has 1 input field which relates to factor affecting evaluation of three AODV, DSDV and DSR routing protocols and three classes i.e. min, normal and max verbal words have been as-signed to each factor and 3 output fields which show efficiency of three AODV, DSDV and DSR rout-ing protocols and the output has been classified into three groups and low, normal and high verbal words have been assigned to each factor. In Fig-ure 3, one of the membership functions of input and output parameters are shown.

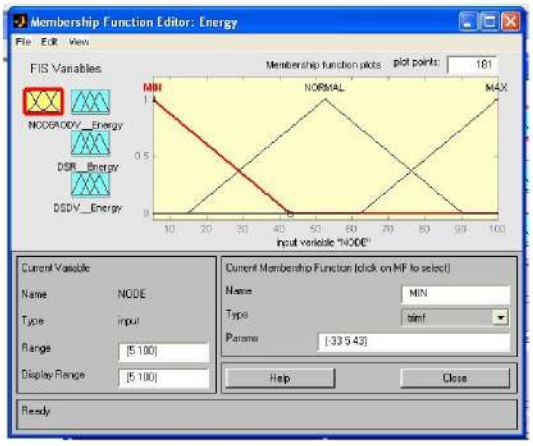

Fig.3. Membership function relating to input of the number of node

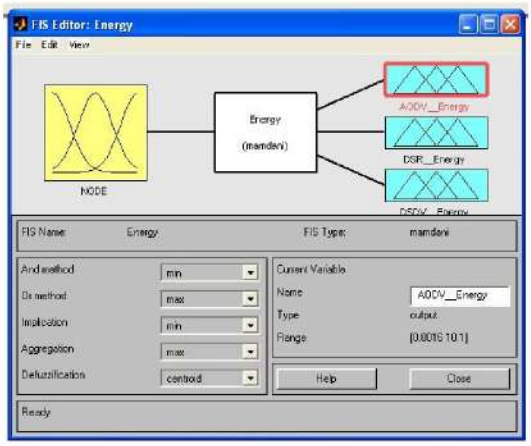

Fig. 4. General model of fuzzy expert System for evaluation of three routing Protocol

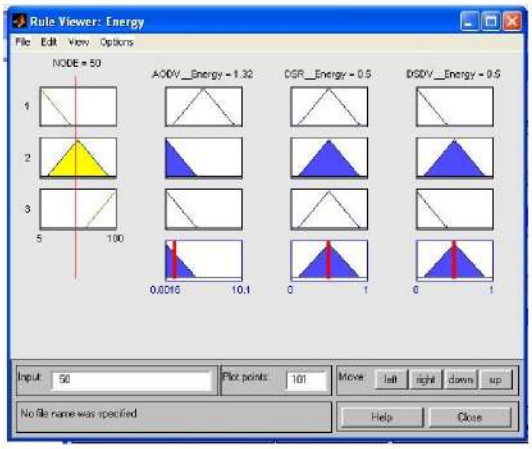

Fig. 5. Result of simulation with 50 nodes

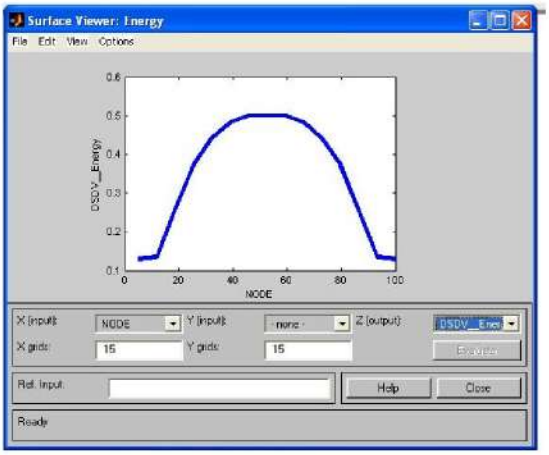

Fig. 6. Effect of number of node on output of energy in DSDV protocol

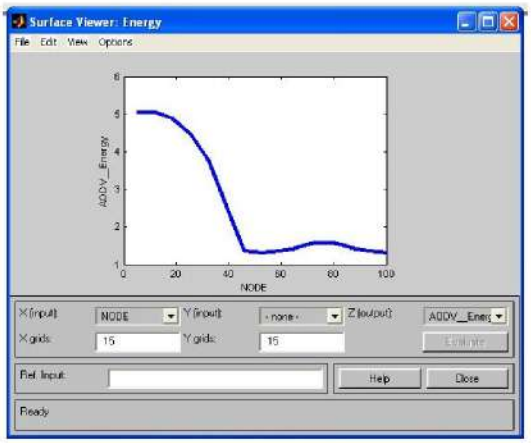

Fig.7. Effect of number of node on output of energy in AODV protocol

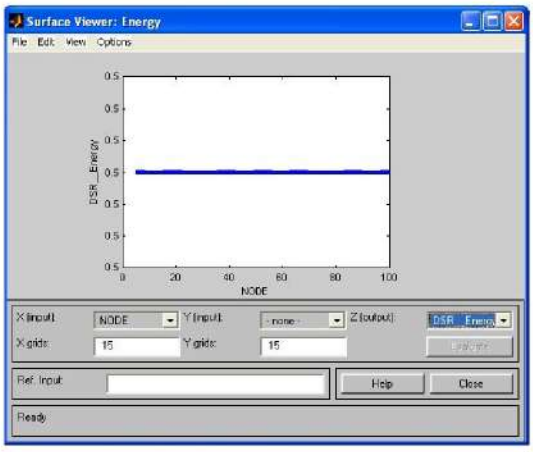

Fig. 8. Effect of number of node on output of energy in DSR protocol

IV. RESULT DISCUSSION

In the following section, the results obtained from our simulations are elaborated. One different performance variable is reported; low capacity radios utilization and the average number of hops required establishing energy consumption.

The utilization of low capacity packet is the ratio of total links established over low capacity channels compared to the total links. Average hop count is an average of end-to-end hops required to reach destination.

As mentioned above, MATLAB software which is a suitable medium for simulation of such systems has been used. Simulation of one case of tests with 50 nodes is given in Fig. 3., Fig. 4. and Fig. 5. The result obtained from effect of the number of node on out-put as 2D

which has been obtained in the simula-tion model. Fig. 5. shows the effect of number of node on output of energy consumption in AODV, DSDV and DSR protocol. Fig. (6,7, 8) shows the effect of number of node on output of energy consumption in AODV, DSDV and DSR protocol. After analysing the above figures we may conclude that AODV and DSR shows the better result in case of energy consumption but DSDV protocol shows the best result in energy consumption parameters if we increase the number of nodes.

In Das et al [14], they tried to extend the lifetime of ad-hoc network with respect to energy efficient multicast routing by calculating route lifetime values for each route. Based on the comprehensive simulation of Fuzzy Based Energy Efficient Multicast Routing using MATLAB and NS2 and comparative study of same with other existing protocols, it is observed that proposed routing protocol contributes to the performance improvements in terms of energy efficiency.

In Razouqi et al. [15] , they concentrated on routing protocols which are widely used in MANET, Destination Sequenced Distance vector (DSDV), Dynamic Source Routing(DSR) and Ad hoc on demand Distance Vector(AODV) routing protocols that are widely simulated in this paper using different scenarios in terms of different traffic types, constant bit rate(CBR), variable bit rate(VBR) then combining both classes in one scenario to scrutinize the impact of this combination. Routing protocols are analyzed against several performance metrics, average energy consumption, average throughput, normalized routing load (NRL), packet delivery fraction(PDF) and total dropped packets(TDP). Combined traffic results shows that DSR and AODV exhibit better behaviours on overall performance metrics examined. For energy consumption, DSDV shows potent response over other protocols when CBR and VBR applied separately, while for shared traffic scenario it shows better performance for lower nodes mobility.

In Goswami et al [10], they evaluated the performance of various ad hoc routing protocols such as DSDV and AODV in terms of energy efficiency by varying pause time, node velocity and packet sending rate. Simulation was done using NS-2. They found that DSDV routing protocol consume 99% energy when node speed is 50 m/s and AODV routing protocol energy consumptions was 100 % when node speed remained same.

In this paper we compare two routing protocol i.e Reactive(AODV & DSR) and Proactive(DSDV) in term of energy consumption behaviour for the three routing algorithms over a wide variety of scenarios and traffic models resulting varying one of the three selected parameters i.e. node velocity, packet

sending rate and pause time. It shows that when the traffic sources numbers increase from 10 sources to 20 sources, routing energy consumption grows 88% in AODV , 78% in DSR & 58 % in DSDV. However, when this factor moves from 20 sources to 30 sources, routing energy consumption grows 70% in AODV , 68 % in DSR & 50 % in DSDV. We observe that DSDV routing protocol consume less energy.

Table 1: Fuzzy value of Input Output Parameter

Groups	*Hop Count*	*Bandwidths (mbps)*	*Battery life (jl)*	*Mobility speed*
AODV	4	55	800	High
DSDV	4	60	300	High
DSR	4	57	300	Medium

The links in the ad hoc network can be broken due to scarce resources like energy, bandwidth, etc. So fuzzy based energy efficient routing protocol uses fuzzy logic and selects multiple routes with very high and high route selection grade. These routes will be in route cache of nodes. During the route maintenance phase, source node checks its route cache for a valid route to destination and transfers the data without any delay. To illustrate the implementation of routing protocol, a hypothetical network is designed to demonstrate the computation of fuzzy systems.

Erengy Consumption in ANOVA Test

Data Energy Energy consumption refers to the amount of energy that is spent by the network nodes within the simulation time. ANOVA statistical computation shows that we do not reject the null hypothesis. That is, there is no significant difference for the different methods in terms of energy performance ($P-value > 0.05$).

Table 2: Summary of Energy

Groups	*Count*	*Sum*	*Average*	*Variance*
AODV	23	7990941	347432.2	8201779957
DSDV	23	8094695	351943.3	20237752574
DSR	23	7267943	315997.5	5554377965

Table for One way ANOVA test in Appendix A

In this case, $F_{crit} = 3.135918$ at $\alpha = 0.05$. Since $F = 0.778278814 < 3.135918$, the result are significant at the 5% significance level. So we will accept the null hypothesis, and conclusion can be drawn that there is strong evidence that the expected values in the three groups does not differ. The variation is quite small and can be eliminated at this significance level. The $P-value$ for this test is 0.463364.

V. CONCLUSIONS

This research work proposes the performance of the fuzzy scheduler for ad-hoc network. It is observed from the results that priority scheduling helps in effective routing of packets without minimum loss and with less delay. The performance of the scheduler is analyzed using easuring metrics such as protocol energy

consumption behaviour. The comparison is done for the success rate and the energy consumption for the three routing algorithms over a wide variety of scenarios and traffic models resulting varying one of the three selected parameters i.e. node velocity, packet sending rate and pause time. The results obtained from the simulations allow us to conclude the following as far as energy consumption refers. Generally DSDV performs better than AODV & DSR . Thus DSDV routing protocol performs better than AODV & DSR routing protocol as regards to protocol energy consumptions.

References

1. C. E. Perkins, and P. Bhagwat, Highly dynamic distance destination-sequenced-vector routing, *ACM SIGCOMM Computer Communication Review*, 1994, 24(4), 234.
2. C. E. Jones, K. M. Sivalingam, P. Agrawal and J. C. Chen, A survey of energy efficient network protocols for wireless networks, *Wireless Networks*, 2001, 7(4), 343.
3. P. Lettieri and M. B. Srivastava, Advances in wireless terminals, *IEEE Personal Communications*, 1999, 6(1), 6.
4. C. K. Toh, "Maximum battery life routing to support Ubiquitous mobile computing in wireless ad hoc networks", IEEE Comm. Mag., June 2001, pp. 138-147.
5. S. Singh, M. Woo and C. S. Raghavendra, "Power aware routing in mobile ad hoc networks", IEEE/ACM Mobi Com, Oct. 1998, pp. 181-190.
6. R. Jain, A. Puri and R. Sengupta, "Geographical routing for Wireless adhoc networks using partial information", IEEE Personal Comm., Feb. 2001
7. C. Intanagonwiwat, R. Govindan and D. Estrin, "Directd Diffusion: A scalable and robust communication paradigm for sensor networks", IEEE/ ACM Mobicom, 2000, pp. 56-67.
8. D. B. Johnson and D. A. Maltz, "Dynamic source routing in ad hoc wireless networks", Mobile Computing, Kluwer, 1996, pp. 153-181.
9. Goswami, S., Joardar, S., Das, C. B., Kar, S., & Pal, D. K. (2017). Performance Analysis of Three Routing Protocols in MANET Using the NS-2 and ANOVA Test with Varying Speed of Nodes. In *Ad Hoc Networks*. InTech, *DOI: 10.5772/66521, ISBN 978-953-51-3109-0.*
10. Goswami, Subhrananda, Chandan Bikash Das, and Dhriti Sundar Maity. "ENERGY CONSUMPTION OF AODV AND DSDV PROTOCOLS IN MANET USING NS-2." "*Tamralipta Mahavidyalaya Research Review*A Peer Review National Journal of Interdisciplinary Studies" ISSN: 2456-1681 Vol-1,pp 60-65,(2016).
11. C. E. Perkins, *Ad Hoc Networking*,

Addison-Wesley, 2001.
12. L. M. Feeney, "An Energy Consumption Model for Performance Analysis of Routing Protocols for Mobile Ad Hoc Networks", *Mobile Networks and Applications*, Vol. 6, pp. 239-249, June 2001.
13. J. Gomez, A.T. Campbell, M. Naghshineh, and C. Bisdikian, "Conserving Transmission Power in Wireless Ad Hoc Networks", *Proc. ICNP'01*, Nov. 2001.
14. Das, S. K., Tripathi, S., & Burnwal, A. P. (2015, February). Fuzzy based energy efficient multicast routing for ad-hoc network. In *Computer, Communication, Control and Information Technology (C3IT), 2015 Third International Conference on* (pp. 1-5). IEEE.
15. Razouqi, Q., Boushehri, A., Gaballah, M., & Alsaleh, L. (2013, March). Extensive simulation performance analysis for DSDV, DSR and AODV MANET routing protocols. In *Advanced Information Networking and Applications Workshops (WAINA), 2013 27th International Conference on* (pp. 335-342). IEEE.

APPENDIX-A

Erengy Consumption

The one way ANOVA test for Energy is

Table 5: ANOVA of Energy

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.764E+10	2	8.82E+09	0.778278814	0.463364	3.135918
Within Groups	7.479E+11	66	1.13E+10			
Total	7.655E+11	68				

Relative toxicity of Lead and Nickel on acid and alkaline phosphatase in epigeic earthworm *Eisenia fetida* (*Savigny*, 1826)

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ARTICLE INFO

Received: 05.10.2020;
Revised: 02.01.2021;
Accepted: 10.01.2021

Keywords: *Lead, Nickel, Eisenia fetida,acid phosphatase (ACP), alkaline phosphatase (ALP).*

ABSTRACT

Among the soil fauna, earthworms are not only acting as bioindicator to determine soil pollution but also they provided with some specific enzymatic biomarker to decode the soil contamination. In the present heavy metal toxicity study, the LC50 of lead (Pb) and nickel (Ni) were determined in both artificial and natural ground soil by acute toxicity test (14 days) in *Eisenia fetida.* Low observed effective concentration (LOEC) of mixture of both metals (Pb and Ni) were also determined through repetitive experimental acute toxicity test. In the chronic toxicity test (28 days), the experimental set up had been arranged as control (C), T1(1506.25 mg Pb), T2 (3012.5mg Pb), T3 (193.75mg Ni), T4 (387.5mg Ni), T5 (753.125mg Pb and 96.875mg Ni) and T6(1506.25mg Pb and 193.75mg Ni) per Kg of dry soil. After end of chronic periods, specific activity of acid and alkaline phosphatase were determined in the earthworm tissue. The mean difference of recorded specific activity values of both enzymes were significant ($P<0.05$) and also showed a significant negative correlation ($P<0.05$) between the specific activity both enzymes.

1. Introduction

Several kinds of anthropogenic activity and use of agrochemicals greatly affects the normal ecological health of soil that is correlated with physiological health of terrestrial biome. Heavy metal pollution is an ecophysiological threat to paedo-fauna that increases in now days.This type of pollution of soil is responsible for beginning of bioaccumulations followed by biomagnifications in terrestrial ecosystem. In the last decades, soil pollution has been enhanced enormously due to industrial activities, urban sewage, intensive use of biocides and chemical fertilizers. Diffusible heavy metals contamination occurs in soil from variable sources, such as agricultural field,

industrial wastes, mining and sludge residues (Wuana et al., 2011). Mandal and Sengupta (2002) reported that the coal fly ash contains heavy and trace metals like arsenic, beryllium, cadmium, chromium, copper, cobalt, nickel (Ni), lead (Pb) etc. Heavy metals released from coal-fired thermal power station and deposited in soil, significantly responsible for environmental degradation of surrounding areas (Khillare et al., 2012). Heavy metals do not decompose or disappear from soil although their release to the ecosystem can be controlled by specific way of treatment (Brusseau, 1997). The sources of metal pollution include natural sources (Hobbelen et al., 2006), agricultural activities, municipal, industrial waste, sewage sludge etc (Salehi and Tabari, 2008), and also comes from particulates from aerial deposition of vehicular emission (Ward and Savage, 1994). The heavy metals are diffused in the soil rapidly (Lanno et al., 2004). Previous studies exhibited that earthworms accumulate metals, such as Cd, Cr, Cu, Co, Ni, Pb, Zn from soil under both field and laboratory conditions (Li et al., 2010). Kammenga et al. (2000) reported that earthworms acts as good sentinel organisms of soil pollution because they contact with soil pore water directly. In the environment, earthworms are frequently exposed to heavy metals and so their efficient detoxification systems maintain certain molecules that acts as molecular biomarkers of soil contamination. The earthworm acts as good bioindicator in terrestrial ecosystem due to interest of pollution (Suthar et al. 2008). Different kinds of soil bioindicator and biochemical indicator has been worked out in soil fauna by many researcher. Enzymes are called as biological catalysts that catalyzed several reactions on its substrate molecule to produce product, in association with specific specificity and kinetics under suitable environmental (external and internal) parameters. Biochemically, the enzymes are simple or complex protein molecules produced in the living cells where they were performing various physiological reactions. The assessment of any the environmental effect of a pollutant on mortality test of animal was a colloquial measure. However, sublethal effects of heavy metals acts as gradual and indicative physiological modification can be found as detrimental as mortality to the animal's survivability (Sonawane, 2017).Quantitative assessment of biocatalyst is a plausible indicator of environmental pollution such as heavy metal contamination that affect physiological and biochemical condition of organisms. Physiological activity of many enzymes including lysosomal hydrolytic enzymes are inhibited by heavy metals(Cheng, 1983).Lysosome contains different kinds of acid hydrolases are responsible for intracellular digestion (Berhet, 1965; de Durve and

Wattiaux 1966).The distribution of both acid phosphatase (ACP) (EC 3.1.3.2) and alkaline phosphatase (ALP) (EC 3.1.3.1) are differing in the cellular and sub cellular localization. Lysosomal enzyme, ACP catalyzes the phosphomonoester substrates in acidic condition pH, 4.8. ALP ismultifunctional enzyme, catalyzes phosphomonoester substrates in alkaline medium pH, 9.8 (Bhargavan, 2010; Sadasivam and Manickam, 2015). The specific activity of acid phosphatase and alkaline phosphatase is a biochemical indicator during hyperactivity of lysosomal digestion process that also involving with the metallic stress (Bhargavan, 2010). ACP is an important lysosomal marker enzyme and ALP is frequently occurs in the fraction containing plasmalemma. These enzymes are associated with the cell differentiation and growth of organisms. (Ram and Sathyanesan, 1985). Bhattacharya et al. (1975) and Anees (1976) reported that the activity of ACP and ALP enzymes being changed with the treatment of endrin and heavy metals like Cu, Hg and Zn on fish *Clarius batrachus* and in the fish *Channa punctatus* respectively. The enzymatic efficiency become changed by metallic exposure in some way associated with the response of physical and physiological parameters, co-factors, and also its Michaelis-Menten constant (K_m) (Jackim, 1974). The inhibition of acid phosphatase activity and the acceleration of the alkaline phosphatase activity were found after fungicide exposure (Chakravorty et. al., 2017). Ukpabi et al. (2013) reported that the activity of ALP and ACP could be used as biomarkers for detection of cellular damage after the treatment of metal contaminated fertilizer in *E. fetida* or for soil contamination surveys.

This experiment a attempt to study the effect of selected heavy metals on the activity of acid phosphatase and alkaline phosphatase. The present work has been done for evaluating the LC50 of Lead and Nickel in acute toxicity followed by chronic toxicity that determine the specific activity of acid phosphatase and alkaline phosphatase in *Eisenia fetid* after exposure with sublethal doses of lead, nickel and their combination of lowest observed effective concentration (LOEC) value.

2. Materials and methods

2.1. Sample collection and culture

The sample specimens *(Eisenia fetida,* red wiggler worm*)* was collected from WBCADC (*West Bengal Comprehensive Area Development Corporation*) Tamluk Project that is regulated under Panchayet & Rural Development Department, Govt. of West Bengal, India. The specimens has been cultured in vermicomposting pit in Raja Narendra Lal Khan Women's College. Gope Palace, P.O- Vidyasagar University, Dist: Paschim Midnapore, Pin: 721102. The vermiculture

unit located in natural environment with shadow shade area. The vermicomposting cum cultural pit had been covered by fine meshed iron net to avoid unwanted contamination. The substrate medium consists of pesticide free fine ground soil with dried cow dung manure. Finely grinded soil particles was mixed with cow farmyard manure as 1:1 ratio and was used as the culture medium for the specimens (Ismail, 1997). The hand sorted cocoons became separated and cultured in a separate pots, those were used as experimental specimens later. The refined water has been used for maintaining moisture. The dust cow dung manure has been utilized as food for the growing test specimen and gave at a specific interval of time. The compost use as biofertilizer in the flower garden of this college. Chakravorty et al., (2017) pointed out that *Eisenia fetida* are easily breed and culture at the Environmental Test Chamber in laboratory with different kinds of organic medium. Therefore, the species is very scientifically specific for ecophysiological and toxicological studies.

2.2. Acute toxicity test

Adult age synchronized *Eisenia fetida* worms (with visible clitellum) were blotted on filter paper and weighed individually. The worms were acclimated for 24 hrs after removal from the mother culture prior to experimental use, washed with redistilled water and then hold on wet filter paper in the dark environmental chamber at desirable temperature (28°C ±0.5°C) and humidity (60-65%). This process was allowed to defecation of gut contents (Rathi et al., 2011). Acclimated *Eisenia fetida* was used for the acute toxicity test in both artificial soil and natural ground soil medium. Age synchronized individual worm weighing about 270-290 mg was selected for exposure in this test. Experimental culture of specimens were done in inert polythene boxes (16 X 12 X 1 cm, total area, 192 cm^2). The artificial soil was comprised (by dry weight) of 70% quartz sand, 20% kaolinite, and 10% finely sieved paddy husk, with the pH adjusted to 6.0 ± 0.5 by the addition of calcium carbonate ($CaCO^3$) (De Silva *et al.*, 2009; Sanyal et al., 2015). The LC50 of lead (Pb) and nickel (Ni) was also determined in the ground soil. The LC50 of lead (Pb) and nickel (Ni) were determined in both artificial and natural ground soil as per acute toxicity test, OECD guideline 207. From pesticide free grassland, the collected soil samples were sun-dried, grinded and sieved as particle size of 0.25 mm that acts as the test medium or substrate. Individually Lead acetate trihydrate [$(CH_3COO)_2Pb._3H_2O$] and *Nickel acetate tetrahydrate* [$(CH_3.COO)2Ni.4H_2O$)] are used for contamination of the experimental soil. Three replicates was kept up for each set of experiments together with control set simultaneously. The experimental inert boxes

were remained as undisturbed for 48 hours before inoculation of worms for softening of medium and thermo-stabilization. Defecated and acclimated earthworms (10 pieces per box) were inoculated in each experimental boxes.

The experimental set up was performed in an Environmental Test Chamber at a constant temperature of 28 ± 0.5°C and 60-65% relative humidity. The entire experiment was performed for three times (Dasgupta et al., 2010).

The physiochemical parameters of both artificial soil and natural soil such as Organic carbon Content, moisture content, and pH were determined in constant room temperature and moisture content. Infrared Torsion balance moisture meter utilized for determination of moisture content of the soil (Chakravorty, 1990). The pH and organic carbon content of both soil were determined by the method of Piper (1942) in stable temperature and moisture. Those specimens showed no observable evidence of life after every 7days of interval, even when poked with a blunt needle, were considered as dead and were removed from the box due to avoid unwanted contamination. The soil was checked at specific regular interval (weekly) for detection of moisture loss by weighing the test containers and replenished with redistilled water if required. After end of the study (14 days), the mortality were assessed by EPA probit analysis program 1.5 (US EPA, 2006).

2.3. Chronic toxicity test

The sublethal doses of LC50 of two selected heavy metals (Pb and Ni) were used individually and jointly for chronic toxicological study of bioaccumulation and metallothionein response in the above described specimen. The chronic toxicity test was performed in similar way as described above in acute toxicity test in ground soil for a period of 28 days of study. Very finely grinded (0.25 mm) and dried cow dung manure (5 g dry weight)) was added to the test soil medium weekly to provide food for the growing worms (Chakravorty et al., 2017). Two individual sub-lethal doses of lead(Pb), nickel(Ni), and combination of lead with nickel (Pb-Ni) were applied in ground soil, along with control (C) for determining the bioconcentration factor and metallothionein response. 25% (T1 -1506.25 mg) and 50% (T2 - 3012.5 mg) of the LC50 values of the lead(Pb) and 25% (T3 – 193.75 mg) and 50% (T4 – 387.5 mg) of the LC50 values of the nickel(Ni) were applied on garden soil (Kg) for metallic exposure. The test concentrations of heavy metals combination were determined after repetitive conduction of preparatory trial experiment. The lowest observed effective concentration (LOEC) for each metals was chosen for final experimentation (Todorova et al. 2015). The mixture of both heavy metals,

lead and nickel was applied on soil (Kg) as 12.5% (T5 - 753.125 mg Pb and 96.875 mg Ni) and 25%(T6 – 1506.25 mg Pb and 193.75 mg Ni) of the LC50 values of the respective lead and nickel metals. The specimens were introduced in the experimental boxes, after that the boxes are placed in an Environmental Test Chamber and maintaining constant temperature of 28°C (±0.5°C) and 60-65% relative humidity. The earthworms were removed from the container after the end of chronic experimental period. The worms were cleanup with redistilled water followed by blotting with paper towels and then the worms were subjected for experimental findings.

Instruments Used:

Instruments	Company and Model N
ectronic Balance	Mettler Toledo (New Classic MS)
Environmental Test Chamber	IIC-INSTIND
Homogenizer	Remi Electrotechnik L (Type RQP-127/A).
Centrifuge	Remi Cooling Centrifu (C-24BL).
ectrophotometer	Systronics (UV-VIS Spectrophotometer 11

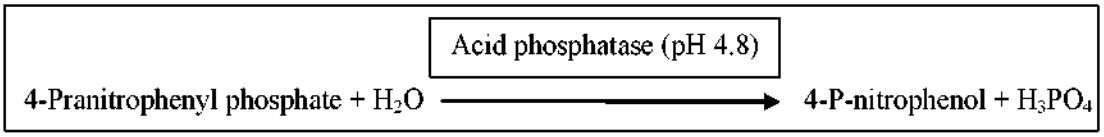
2.4. Determination of acid phosphatase activity (ACP)

The activity of acid phosphatase was measured as described by Walter and Schutt, (1974). 250 mg fresh earthworm tissue was taken in homogenizing tube and subjected to homogenization in 5 ml normal saline. The homogenized sample was then centrifuged at 10000 rpm for 10 minutes at normal room temperature. 0.2 ml supernatant was taken in a test tube in which 1 ml acid buffer was added and mixed thoroughly. A blank was prepared by giving 0.2 ml of 0.7% saline to 1 ml acid buffer. The test tubes were keep in the incubator for 30 minutes at 37° C. After the incubation period, 2 ml of 0.1 N NaOH solution was added to the test tubes and were mixed absolutely. The amount of liberated p-nitrophenol in tissue sample mixture gives an intense yellow colour that was measured spectrophotometrically at 405 nm wave length after adjusting the absorbance of the blank. A standard curve was drawn with the known amount of paranitrophenyl phosphate in the same procedure and the values of liberated p-nitrophenol were determined from the standard curve.

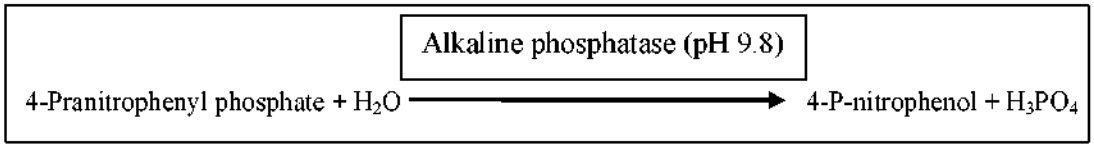
Protein concentration of each sample was quantified by the Lowry's method. A standard curve was drawn using BSA and the amount of protein in our earthworm tissue was calculated from the liner regression equation based on the standard curve (Lowry et al., 1951). The acid phosphatase activity was finally expressed in µg pnp/mg of protein/30 mins.

2.5. Determination of alkaline phosphataseactivity (ALP)

The activity of alkaline phosphatase was measured as described by Walter and Schutt,



(1974). 250 mg fresh earthworm tissue was taken in homogenizing tube and subjected to homogenization in 5 ml normal saline. The homogenized sample was then centrifuged at 10000 rpm for 10 minutes at normal room temperature. 0.05 ml supernatant was taken in a test tube in which 2 ml alkaline buffer was added and mixed thoroughly. A blank was prepared by giving 0.05 ml of 0.7% saline to 2 ml alkaline buffer. The test tubes were keep in the incubator for 30 minutes at 37° C. After



the incubation period, 10 ml of 0.05 N NaOH solution was added to the test tubes and were mixed absolutely. The amount of liberated p-nitrophenol in tissue sample mixture gives an intense yellow colour that was measured spectrophotometrically at 405 nm wave length after adjusting the absorbance of the blank. A standard curve was drawn with the known amount of p-nitrophenol phosphate in the same procedure and the values of liberated p-nitrophenol were determined from the standard curve.

Protein concentration of each sample was quantified by the Lowry's method. A standard curve was drawn using BSA and the amount of protein in our earthworm tissue was calculated from the liner regression equation based on the standard curve (Lowry et al., 1951). The alkaline phosphatase activity was finally expressed in µg pnp/mg of protein/30 mins.

3. Results

In our experiment, some important physicochemical parameters of both artificial and artificial soil had been determined which are given in table 1. The pH and organic carbon content of natural ground soil was slightly higher than artificial soil. But moisture content of ground soil was lower than artificial soil.

The acute toxicity test was performed for 14 days to determine the LC50 of lead and nickel in both natural garden soil and artificial soil and the LC50 value were given in table 2. In this experiment nickel shows more toxic than lead in both soil experiment. On the other hand, both metals revealed more toxic in ground soil than artificial soil.

The acid phosphatase, alkaline phosphatase and acetylcholinesterase activity of *E. fetida*

Table 1: Physicochemical parameters of the artificial soil and natural soil acts as test medium used in our experiment.

Soil parameters	Artificial soil	Natural soil
P^H	6.40	6.80
Organic Carbon Content	0.76%	0.88%
Moisture	62.2%	61.4%

were determined after exposed to control (C), sub-lethal doses of Pb (T1 & T2), sub-lethal doses of Ni (T3 & T4), sub-lethal doses of Pb with Ni (T5 & T6). The activity of acid and alkaline phosphatases were significantly different among all sublethal doses of metallic treatments ($P<0.05$). Activity of acid and alkaline phosphatases per mg of protein in all different treatment of soil was graphically represented in figure 1 and figure 2 respectively. The highest level of acid phosphatase activity was recorded in T4 and lowest was recorded in control set (C). Induction of acid phosphatase activity in worm was greater in nickel treated soil than the lead. The alkaline phosphatase activity was decreased in all metallic exposure than control set (C). The highest and lowest level of activity of alkaline phosphatase had been recorded in control set (C) and in T6 respectively. Inhibition of alkaline phosphatase activity in worm was greater in nickel treated soil than the lead. The activity of both phosphatases were more affected by combined treatment of

Table 2: LC50 values of the two heavy metals (mg/kg) used in the Acute Toxicity test.

Heavy metals	Commercial compound	LC50(14 days) (mg/kg)					
		Artificial soil			Ground soil		
		LC50	95% Confidence Limit		LC50	95% Confidence Limit	
			Lower	Upper		Lower	Upper
Lead(Pb)	Lead acetate trihydrate $[(CH_3COO)_2 Pb.{}_3H_2O]$	6250	1.065	1.484	6025	0.755	1.994
Nickel(Ni)	Nickel acetate Tetrahydrate $[(CH_3.COO)_2 Ni.4H_2O)]$	790	0.137	0.183	775	0.135	0.179

lead and nickel comparatively than the treatment of metal intoxication individually, lead or nickel.

4. Data analysis:

The LC50 of individual metal was determined through probit analysis by EPA probit analysis program, version 1.5 (US EPA; 2006) in 95% confidence limit of each metal. Statistical analyses for other measurements were performed by Statistical Package for Social Sciences (SPSS) version 20.0.After metallic exposure, the mean activity of acid and alkaline phosphatases were different significantly by the analysis of variance (ANOVA) followed by Post Hoc test ($P<0.05$). The values of both phosphatases of earthworm tissue after the metallic exposure were analyzed by correlation and showed negative type of correlation ($P<0.01$).

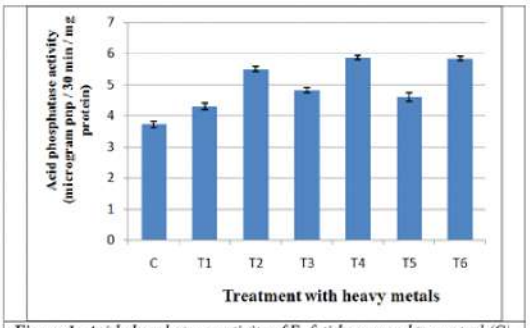


Figure 1: Acid phosphatase activity of E. fetida exposed to control (C), sub-lethal doses of Pb (T1 & T2), sub-lethal doses of Ni (T3 & T4), sub-lethal doses of Pb+Ni (T5 & T6). Error bar represent the standard deviation.

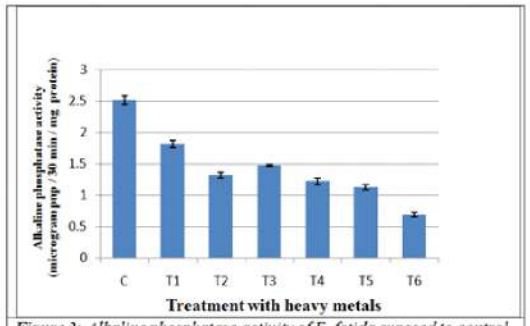


Figure 2: Alkaline phosphatase activity of E. fetida exposed to control (C), sub-lethal doses of Pb (T1 & T2), sub-lethal doses of Ni (T3 & T4), sub-lethal doses of Pb+Ni (T5 & T6). Error bar represent the standard deviation.

5. Discussion:

Digestion and metabolism are essential part in the nutrition of animals. Both are sequential processes and maintained by several types of extra and intra cellular enzymes. Investigation of the physiological deviation from normal state made by the pollutants such as heavy metal can alter the enzymatic activity. Recent studies showed that environmental monitoring had been assessed quickly by use of enzymatic biomarkers and the increase or decrease of the activity of specific enzymes could detect a possible environment stress. The enzymatic activity acts as the excellent hallmark for potential biomonitoring out of the many biological devices to assess the metabolic alteration caused pollutants (Filimon et al., 2013). Several research had been explain alterations in the enzymatic activity were occurred (superoxide dismutase, catalase, gluthathione reductase and gluthathione peroxidise etc) in several organisms under metallic stress conditions and use of

enzymeacts as efficient molecular biomarkers in evaluation of environmental impacts associated with heavy metals. (Bocchetti et al., 2008; Cogo et al., 2009).Heikens et al. (2001) pointed out a review to explain the concentration of heavy metals in most groups of soil invertebrates were happened in the order as Pb>Cd>Cu. Alnuaimi et al. (2012) crried out the activity of acid and alkaline phosphatase in the clam, *Scrobicularia plana* were altered with the treatment of heavy metals. Elevation of acid phosphatase activity indicates hyper activity of Lysosome that generally occurs in prenecrotic changes (Novikoff 1961; De Duve 1968). Khan et al. (2014) showed that lead acetate induce the histopathological changes in brain and hepatocytes in crucian carp along with the inhibition of neural enzymes. Alam (1984) reported that the acute and chronic exposures with metals, the activity of acid phosphatase became enhanced and decrease in alkaline phosphatase activityin *Viviparous bengalensis*. Dalela et al. (1980) opined that oxidative phosphorylation became uncoupled with the inhibition of acid and alkaline phosphatases. Acid and alkaline phoasphatase became decreased and increased respectively after the exposure of insecticide and herbicide (Chakravorty et al. 2015 and 2017). But Sonawane reported that (2017) after the heavy metal stress (copper, mercury and cadmium), the activity of acid and alkaline phosphatase showed increased and decreased level respectively than control set of experiment on fresh water bivalve, *Lamellidens marginalis* and this observation support the present investigation. The both enzymes, acid and alkaline phosphatases were showed a reverse image on their specific activity after the exposure of insecticide or herbicide and heavy metals (lead and nickel). In this experimental findings, it was clearly indicated that LC50 value of lead was higher than nickel, that means nickel was more toxic than lead. After the metallic exposure, it has been also established a negative correlation between the specific activity of both acid and alkaline phosphatases of earthworm's tissue. The activity of both acid and alkaline phosphatases of earthworm's tissue were increased and decreased respectively after treatment the soil with heavy metals and acts as biochemical and physiological biomarker to identify soil pollution. The effect of nickel was more effective than lead on both phosphatases. The combination of heavy metals had synergistic effect to the activity of both phosphatases than individual metal also.

Conflict of interest: The authors declare that there are no conflicts of interest in the above specified investigation.

Acknowledgement:

Atanu Dey and Partha Pratim Chakravorty thankfully acknowledge the Principal of Raja

N. L. Khan Women's College, Midnapore, West Bengal, India for providing us with the necessary laboratory facilities and other support those are associated with this regards. Atanu Dey acknowledge the *University* Scientific Instrumentation Centre (*USIC*) in Vidyasagar University, Midnapore, West Bengal, India for laboratory instrumental support.

References:

Alam, S.M. Some aspects of physiology of *Viviparus bengalensis*. *Ph.D. Thesis*. Marathwada University.Aurangabad (M.S., India), 1984.

Ali, S. and Kashem, M.A. Life Cycle of Vermicomposting Earthworms *Eisenia fetida* and *Eudrilus eugeniae* Under Laboratory Controlled Condition. *Biomed J Sci & Tech Res,* 2018; 10(5): 8110-8113.

Alnuaimi, M.M., Saeed, I.A. and Ashraf, S.S. Effect of various heavy metals on the enzymatic activity of E. coli alkaline phosphatase. *Int J Biotechnol Biochem, 2012;* 8:47–59.

Anees, M.A. Intestinal pathology in fresh water teleost *Channa punctatus* (Bloch) exposed sublethal and chronic levels of three organophosphorus insecticides. *Acta. Physiol. Latino – Amer.* 1976; 26(1): 63-67.

Berhet, J. La digestion intracellulaire et les lysosomes. *Archs. Biol.* (Liege), 1965; 76: 387-388.

Bhargavan, P.and Salih, K. Haematological responses of green mussel Perna viridis (Linnaeus) to heavy metals copper and mercury. *Ph.D thesis submitted to Cochin University of Science and Technology*, Kochi, India. 2010; pp 22-23.

Bhattacharya, S., Mukherjee, S. and Bhattacharya S. Toxic effects of endrin on hepatopancreas of the fish Clarius bactrachus (Lin.) *Ind. J. Exp. Biol.* 1975; 13: 185-186.

Bocchetti, R., Fattorini, D., Pisanelli, B., Macchia, S., Oliviero, L., Pilato, F., Pellegrini, D. and Regoli, F. Contamination accumulation and biomarker responses in caged mussels, Mytilus galloprovincialis, to evaluate bioavailability and toxicological effects of remobilized chemicals during dredging and disposal operations in harbour areas. *Aquatic Toxicology.* 2008; vol.89; 257-266.

Brusseau, M.L. Transport and fate of toxicants in soils.*Soil Ecotoxicology*, J. Tarradellas, G. B., and D. Rossel, Eds., Lewis Publishers, New York, NY, USA, 1997; pp.33–53.

Chakravorty, P.P. Studies on the ecological hazards and residual toxicity of some insecticide on non-target soil micro

arthropod fauna. *PhD Thesis*. Visva-Bharati University, Santiniketan. 1990; 268-270.

Chakravorty, P.P. Chronic toxicity of cypermethrin, a pyrethroid insecticide to epigeic earthworm eisenia fetida. *International Journal of Current Research*. 2015; 07. 17693-17696.

Chakravorty, P. P., Mandal. S. and Kundu. J. K. Relative Toxicity of two Selected Fungicides on Acid Phosphatase and Alkaline Phosphatase activity of Epigeic Earthworm Eisenia Fetida (Oligochaeta) *World Wide Journal of Multidisciplinary Research and Development* . 2017; 4(2): 14-17.

Cheng, T. C. Effects of *in vivo* exposure of *Crassostrea virginica* to heavy metals on hemocyte viability and activity levels of lysosomal enzymes. *Pathol. Mar. Sci.* 1990; 513–524.

Cogo, A.J.D., Siqueira, A.F., Ramos, A.C., Cruz, Z.M.A. and Silva, A.G. Utilização de enzimas do estresse oxidativo como biomarcadoras de impactos ambientais. *Natureza on line*. 2009; 7(1); 37-42.

Dalela, R.C., Rani, S. and Verma, S.R. Physiological stress induced by Sublethal concentrations of Phenol and Pentachlorophenol in Notopterus notopterus; Hepatic acid and alkaline phosphate axes and a succinic dehydrogenase. *Environ. Pollut.* 1980; 213-218.

Dasgupta, R., Chakravorty, P.P. and Kaviraj, A. Studies on relative toxicities of six insecticides on epigeic earthworm, Perionyx excavates. *Bull. Environ. Contam. Toxicol.* 2010; 85, 83-86.

De Duve, C. and Wattiaux, R. Functions of Lysosomes. *A Rev. Physiol.* 1966; 28: 435-492.

De Duve, C. In lysosomes a new group of cytoplasmic particles. Subcellular particles edited by T. Hayashi, *Renald Press*, New Yo4k, 1988; 128.

De Silva, P.M.C.S. and van Gestel, C.A.M. Development of an alternative artificial soil for earthworm toxicity testing in tropical countries. *Applied Soil Ecology*. 2009; 43: 170–174.

Filimon, M.N., Nica, D.V., Ostafe, V., Bordean. D.M., Borozan, A.B., Vlad, D.C. and Popescu. R. Use of enzymatic tools for biomonitoring inorganic pollution in aquatic sediments: a case study (Bor, Serbia). *Chem Cent J.* 2013; 7(1): 59.

Heikens, A., Peijnenburg, W.J.G.M. and Hendriks, A.J. Bioaccumulation of heavy metals in terrestrial invertebrates.*Environmental Pollution*. 2001; 113 (3): 385- 393.

Hobbelen, P.H.F., Koolhaas, J.E., Van Gestel,

C.A.M., Bioaccumulation of heavy metals in the earthworms Lumbricus rubellus and Aporrectodea caliginosa in relation to total and available metal concentrations in field soils. *Environ. Pollut.* 2006; 144 (2): 639–646.

Ismail, S.A. *Vermicology-the Biology of Earthworms*. Orient Longman, 1997.

Jackim. F. Enzyme responses to metals in fish. In : Pollution and physiology of marine organisms. *Eds. Vernberg F.J. and Vernberg, W.B., Academic Press, New York and London*, 1974.

Kammenga, J.E., Dallinger, R., Donker, M.H., Köhler, H.R., Simonsen, V., Triebskorn, R. and Weeks, J.M. Biomarkers in terrestrial invertebrates for ecotoxicological soil risk assessment. *Rev. Environ. Contam. Toxicol.* 2000; 164: 93–147.

Khan, S. A., Liu. X. and Shah, B. R. Impact of acute toxicity of lead acetate on the level of essential trace metals and histopathological changes in crucian carp. (*Carassius auratus gibelio*) *The Journal of Animal & Plant Sciences*. 2014; 24(5): 1018-7081

Khillare, P.S., Jyethi, D.S. ad Sarkar, S. Health risk assessment of polycyclic aromatic hydrocarbons and heavy metals via dietary intake of vegetables grown in the vicinity of thermal power plants. *Food Chem. Toxicol.* 2012; 50: 1642–1652.

Lanno, R., Wells, J., Conder, J., Bradham, K. and Basta, N. The bioavailability of chemicals in soil for earthworms. *Ecotoxicol. Environ. Saf.* 2004; 57(1): 39–47.

Li, L., Xu, Z., Wu, J. and Tian, G. Bioaccumulation of heavy metals in the earthworm *Eisenia fetida* in relation to bioavailable metal concentrations in pig manure.*Bioresource Technology*, 2010; 101(10): 3430–3436.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* 1951; 193: 265-275.

Mandal, A., and D, Sengupta., Characterization of coal and fly ash from coalfired thermal power plant at Kolaghat possible environmental hazards. *Indian J EnvironProt*, 2002; 22 (8): 885-891.

Mazorra, M.T., Rubio, J.A., and Blasco, J. Acid and alkaline phosphatase activities in the clam Scrobicularia plana: kinetic characteristics and effects of heavy metals.*Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* 2002; 131: 241–249.

Novikoff, A.B. The cells (Eds. J. Brachent and A.P. Mirsky) *New York. Academic Press*, 1961; 423.

OECD, Guideline for testing of chemicals No. 207, Earthworm Acute Toxicity Test.

Organization for Economic Co-operation and Development. Paris, France, 1984.

OECD, Guideline for testing of chemicals No. 222, Earthworm Reproduction Test (*Eisenia fetida/andrei*). Organization for Economic Co-operation and Development. Paris, France, 2004.

Piper, C.S. Soil and Plant Analysis. Hans Publ. Bombay. 1942; p.368.

Ram, R. N. and Sathyanesan, A. G. Mercuric chloride, cythion and ammonium sulfate induced changes in the brain, liver and ovarian alkaline phosphatase content in the fish *Channa puntae- tus. Envir.Ecol.* 1985; 3:263-268.

Rathi, V., Sambyal, S.S., Kulshreshtha, H. and Satvat, P.S. Heavy metal bioaccumulation by *Eisenia fetida*, cynodon dactylon and Vigna radiata in single, bi and tri-metal soil systems.*International Journal of Technology And Engineering System(IJTES) 2011;* 23.

Sadasivam, S., Manickam, M. Biochemical methods. *New age international publishers*. Third edition. 2015; 111.

Salehi, A. and Tabari, M., Accumulation of Zn, Cu, Ni and Pb in soil and leaf of Pinus elderica Medw. Following irrigation with municipal effluent. *Res. J. Environ. Sci.* 2008; 2(4): 291–297.

Sanyal, S., Dasgupta, R., Kaviraj, A., Chakravorty, P.P., Comparative toxicity studies of four pesticides on the epigeic earthworm *Perionyx excavatus* in two different soil media. *EM International.* 2015; 34(1): 47-51.

Sonawane, S.M. Effect of Heavy Metals on Lysosomal Enzyme Acid Phosphatase Activity of Bivalve L. marginalis. *International Journal of Engineering Science Invention.* 2017; 6 (8): 65-70.

Sonawane S M Effect of Heavy Metals on Lysosomal Enzyme Alkaline Phosphatase Activity of Bivalve *L. marginalis. IOSR Journal Of Pharmacy.* 2017; 7: 53-58.

Suthar, S., Singh, S. and Dhawan, S. Earthworm as bioindicators of metals (Zn, Fe, Mn, Cu: pb and Cd) in soils: is metal bioaccumulation affected by their ecological categories. *Ecol. Eng.* 2008; 32: 99–107.

Todorova, K., Velcheva, I., Yancheva, V., Stoyanova, S., Petrova, S., Georgieva, E., Effects of nickel and its combination with other heavy metals (cd, pb, zn) on common carp (*Cyprinus carpio* Linnaeus, 1785). *Trakia Journal of Sciences*, 2015; 13(2): 324-328.

Ukpabi, C. F., Akubugwo, E. I., Ibiam, U. A., Agbafor, K. N., Ezeigbo, R. O. and Wogu, C. Fertilizer Application Effect on Heavy Metal Composition and Biochemical Responses of *Earthworm Eisenia Fetida* (Savigny) in a Sandy Farm

Soil. *American Journal of Biochemistry*. 2013; 3 (3): 74-79

US EPA, Statistical analysis for biological methods. Available at http:// www.epa.gov/nerleerd /stat2.htm . Ecological exposure research division, US EPA. Available via DIALOG, 2006.

Walter, K. and Schutt, C. Acid and alkaline phosphatase in serum. In: Bergmeyer, H. U. (ed.), Verlag Chenie Weinheim, Methods of Enzymatic analysis. Academic Press Inc, New York, 1974; 856-864.

Ward, N.I. and Savage, J.M. Elemental status of grazing animals located adjacent to the London Orbital (M25) motorway. *Sci. Total Environ.* 1994; 147: 185–189.

Wuana, R.A. and Okieimen, F.E. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *International Scholarly Research Notices: Ecology - Hindawi*, Article ID 402647, 2011; 20.

Effect of *Citrus sinensis* peel on the blood sugar level in Diabetic White Rat

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ARTICLE INFO

Received: 01.10.2020;
Revised: 01.01.2021;
Accepted: 10.01.2021

Key words: *Citrus sinensis,* orange peel, Blood sugar level.

ABSTRACT

Citrus sinensis is a citrus orange belonging to the family Rutaceae. The peel contains fibre, Vitamin C, polyphenols, provisional A, folate, riboflavin, thiamine, Vitamin B6 and calcium. The peel extract was given to twenty white rats weighing between 140-200g. They were divided into four groups. Group 1 was control whereas the experimental rats (groups 2 to 4) received 10mg/kg, 20mg/kg, 30mg/kg doses of *Citrus sinensis*peel extract, group 2 rats treated with alloxan monohydrate to induce Diabetes mellitus. Fasting blood sugar levels were obtained and compared between groups. A significant difference ($p<0.05$) in relative blood sugar level among groups (control, medium dose and high dose) was absen except in low dose group. A statistically significant difference was observed for blood sugar levels across the groups compared to control. Thus, consumption of *Citrus sinensis peel* extract caused a dose dependent effect on blood sugar level in white rats.

Introduction

The orange is a citrus fruit originated in a region comprising Southern China, North east India and Myanmar. Orange trees are widely grown in tropical and sub-tropical climate for their sweet fruit. The fruit of the orange tree can be eaten fresh or processed for its juice or fragrant peel. As of 2012, sweet oranges accounted for approximately 70% of citrus production. 73 million tons of oranges were grown worldwide, with Brazil producing 24% of the world total, followed by China and India.

Orange peel, which is the primary waste fraction in the production of orange juice, contains flavonoids associated with antioxidant activity (Kanaze et. al. 2008). The glycosides hesperidin and naringin are mainly responsible for the purported antioxidant activity of citrus peel extract.

The citrus peel extract, polymethoxylatedfl avones (PMFs) has been reported to have beneficial effects on cholesterol level. The

PMF restores insulin sensitivity. PMF are similar to other plant pigments found in citrus fruits that has been increasingly linked to health benefits, including protection against cancer, heart disease and inflammation. The main PMF in the extract are tangeretin and nobiletin as well as small amount of synephrine.

The major ingredients of the orange peels are flavonoids, that mainly consists of terpenoid such as limolene, linalool and other volatile oils. The peels contain a large amount of Vitamin C. Besides it also provides Vitamin A and B complex and minerals such as Calcium, Magnesium, Selenium and Zinc (Morton 1987).

Diabetes mellitus is a metabolic disorder that has caused significant morbidity and mortality (Patel et. al. 2012). This is a chronic disease caused when either the body can not use the insulin effectively produced by the body or when the pancreas does not produce enough insulin. This disease prevents the body from properly using the energy from the food taken. The blood vessels and the blood are the highways that transports sugar from where it is either taken in (the stomach) or manufactured (in the liver) to the cells where it is stored (Fat). The pancreas releases insulin into the blood which serves as the helper, or the "key" that lets sugar into the cells for use as energy. When sugar enter the cells, the blood sugar level is lowered. Without insulin the sugar cannot get into the body cells for use as energy. This causes a rise in the blood sugar level. Too much sugar in the blood is called "Hyperglycemia" or commonly known as 'High Blood Sugar'. The orange peelsarea source of health promoting carbohydrates. Orange peel and pulp contain hesperidin, a flavonoid that helps to lower cholesterol and triglyterides. Orange peel being rich in pectin which is a natural fibre helps to reduce cholesterol levels (Youssef, et. al. 2013) also.

Present study aimed to evaluate the effect of sweet orange peels on the blood glucose level in diabetic rats.

Materials and methods

Materials

Orange Peels:

The *Citrussinensis* is the variety of orange used. The peels were obtained from fresh orange. The peels were cleaned and washed with tap water. Then air drier oven was used to dry the peels. The oven was set at 45 degree Celsius for 48 hours. A Multi Mill apparatus was used, and the peels were passed through fine mesh sieve and fine powder was obtained.

Rats:

Twenty healthy adult male albino rats 140-200 grams were taken for the research work.They were kept in single wire cages with wire bottoms under hygienic conditions and controlled laboratory condition of temperature (26 degree Celsius), lighting and ventilation.

Food and tap water were provided and checked daily.

Diet:

The vitamin mixtures and salt mixtures were prepared according to (AIN, 1977).

Experimental Design:

The adult male albino rats were fed on standard diet for 10 days for adaptation with the environment. They were then divided into four groups (n=5). The first group (1) was given standard diet only and served as control group. The remaining three groups i.e. group 2, group 3 and group 4 were the diabetic groups. Diabetes was induced in the normal healthy rat by injecting alloxanmonohydrate in the dose 150 mg per Kg body weight according to the method described by Desai &Bhide (1985). Six hours after the injection of the alloxan monohydrate, fasting blood samples were obtained by retro-orbital method to estimate the fasting serum glucose. When the fasting serum glucose was more than 200 mg per dl, the rats were considered to be diabetic (NDDG, 1994). The groups 2, 3 and 4 were given basal diet plus 10 mg per kg, 20 mg per kg, and, 30 mg per kg orange peel extract respectively and replaced by equal amount of starch for 60 days. At the end of the experiment period, the animals were sacrificed after being fasted (overnight) under anesthetized condition. The blood samples were collected from the hepatic portal vein in dry centrifugetubes. The serum was separated by centrifugation of the blood at room temperature in a centrifuge machine by revolving at a speed of 4000 rpm (revolutions per minute) for 20 minutes and kept in plastic vial at -20 degree Celsius till analysis.

Method

Chemical Analysis of Peels

The crude protein, fibre, fat and ash content were determined by following the method described by (AOAC, 1995).

Biochemical Parameters

The enzymatic calorimetric method was used to determine the serum glucose level according to Kaplan (1984).

Statistical Analysis

The data were expressed as mean $\pm$ standard deviation (mean $\pm$ SD). The one-way analysis of variance (ANOVA) ($P<0.05$) was used for testing all variables for normal distribution. When the group showed significant differences, Turkey's multiple comparison test was performed with Snedecor and Cochran (1972).

Results and discussions

The data in Table 1, indicated that the fibre was 9.35 gram per 100gram dried orange peels represents approximately one third of the recommended daily intake. The dietary fibre is fundamental and remains intact in fibre-rich food (example fruit, vegetables, legumes, whole grains) is widely known to have

beneficial effects on the health, when consumed at recommended levels (25 grams per day for adult women and 38 grams per day for adult men) (According to Mc Rorie 2015). The risk of cardiovascular disease can be prevented by intake of high dietary fibre (Johanssan et. al.).

The Table2 showed that the blood glucose level gradually decreased in diabetic rats after 2 months (as an experimental period) with the increase of supplement dose. These results are in agreement with those reported by Youssef et. al. (2013) who reported that, the orange peels marked protection and it brought down the level of blood sugar. Chifai et. al. (2003) suggested that, glucose lowering effects are most often associated with the *viscous* fibre that lies in the soluble dietary fibre content of peels. Spandana et. al. (2016) found that orange peel and orange peel extract can provide benefits to diabetic patients and may reduce overeating, which were in accordance with the result. This mainly occurs due to the presence of natural fibre in the orange peels as a natural source of pectin, which helps in reducing blood sugar.

Table 1 Chemical composition of dried orange peels (g%)

Constituent Material	**Protein (g)**	**Fat (g)**	**Fibre (g)**	**Ash (g)**	**T. Carb. (g)**	**Total (g)**
Orange Peels	1.43	2.0	9.35	6.75	80.47	100

Table 2 Effect of feeding of different doses of orange peels on glucose level in diabetic rats (mg per dl)

Group Item	**Group 1**	**D.Group**	**Group 2**	**Group 3**	**Group 4**
Blood Glucose Level	89.80 ± 0.87	389.20 ± 4.50	289.40 ± 3.50	258.90 ± 1.70	218.50 ± 3.50

Data are expressed as mean ± SD. Values within a row having different superscript are significantly different ($P \leq 0.05$).

Conclusion

From this study we came to a conclusion that the orange peels are a rich source of fibre that is intrinsic and intact in whole food and antioxidant as active components. This study observed a significant reduction in the blood glucose level after high dose intake of orange peels.

References

American Institute of Nutrition (AIN), Committee on Standard Nutritional Studies. J. Nutr. 1977; 107:1340-1348.

American Institute of Nutrition (AIN), Purified diet for Laboratory Rodent.J. Nutr.1993;123:1939- 1951.

Official Methods of Analysis, Association of Official Analytical Chemists.1995;16th Ed., Washington D.C.

In vitro hypoglycemic effects of different insoluble fiber-rich fractions prepared from the peel of Citrus sinensis L. cv. Liucheng. Journal of Agricultural and Food Chemistry.ChifaiC;Huang,Y. andLee, M, 2003; 51(22):6623-6626.

Hypoglycemic effect of hanitioniasuaveolensIndian. J. Med.Desai,A. andBride, M. 1985;81: 86-91.

A high intake of dietary fiber influences Creactive protein and fibrinogen, but not glucose and lipid metabolism, in mildly hypercholesterolemic subjects,JohanssonPerssonA ; UlmiuslM; Cloeten s L; Karhu T; Herzig KH and Önning G European Journal of Nutrition, 2014; vol. 53, Issue 1, pp 39–4.

Determination of blood glucose.Cline.chem.The C.V. Mosby.Co. St Louis Toronto. Princeton,Kaplan, A. 1984; pp. 1032 – 1036.

Evidence-Based Approach to Fiber Supplements and Clinically Meaningful Health Benefits,McRorieJW. 2015; Part 1, Nutr Today. 50(2): 82– 89.

Orange. In: Fruits of warm climates. Julia F. Morton Miami, FL. 1987; p. 134-142.

A list of the benefits of orange peels http:// www.livestrong,co m/article/440499-a-listof-the-benefits-oforange-peels/ ixzz21 fucox HV. Myers, C. 2011.

Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance.Diabetes,NDDG, National Diabetes Data Group. 1994; 28:1039-1057.

An overview on antidiabetic medicinal plants having insulin mimetic property.Asian Pac J Trop Biomed,Patel DK, Prasad SK, Kumar R and HemalathaS, 2012; 320–330.

Statistical methods 6th Ed.The Iowa State University Press. Ames. IA.Snedecor, G.

W. and Cochran, W. G. 1972.

Effect of hespertin isolated from orange peels on cisplatinindused nephrotoxicity. Spandana U., Sreedvi A, HavilahEsther.S and Lakshmalah. Int J Pharm Bio Sci 2016 Oct ; 7(4): (P) 15 – 21.

Evaluation of antihyperglycaemic activity of citrus peels powders fortified biscuits in Albino induced diabetic rats. Food and Public Health.Youssef, M. K. E.; Youssef, H. M. K. E.; and Mousa, R. M. A, 2013; 3:161- 167.

Role of *Laternula truncata* (Lamarck, 1818) in maintaining of biological integrity of West Bengal coastal region

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ARTICLE INFO

Received: 02.10.2020;
Revised: 03.01.2021;
Accepted: 10.01.2021

Key words:
Laternula truncata, mudflat, bioturbation, food chain.

ABSTRACT

Laternula truncata, a truncate lantern calm has been recognized as a benthic species in a mudflat of degrated mangrove oriented coastal region of West Bengal with Bay of Bengal. By virtue of their bioturbatory activities like nest building, burrow formation, reconstruction and rebuilding of their own habitat they help juveniles of *Lingula anatina, Glauconome sculpta, Macoma birmanica*, *Uca* sp., *Parachondylactis* sp., *Virgularia* sp., *Glycera* sp. etc for settlement and recolonization. Their burrow strongly form a microhabitat with different complex food chains and food webs starting from phytoplankton to higher vertebrates made this ecosystem more dynamic. Water and oxygen influx within the burrow constructed by them enrich soil texture with nutrients made possible for successful succession of new mangrove vegetations in this habitat. The present work aims to highlight on the biological and ecological activities of studied species for maintenance of biological integrity of mangrove estuarine ecosystem of West Bengal coastal region with Bay of Bengal.

Introduction

The mangals of West Bengal coastal region near Subarnarekha estuary have undergone significant human induced ecological changes. With ongoing threats to this ecosystem it remains imperative that there be a continued and expanded research effort to understand this versatile ecosystem and their associated biota. Mangrove habitats of East Midnapore coastal region, West Bengal although offer a rich biota and an important fishing habitat, yet remain biologically poorly known with little information available about even some of the more common taxa. One such group includes laternulid bivalves. Field studies of laternulids from Asia as well as from india are uncommon. Although some studies on eco- biology of some selected bivalves has been conducted throughout last few years from west Bengal coastal region and associated mangrove

ecosystems, no studies on *Laternula truncata* have been done so far from this region . The present paper emphasizes on the Role of *Laternula truncata* in maintaining of biological integrity of West Bengal coastal region

Material and methods:

Physiography of the study site.

The ecologically significant mudflats of a mangrove ecosystem near Subarnarekha estuary (Longitude 87°52 E, Latitude 20°302 N and Longitude 87°452 E, Latitude 21°472 N)) has been selected for the present study during July, 2018 to June, 2019.

Procedure for biological samplings.

Random samplings of *Laternula truncata*, were made from the study site through twelve months and three seasons in a year during low tide level. For quantitative estimation, five quadrates having an area of 1 m^2 were placed randomly and bottom dwelling faunal components especially the laternulids were unearthed, counted and expressed as No/m^2. Specimens were grouped into different size classes (0-2 cm, 2- 4 cm, and 4- 6 cm) and their mean size classes (lengths) were recorded with the help of slide calipers and with a plastic scale.

Identification of collected specimen.

L. *truncata* after collection from study site was identified following standard literature of Prezant et al., 2008.

Burrowing behavior :

Burrowing behaviour was examined in the laboratory using recently collected specimens of *Laternula truncata* during study period. Larger sized specimens of L. *truncata* were placed in a small aquarium that held 10 cm of field-collected sediment covered with 5 cm of field-collected water to monitor burrowing behaviour and/or differences in rate of burial between size classes. Specific behavioural landmarks were monitored by first foot probe of sediment, movement of valves and fully burrowed with only siphons exposed.

Results:

The present study site is an extensive shallow mudflat with soft muddy to sandy sediments dotted with degraded mangrove vegetations. The flats are heavily fished by hand by local residents for edible bivalves, crabs, and mudskippers. The majority of plants are species of Acanthus, Avicennia, Salicornia, Ipomea etc. The mudflats are well drained, oxygenated, and the salinity can be brackish.

The animal is particularly prevalent in estuarine tidal mudflats of studied area and is most commonly found within burrow as a macrobenthic animal. They are lying vertically in their habitat with the siphon pointing upwards.

All living *Laternula truncata* (Figure- 1 and Figure- 2) collected during study period showed gradations of sizes from very small specimen (1.6 cm length) to the largest (7.6 cm length). There was some indication of an

age-related die-off in the largest size range because the number of clams decreased rapidly. Prominent low and tightly positioned rings were present on the shells of L. *truncata*. These regular concentric rings were interspersed with concentric, irregularly placed but more prominent growth stoppage rings. All such rings seamlessly traversed across the umbonal slit typical of the valves.

Specimens of *Laternula truncata* were often found buried 6–14 cm deep in highly compacted sand layers beneath clay canopy. The open sand sites between these mangrove islands held few specimens.

Sediments in study site along my transect was dominated by fine sand following silt and clay . Sand, silt and clay content of the habitat recorded during present study was 61.73 ± 0.81%, 28.81 ± 0.29% and 8.49 ± 0.61% respectively. Organic carbon content of Soula mudflat sediments along the transect was relatively low, averaging 1.14 ± 0.08% as found during present study. The average value of salinity of interstitial water as recorded during present study was 21.72 ± 1.62 ppm (Figure- 3).

The highest concentrations of *Laternula truncata* along the transect were found within quadrats during post monsoon period and average value of population density of studied species was 17.16 ± 4.49. Average shell length, width and height of the studied species as found during present study was 5.6 ± 4.91 cm, 2.3 ± 0.62 cm and 1.6 ± 0.71 cm respectively (Table-1).

Quite straight forward burrowing behavior of *Laternula truncata* has been observed during present research work. Smaller specimens of *studied species* probed more readily in the sediment with their foot. Among all studied specimen it was observed that some reburrowed fully and majority reburrowed partially when removed from sediment. After lying on one valve, the foot emerges and immediately probes the sediment for an extended period of time and similar observation has been documented by Prezant et al., 2008 also. On the average, larger clams burrowed slowly than smaller calms and depth of burial was usually related to siphonal length of the studied species.

Discussion

Marine- coastal- estuarine- mangrove ecosystem represents the most productive and dynamic ecosystem of the world. It supports innumerable number of fora and fauna in its diversified habitats and ecological niches (Chakraborty, 2017). Midnapore (East) coastal belt in West Bengal, India, just on the southern part of Sundarban Mangrove Estuary, encompasses a diversified habitats and niches which accommodate a good number of faunal components in the form of pelagic and benthic form (Samanta et al., 2015). Among these,

truncated bivalvia- *Laternula truncata* has been reported from an mudfl at Saula estuary with the Bay of Bengal, West Bengal coastal belt, India.

Among various mangrove- estuarine bivalves *Laternula* is currently the sole accepted genus within the family Laternulidae (Prezant et al., 2008). Although Morton (1976) noted eight species within the family, there is no clear delineation as to the total number of species (Prezant & Smith, 1998). In 1980, Piyakarnchana, in an overview of mangals of the Thiland noted only *Laternula truncata* among laternulids. Although two species under the genus Laternula have been reported by Tudu et al. from Odisha, India coastal zone in the year 2018 no such report on biology and ecology of *Laternula truncata* has been published from West Bengal Coastal zone, India.

The intertidal belts along with mangroves-estuarine system function like a sponge with complex network of biogenic structures which foster to and fro movement of interstitial water, nutrients and gases both vertically and laterally coupled with tidal advection and drainage.(Chatterjee et al, 2008). In estuarine environment, macrobenthic communities generally are characterized by low diversity with high abundance (Attrill et al., 1996). But low population density of studied species as recorded during present investigation clearly indicating that their existence in this area is in danger or threatened condition. Human induced activities like fishing, mangrove destruction for collection of fuel materials, oil leaching from fishing boat made this highly productive ecosystem into a stressed ecosystem which in turn threatened the existance of macrobenthic fauna in this habitat. Changes in the population density of studied truncate bivalve could be due to changes in the sediment structure and composition.

From an ecological perspective, bioturbation is coupled to physical processes and associated chemical changes related to movement of particles or water. The important biological and biogeochemical consequences of bioturbation must be considered in a larger context, such as within the framework of ecosystem engineering as pointed out by (Meysman et al., 2006).Vertical tube formation in the sediment through bioturbatory activities by bivalves such as *Laternula truncata* made the environment more comfortable for juvenile settlement of various faunal component of this area. their burrowing activities can directly break and transport sediments, decrease the hardness of the soil (Botto et al., 2005) burrowing also affects soil chemistry and associated microbial processes, increases soil oxygenation, and alters pore water salinity (Fanjul et al., 2007). Burrowing activities by laternulids significantly affect belowground processes that

can impact marsh plants which have been noticed by Smith et al. 2009 in fiddler crabs. Burrowing by them increases the passage of liquid and gas between the soil and environment , increasing soil oxidation (Weissberger et al., 2009) and the decomposition rate of organic debris (Fanjul et al., 2007). Burrows of studied species can selectively trap sediments that have high organic matter concentrations, finer grain size and low density through the interactions of the burrow openings with tidal water, which facilitate organic matter decomposition, which in turn increase nutrient availability and thus, promote their growth (Botto et al., 2005). Excavation by L. *truncata* transport soils and nutrients from deep layers to the marsh surface which might accelerate the turnover of soils and nutrients. It seems that above mentioned different unique structures of different species have diverse functional roles, but more intensive study is required to understand their functional as well as ecological significance.

The relatively aerobic sediment, lower organics, and ability to the fine sand substratum, could be a requisite sedimentary habitat for *L. truncata*. Periodic deposition of silt/clay into the upper substratum, on the other hand, could inhibit initial larval settlement. The abundances of *Laternula truncata* are partially related to predation, which in turn are related to prey accessibility. How accessible an infaunal bivalve is to a surface or near-surface dwelling predator is related to depth of burial, rate of burial, and/or the mechanical ability of a predator to "reach" the prey (Prezant et al., 2008).

The author hypothesizes that the burrowing cycle of studied species can be divided into four phases: (i) foot extension (ii) penetration of foot into the sediment, (iii) anchorage by foot tip dilation, and (iv) insertion of shell into the sediment by contraction of pedal retractors. Zamorano et al. (1986) also reported unusual siphonal activity associated with locomotion and burial. This process consisted of levering the tips of the siphons against the substratum to lift the shell and then "looping" the siphons in a process that allows shell rotation while forcing water from the exhalent siphon to create jetting. Zamorano et al. (1986) suggested that this method was used by *L. elliptica* to move the bivalve to another favorable position for reburying.

Burroing activities performed by the studied species makes top soil of the habitat more smooth and comfortable for larval deposition and settlement of Lingula sp. and Glauconome sp.It is hypothesized that for this reason high population density of these two associated faunal component have been observed during field work.

Laternulid bivalve- *Laternula truncata* is an important part of the biota in the mangals of

the Soula, Midnapore (East), West Bengal, India seaboards and little is known about this organism in the studied threatened mangrove forest. Hence in future more extensive studies on *Laternula truncata* should be organized by the researchers.

References:

Attrill, M. J., Ramsay, P.M., Thomas, R.M. and Trett, M. W. An estuarine biodiversity hot-spot. J. Mar. Biol. Ass. U. K. 1996; 76(1): 161-175.

Botto, F., Valiela, I., Iribame, O., Martinetto, P. and alberty, J. Impact of burrowing crab on C and N sources, control and transformation in sediments and food web of S W Atlantic estuaries. Marine Ecology Progress Series. 2005; 293: 155-165.

Chakraborty, S. K. Ecological services of intertidal benthic fauna and the sustenance of coastal wetlands along the Midnapore(East) coast, West Bengal, India. In book;p Coastal wetlands: alternation and remediation. 2017. pp. 777- 866.

Chatterjee, S., G. Bhunia and S. K. Chakraborty. Bioturbation of Brachyuran crabs and its impact on coastal ecosystem of Midnapore district, West Bengal, India. George A. Sorial and Jihua Hong Eds.), In proceedings of Forth International Conference on Environmental Science and Technology,2008; Vol. 2, Houston, Texas U.S.A P. 133-148.

Chatterjee, S., D. Mazumder and S. K. Chakraborty. Ecological role of fiddler crabs (Uca spp.) through bioturbatory activities in the coastal belt of East Midnapore, West Bengal, India. J. Mar. Biol. Ass. India, 2014, 56; (2):16- 25.

Fanjul, E., M. A. Grela and O. Iribarne. Effects of the dominant SW Atlantic intertidal burrowing crab Chasmagnathus granulates on sediment chemistry and nutrient distribution. Mar. Ecol. Prog. Ser., 2007, 341:177-190.

Meysman, F. J. R., Middelburg, J.J. and Heip, C. H. R. Bioturbation: a fresh look at Darwin's last idea. Trends Ecol Evo. 2006, 21: 688–695.

Morton, B. The structure, mode of operation and variation in form of the shell of the Laternulidae (Bivalvia: Anomalodesmata: Pandoracea). *Journal of Molluscan* Studies,1976; 42: 261–278.

Piyakarnchana, T., *The Present State of Mangrove Ecosystems in Southeast Asia and the Impact of Pollution. Thailand.* Project report SCS/80/WP/94E. Food and Agriculture Organization of the United Nations and United Nations Environment Programme, South China Sea Fisheries Development and Coordinating Programme, Manila, 1980; 138 pp. Online

version http://www.fao. org/docrep/fi eld/ 003/AB751E/AB751E00.htm.

Prezant, R. S., Sutcharit, C., Chalermwat, K., Kakhai, N., Duangdee, T. and Dumrongrojwattana, P. Population study of Laternula truncate (Bivalvia: Anomalodesmata: Laternulidae) in the mangrove sandflat of Kunkrabaen Bay ,Thiland, With notes on Laternula. Cf. Corrugata. The Raffles Bulletine of Zoology, 2008; 18: 57- 73.

Samanta, S., Choudhury, A and Chakraborty, S. K. Eco-biology of a Precambrian intertidal benthic brachiopod, Lingula anatine from the confluence of Subarnarekha estuary with Bay of Bengal, India. J. Mar. Biol. Ass. India, 2015; 57 (1), January-June 2015.

Smith, N. F., C. Wilcox and J. M. Lessmann. Fiddler crab burrowing affects growth and production of the white mangrove (Laguncularia racemosa) in a restored Florida coastal marsh. Mar. Biol., 2009; 156: 2255- 66.

Tudu, C.P., Yennawar, P., Ghorai, N., Tripathy, B. and Mohapatra, A. An updated checklist of marine and estuarine mollusk of Odisha coast. Indian Journal of Geo- Marine Sciences, 2018, 47; (08): 1537- 1560.

Weissberger, E. J., L. L. Coiro and E. W. Davey. Effects of hypoxia on animal burrow construction and consequent effects on sediment redox profiles. J. Exp. Mar. Biol. Ecol., 2009; 371: 60-7.

Zamorano, J. H., W. E. Duarte and C. A. Moreno, Predation upon *Laternula elliptica* (Bivalvia, Anatinidae): a field manipulation in South Bay, Antarctica. *Polar Biology*, 1986; 6: 139–143.

Figure- 1: Freshly collected specimens of *Laternula truncata.*

Figure- 2: *Laternula truncata* after opening of shell by dissection.

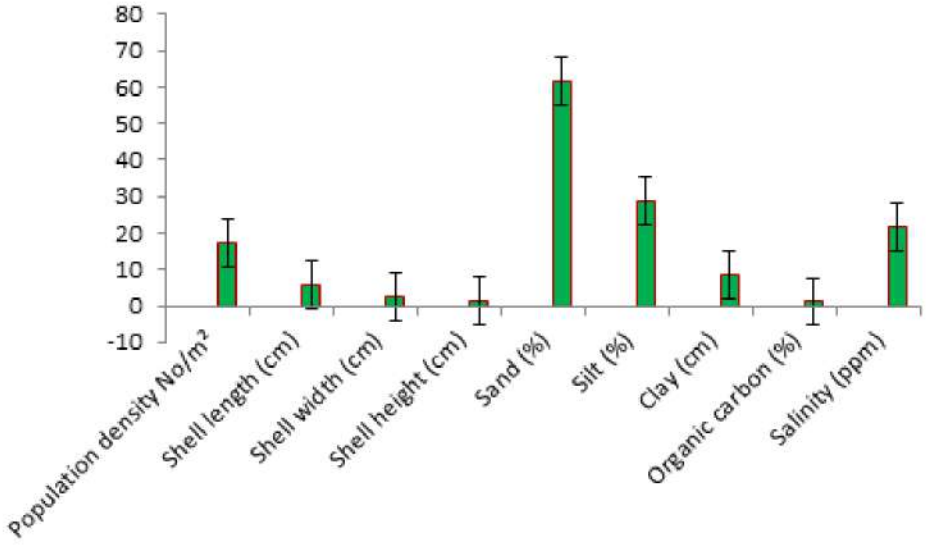

Table-1: Graphical representation of different biological and hydroedaphic parameters.

Storage Potentiation of a Grass pea Seed species using Selected Plant Extracts

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ARTICLE INFO

Received: 01.10.2020

Revised: 01.01.2021

Accepted: 10.01.2021

Key words

Grass pea, *Ocimum,Aegel,* storage potentiation, accelerated ageing.

ABSTRACT

Pretreatment of grass pea (*Lathyrus sativus* L.) seeds with aqueous solutions of leaf extracts of tulsi (*Ocimum sanctum*) and bel (*Aegel marmelos*), 25g in 1000 ml distilled water of each for 2 hours and then dried back to the original dry weight of the seeds before accelerated ageing treatment (99.5% RH and 32±2^{0}C) for different durations (0 to 30 days) slowed down the rapid loss of germination and reduced the time (h) required for 50% germination (T_{50}) of seeds. The plant extracts also significantly arrested the reduction of protein, insoluble carbohydrate as well as activity of catalase enzyme of seed kernels during forced ageing period. Conversely, ageing-induced stimulation of the activity of amylase enzyme was alleviated by the seed pretreating agents. Thus, the promising effects of the experimental plant extracts on storage potentiation of the grass pea seeds are apparent in this investigation.

Introduction

Pulses constitute groups of crops of the legume family which with the help of *Rhizobium* like bacteria in their root nodules fix atmospheric nitrogen and improve the soil fertility. These crops are generally included in rotation in most of the areas in India and help to keep soil alive and productive. The pulse crop like grass pea (*Lathyrus sativus*) is considered as almost zero management crops requiring little attention for raising yield (Sundararaj and Thulasidas, 1993).

Maintenance of vigour and viability of seeds in tropical countries like India is a matter of serious concern to the crop growers because of high temperature and high relative humidity (RH) prevailing in major parts of the country almost throughout the year. These two environmental factors strongly impair seed and seedling health and cause to reduce percent seed germinability and seedling performance at a rapid rate (Basu,1994, Copeland and McDonald, 1995, Bhattacharjee, 2001, Mishra, 2006, Pati and Bhattacharjee,2013). Keeping in mind the problem of seed storing in our country, an attempt has been made in this

investigation to prolong the storage life of the grass pea seeds having viability problems using tulsi (*Ocimum sanctum*) and bel (*Aegle marmelos*) leaf extracts. Experiments of this investigation were carried out under accelerated ageing condition to obtain more or less uniform and expeditious results (Heydecker, 1972, Pati and Bhattacharjee, 2016,Pati,2019) and this mimics the natural ageing process.

Materials and Methods

After surface sterilization (0.1% $HgCl_2$ for 90 seconds) the seed sample of grass pea (*Lathyrus sativus* L.) was separately presoaked in the aqueous leaf extracts of tulsi (*Ocimum sanctum*) and bel (*Aegle marmelos*), 25g/ 1000ml each, or distilled water for 2 hours (h) and then dried back to the original dry weight of the seeds. This was repeated twice allowing maximum penetration of the chemicals present in the aqueous solution. The pretreated seed lots were taken in separate cloth bags and thus stored in a desiccator in which 99.5% relative humidity (RH) was preimposed by keeping 1.57% H_2SO_4 within it. This experimental set up was kept at 32±2°C for 30 days allowing the seeds to experience forced ageing treatment and H_2SO_4 was changed at 15 day intervals to restore the desired RH within the desiccator for 30 days.

To analyse the percentage germination seeds of each treatment were transferred to separate Petri dishes containing filter paper moistened with 10ml distilled water. Germination data were recorded after 96 h of seed soaking following the International Rules for Seed Testing (ISTA, 1976). The time for 50% germination of seeds (T_{50}) was determined following the method described by Coolbear *et al.* (1984).

Protein, insoluble carbohydrate contents as well as activities of catalase and amylase enzymes were analysed from seed kernels of each sample. Quantification of insoluble carbohydrates was done following the method of McCready *et al.* (1950). Protein levels was estimated as per the methods of Lowry *et al.* (1951). Extraction and estimation of the enzyme catalase was made following the method of Snell and Snell (1971) as modified by Biswas and Choudhuri (1978). Amylase activity was estimated as per the method of Khan and Faust (1967). Assaying of these enzymes were done as per the method of Fick and Qualset, 1975.

Data were statistically analysed at the treatment and replication levels and least significant difference (LSD) values were calculated at 95% confidence limits (Panse and Sukhatme, 1967).

Results and Discussion

Data clearly revealed that pretreatment of the seed species with bel and tulsi leaf extracts significantly alleviated the accelerated ageing-induced loss of germination and reduced T_{50}

hours (Table 1), alleviated the loss of protein and insoluble carbohydrates (Table 2) as well as ageing-induced reduction of activity of catalase enzyme (Table 3) and stimulation of the activity of amylase enzyme (Table 3).

The results therefore point out that although deterioration is a common phenomenon in treated and control samples of the seed species, the catabolic processes within the treated seed samples remained somewhat subdued, thereby rendering them tolerant against unfavourable storage environment. Available reports show that during seed ageing a loss of some vital cellular components including protein, carbohydrates occurred (Abdul Baki and Anderson, 1972, Kole and Gupta, 1982). Catalase is regarded as a scavenger enzyme (Fridovich, 1976, Pati and Bhattacharjee, 2017, Ojha *et al.*,2020) and higher activity of this enzyme is indicative of higher plant vigour (Sarkar and Choudhuri, 1980). In this investigation, the plant extract-induced arrestation of rapid loss of the enzyme activity is indicative of strengthening the defense mechanism by the herbal extracts under adverse storage condition.

It can be concluded from the results of this investigation that tulsi and bel leaf extracts are effective in enhancing storage potential of grass pea seed species. Thus, invigouration property of the present seed pretreating agents seems to be apparent from the experimental results.

Table 1

Effect of seed pretreatment with leaf extracts of *Ocimum* sp. and *Aegle* sp. (25g/1000ml each) on percentage seed germination and T_{50} (time required for 50% germination) values of grass pea seeds.

Seed sample	Treatments	Percentage seed germination			T_{50} of germination		
		Days after accelerated ageing					
		0	**15**	**30**	**0**	**15**	**30**
Grass pea	Control	100	78	38	12	36	NA
	Ocimum sp.	100	84	57	12	24	72
	Aegle sp.	100	80	52	12	24	84
	LSD (P = 0.05)	NC	5.60	4.58	NC	2.50	6.05

NC: Not calculated; NA : Non attainment of 50% germination.

Table 2

Effect of seed pretreatment with leaf extracts of *Ocimum* sp. and *Aegle* sp. (25g/1000ml each) on protein (mg/g fr. wt.) and insoluble carbohydrates (mg/g fr. wt.) levels of grass pea seeds.

Seed sample	Treatments	Protein			Insoluble carbohydrates		
		Days after accelerated ageing					
		0	**15**	**30**	**0**	**15**	**30**
Grass pea	Control	32.0	22.6	11.9	23.10	18.50	10.19
	Ocimum sp.	32.2	28.2	22.0	23.17	21.82	18.02
	Aegle sp.	32.7	29.9	28.9	23.16	20.19	17.07
	LSD (P = 0.05)	NS	1.05	1.15	NS	1.01	0.08

NS : Not significant.

Table 3

Effect of seed pretreatment with leaf extracts of *Ocimum* sp. and *Aegle* sp. (25g/1000ml each) on activities of enzyme catalase (unit/h/g fr. wt.) and amylase (unit/h/g fr. wt.)) of grass pea seeds.

Seed sample	Treatments	Catalase			Amylase		
		Days after accelerated ageing					
		0	**15**	**30**	**0**	**15**	**30**
Grass pea	Control	40.4	26.2	16.9	37.1	50.0	67.8
	Ocimum sp.	40.2	32.1	27.9	37.2	40.1	51.7
	Aegle sp.	40.0	30.9	25.0	37.0	41.2	53.4
	LSD (P = 0.05)	NS	2.50	1.35	NS	3.95	4.80

NS : Not significant.

References

Abdul-Baki, A.A. and Anderson, J.D. (1972). Physiological and biochemical deterioration of seeds. In *Seed Biology* (ed. T.T. Kozlowski), Vol. **2**, pp. 283-315, Academic Press, New York.

Basu, R.N. (1994). An appraisal of research on wet and dry physiological seed treatments and their applicability with special reference to tropical and sub-tropical countries. *Seed Science and Technology,* **22**, 107-126.

Bhattacharjee, A. (2001). Influence of a growth retardant and a volatile oil on enhancement of storage longevity of two low vigour seed species. *Journal of National Botanical Society.* **55**, 55-61.

Biswas, A.K. and Choudhuri, M.A. (1978). Differential behaviour of the flag leaf of intact rice plant during ageing. *Biochemie. und Physiologie der Pflanzen.* **173**, 220-228.

Coolbear, P., Francis, A. and Grierson, D. (1984). The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *Journal of Experimental Botany*, **35**, 1609-1617.

Copeland, L.O. and McDonald, M.B. (1995). In *Principles of Seed Science and Technology* (3rd edn.) Chapman and Hall, New York.

Fick, N.G. and Qualset, C.O. (1975). Genetic control of endosperm amylase activity and gibberellin responses in standard height and short statured wheat. *Proceedings of National Academy of Science.* USA, **72**, 892-895.

Fridovich, I. (1976). Oxygen radicals, hydrogen peroxide, and oxygen toxicity. In *Free Radicals in Biology*, (ed. W.A. Prior), Vol. **1** pp. 239-277, Academic Press, New York.

Heydecker, W. (1972). *Vigour in Viability of Seeds* (ed. E.H. Roberts), pp. 209-252. Chapmann and Hall Ltd., London.

International Seed Testing Association (1976). International Rules for Seed Testing. *Seed Science and Technology.*, **4**, 51-177.

Khan, A.A. and M.A. Faust (1967). Effect of growth retardants on a-amylase production in germinating barley seeds. *Physiologia Plantarum.* **20,** 673-681.

Kole, S. and Gupta, K. (1982) Biochemical changes in safflower (*Carthamus tinctorius*) seeds under accelerated ageing. *Seed Science and Technology*, **10**, 47-54).

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin-phenol reagent. *Journal of Biological Chemistry*, **193**, 265-275.

McCready, R.M., Guggloz, J., Silvirea, V. and

Ownes, H.S. (1950). Determination of starch and amylase in vegetables. *Analytical Chemistry*., **22**, 1156-1158.

Mishra V.K.2006. Studies on seed vigour and viability of a few pulse crops and devising techniques of seed invigouration. Ph.D. Thesis, Vidyasagar University, India.

Ojha, S., Pati, C. K. and Sen, M.(2020). Allelopathic effect of *Fagopyrum* plant extracts on crop seed species, *Science and Culture*, **86(3-4)**,113-115.

Panse, V.G. and Sukhatme, P.T. (1967). Statistical methods for agricultural workers. 2e., pp. 150-157. *Indian Council of Agricultural Research*, New Delhi.

Pati, C. K. and Bhattacharjee A. (2013).Chemical Manipulation for Storage Potentiation of Crop Seeds.LAP LAMBERT Academic Publishing (ISBN: 978-3-659-38761-6), Germany.

Pati, C. K. (2016). Seed potentiation of a green gram species under storage.*Indian Agriculturist*. 60 (3&4):129-132.

Pati, C. K. and Bhattacharjee, A. (2017). Storage Potentiation of a pea seed species under accelerated ageing condition. *International Journal on Agricultural Sciences*.8 (1):30-34.

Pati, C. K. (2019). Prolongation of seed viability under storage, *World Scientific News*, **132**,256-263.

Sarkar, U. and Choudhuri; M.A. (1980). Glycolate content, glycolate oxidase and catalase activity in intact sunflower plant during ageing and development. *Biochemie und Physiologie der Pflanzen*, **175**, 23-28.

Snell, F.D. and Snell, C.T. (1971). Colorimetric methods of analysis, Vol. **4AAA**. p. 7-145. Van Nostrand Reinhold Co., New York.

Sundararaj, D.D. and Thulasidas, G. (1993). Botany of Field Crops (2nd edn.). Macmillan India Ltd., New Delhi-110002.

Immune responses of the circulating haemocytes of *Lissachatina fulica* (Bowdich, 1822) challenged with the spores of *Rhizopus* sp and *Aspergillus* sp.

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ARTICLE INFO
Received: 20.06.2021
Revised: 07.08.2021
Accepted: 16.08.2021

Key words:
Fungal spores, Gastropoda, Phagocytosis, Pleomorphic fibrillar cell, SEM, Time lag immune response.

ABSTRACT

Light and scanning electron microscopic observations are made on the haemocytes in the haemolymph of *Lissachatina fulica*. Three distinct populations of the haemocytes are recognized. They are Group I, Group II, and Group III haemocytes. Group I cells are round, non-spreading small cells. Group II and Group III cells are spreading haemocytes. Group II cells are categorized into agranulocytes, small granulocytes, and large granulocytes. Agranulocytes are large slightly spreading agranular cells. Small granulocytes are small spreading granular cells. Large granulocytes are spreading granular cells, forming numerous pseudopodia. Group III cells are termed as "pleomorphic fibrillar cells". Group III cells are characterised into three sub-types (Type-A, Type-B, and Type-C) depending on cell morphology. Time lag immune responses of all the haemocytes are studied after challenged with the spores of *Rhizopus* sp and *Aspergillus* sp. Group I cells show their defense response by formation of the cell nodulation to each other. Cell nodulation is very common response of the agranulocytes, small granulocytes, Type-A and Type-B Group III cells against the invading fungal spores. Large granulocytes form numerous pseudopodia and vacuoles. It is observed that the circulating haemocytes in the snail showing more cell surface modifications after challenged with the spores of *Rhizopus* than that of the spore of *Aspergillus*. The study advocates that the immune responses of the circulating haemocytes of *Lissachatina fulica* may depends on the specific immune activator.

Foot notes: The co-authors Dr. Korak Kanti Chaki and Dr. Kamales Kumar Misra were unfortunately expired on June 5th 2021 and Feb 23rd 2021 respectively. This work could not complete without their encouragement and cooperation. We are feeling very sad about their untimely departure and this work is dedicated to their unforgettable memories.

Introduction

Invertebrate immune systems apparently lack immunoglobulins, interactive lymphocyte subpopulations and lymphoid organs but they depend on circulating haemocytes (or immunocytes). Nevertheless, the huge numbers and diversity of invertebrates attests to the efficiency of their host defense. The mucus and tough external skeletons form barriers to invasion in some coelenterates and molluscs, echinoderms, and arthropods. When these barriers are breached, the pathogens are then exposed to a range of interacting cellular and humoral defense reactions like, blood clotting/coagulation, wound healing, phagocytosis, encapsulation responses, natural and inducible antimicrobial factors. Circulating immunocytes are considered as main responsive cells in the immune system of gastropods. Haemolymph of gastropod molluscs contains dissolved haemoproteins and immunocytes which mainly perform phagocytosis (Sminia, 1972, 1981; Yoshino, Granath, 1985; Knaap et al., 1981). Phagocytic cells ingest microbial as well as other immunogenic invaders and encircle larger invaders. Light and electron microscope studies revealed that there are two categories of immunocytes in gastropod molluscs as either agranulocytes or hyalinocytes and granulocytes (Cheng, 1984; Yonow, Renwrantz, 1986; Voltzow, 1994; Mahilini & Rajendran, 2008) or non-spreading, round cells and spreading cells (Ottaviani, 1983; Ottaviani, 1992; Ottaviani & Franchini, 1988; Martin et al., 2007).

The structure and functions of haemocytes are studied in a number of gastropods including pulmonates such as *Lymnaea stagnalis* (Adema et al., 1992; Adema et al., 1994), *Biomphalaria glabrata* (Matricon-Gondran, Letocart, 1999), *Haliotis tuberculata* (Serpentini *et al.*, 2000; Malham et al., 2003), *Megathura crenulata* and *Aplysia californica* (Martin et al., 2007), *Trachea vittata* (Mahilini & Rajendran, 2008) and *Pila globosa* (Mahilini & Rajendran, 2008; Ray et al., 2013), *Bellamya bengalensis* (Kambale & Potdar, 2010; Ray et al., 2013). The types and functions of haemocytes in the molluscs in ambient conditions are necessary in studying basic cell responses to the infections (Beckmann et al., 1992; Medzhitov, Janeway, 2000; Janeway & Medzhitov, 2002; Cochennec-Laureau et al., 2003; Plows et al., 2005; Ottaviani, 2011).

The giant African snail, *Lissachatina fulica* (Bowdich, 1822) (Achatinidae, Stylommatophora, Eupulmonata) is considered as an agricultural pest throughout the World. This species is formerly known as *Achatina fulica* (Gastropoda: Pulmonata) (http://www.marinespecies.org/aphia.php?p=taxdetails & id=881469). Earlier, Adema et al. (1992) reported the presence of two types of haemocytes in *Achatina fulica* i.e., round cells

and spreading cells. The authors also examined 5 other species of gastropods for the haemocytes under light microscope. The detail classifications, structures and functions of circulating haemocytes of *Lissachatina fulica* were not clearly described.

The present study aims to identify and categorize different circulating haemocytes in the haemolymph of *Lissachatina fulica* (Bowdich, 1822) and their responsiveness against the spores of *Rhizopus* sp and *Aspergillus* sp. The characteristic responses of the various haemocytes against these fungal spores are studied using light and scanning electron microscope in different time lag period. The morphology and morphometry of the circulating haemocytes of both control and fungal spore challenged snails are also compared here. The hypothesis of the study is that specific sub-population of circulating immunocytes would exhibit specific defense responses against specific antigen.

Material and methods

Sampling and rearing of experimental animals

Total 120 active, healthy *Lissachatina fulica* were collected from the field around Kolkata during rainy season (June-July). The snails were kept in a wooden box and acclimatized for five days, providing with leafy vegetables and spraying water throughout the study period and controlled temperature (27°C ± 2° C) and humidity (90 ± 10 %) to minimize seasonal stress. The *Rhizopus* was collected from the moist used tea leaves and the *Aspergillus* was collected from the fungi infested breads. These fungi were maintained in two separate glass jars and maintained a moist condition by frequently spraying water.

Work design of the study and collection of the haemolymph

Total 100 snails of similar size group (average shell length 6.75 ± 0.25 cm) were selected for the study and were divided into four wooden

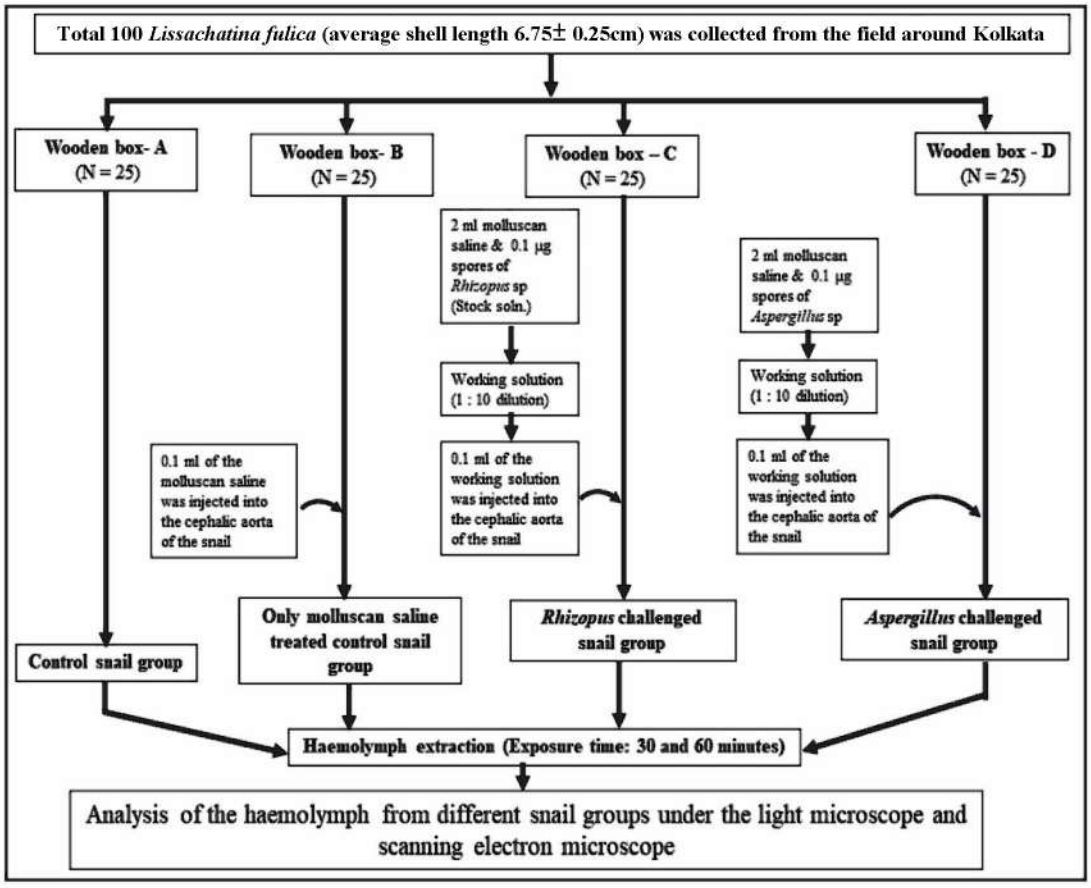


Fig. 1 The work design of the study to investigate the immune responses of the various circulating haemocytes in *Lissachatina fulica* challenged the spores of *Rhizopus* sp and *Aspergillus* sp.

boxes, marked as the control snail group (N = 25), only molluscan saline treated control snail group (N = 25), *Rhizopus* challenged snail group (N = 25) and *Aspergillus* challenged snail group (N = 25) (Fig. 1).

The spores of these two fungi were collected into individual petri dish by simply scrapping the surface of the corresponding tea-leaves and bread with sharp scalpels. An individual stock solution for each fungal spores was prepared (0.1 mg spores: 2 ml molluscan saline) (Adema et al., 1992). A working solution was prepared with 1:10 dilution with molluscan solution from the respective stock solutions and kept into individual glass vial and marked properly (Table 1). The working solution (about 0.1ml)

of the *Rhizopus* spore and *Aspergillus* spore was injected using commercially available insulin syringe into the cephalic aorta of the individuals in both experimental groups; *Rhizopus* challenged snails and *Aspergillus* challenged snails respectively.

The haemolymph was collected from the live individuals of the four snail groups by using individual sterile insulin syringe following a standard method (Oakes, 2008; Bakry, 2009). The needle was inserted into the visceral aorta of the individuals (N = 5) and the desire haemolymph was collected from the four experimental snail groups by slow pulling of the plunger of the syringe on the 30 and 60 minutes of the fungal spore administration (Fig. 1). The haemolymph from all the four snail groups were used to study the haemocytes and their morphological properties under the light microscope (Nikkon 50i) and the scanning electron microscope (Fei, Quanta 200).

Light microscopic study

For the morphological and morphometric studies of the haemocytes both the thick and thin smears of haemolymph were prepared, stained with the Haematoxylin-Eosin and Giemsa stain and examined under light microscope. The analysis of a single drop was done here with two vital stains such as the Janus green and Methylene blue (0.1 %). In the single drop analysis, a drop of haemolymph was kept on a clean glass slide (25 mm × 75 mm) and a tiny drop of a vital stain was pour on the haemolymph drop using a fine needle tip covered by a watch glass and the whole setup was kept in a cold (4°C) chamber for 15 minute to settle as well as staining of the suspended cells. The stage micrometer scale (0.01mm, Erma; Tokyo) and ocular micrometer scale (1 ocular division = 4.35 µm in 400X magnification) were used to measure cellular parameters under light microscope.

Table 1. Different immunogenic components (fungal spores) inoculating into *Lissaachatina fulica*.

Immunogenic components (Fungal spores)	No. of spores in solution		State of immunogen interaction	Time of exposure (Minutes)	Stains
	Stock solution (0.1 mg)	Working solution (ten times dilution)/ (1 ml)			
Spores of *Rhizopus* sp	~ 4250 - 4300	~ 214	*in vivo* & *in vitro*	30 & 60	Jenus green
Spores of *Aspergillus* sp	~ 4190 - 4210	~ 210	*in vivo* & *in vitro*	30 & 60	Methylene blue

Differential count and viability assay of the haemocytes

The population of different haemocytes was evaluated by the thin smear analyses of the haemolymph of both the control and the spore challenged snails. These all smears were stained and more than 200 cells were examined under light microscope with 400X magnification. The haemocytes were categorized depending on relative cell size, spreading properties, cytoplasmic variations, phagocytic ability as well as their staining properties of both the nucleus and cytoplasm.

Additionally, the haemolymph was incubated in Trypan Blue (0.1%) for five minute and counted the dead cells to determine cell viability by Trypan Blue exclusion assay (Accorsi et al., 2013).

Scanning Electron Microscopy (SEM) of the haemocytes

For the ultrastructural studies of the cell surface of the haemocytes, some of the collected haemolymph of the four groups of the *Lissachatina fulica* were used to make the individual thin smears of the haemolymph. An individual thin smear was made on a small clean glass cover slip (5 mm in diameter). After air-dried the smears, all the smear containing cover slips were fixed with Karnovsky's fixative (pH 7.2) for 30 minutes. The smears were washed with phosphate buffer solution (pH 7.4) and dehydrated with ascending grade of the ethanol. The samples were coated with gold using S150 Sputter Coater and examined under SEM (Fei, Quanta 200).

Results

In the present study, it is observed that there are no such significant differences of the haemolymph constituents between the individuals of the control snail group and the only molluscan saline treated snail groups. The following sections are dealt with the comparative morphological characteristics of the haemocytes between the control snail group and two fungal spore challenged snail's groups.

Morphology and morphometry of the haemocytes

The snail possesses three distinct populations of haemocytes on the basis of their size, spreading properties and cytoplasmic variations of the cells. The cell populations are named as Group I (Gr-I) cells, Group II (Gr-II) cells and Group III (Gr-III) cells (Fig. 2). The comparative morphological (Figs. 3"5) and morphometric analysis of different haemocytes along with their staining properties are tabulated in Table 2.

The Gr-I cells are characterised by small sized, round cells (Figs. 3 and 4A). The size of the cell is 12.92 ± 0.09 μm in diameter and its population size is 16 ± 5 % in the circulation. These cells are either non-spreading or very slightly spreading in nature and easily distinguished after vital stain.

The Gr-II cells are pseudopod forming, spreading cells with various sizes. The Gr-II cells are further categorised into two classes depending on cytoplasmic granulations; agranulocyte (ag) (Fig. 4B) and granulocyte (Figs. 4C-D). The agranulocytes (16.92 ± 0.09 µm in diameter) are composed of large nucleus with agranulated cytoplasm (Figs. 4B and 4A). The agranulocyte is slightly spreading cell and the population was 12 ± 3 % in circulation. The granulocytes are characterized by granulated cytoplasm and having two sub-classes; small granulocyte (sg) cells (Fig. 4C) and large granulocyte (lg) (Figs. 4D and lg, in 5A). The small granulocytes (11.35 ± 0.38 µm in diameter) possess circular nucleus, occupies most of the cellular area and was surrounded by thin rim of cytoplasm (Fig. 4C). The large granulocyte (15.44 ± 2.55 µm in diameter) contains large nucleus with no definite shape. The nucleus of large granulocytes is located either acentrically or eccentrically in the cell. The cytoplasm of the large granulocytes consists of distinct small vacuoles (Fig. 4D). The population size of small and large granulocytes is 14 ± 4% and 33 ± 3 % respectively in circulation.

The Gr-III cells are more variable in their shape and size. The cytoplasm and nucleus of these cells are identical in staining characters and fibrous in nature. Although the whole cell body appeared as typical magenta or bluish-purple colour after Giemsa staining (Table 2). In case of haematoxylin and eosin stain the total cell body of these cells were pinkish with a very little blue portion of nucleus. The Gr-III cells are categorized into three subtypes: Type-A Gr-III (Gr-IIIa) cells, Type-B Gr-III (Gr-IIIb) cells and Type-C Gr-III (Gr-IIIc) cells and are designated as "Pleomorphic fibrillar cells" (Figs. 4E-G and 5B-D). The Gr-IIIA cell (15.70 ± 2.16 µm in diameter) is circular in shape and consists of prominent deeply stained circular or kidney shaped nucleus (Figs. 4E and 5B). The Gr-IIIB cells (20.14 ± 4.35 µm in diameter) have no definite shape and comparatively larger than Type-A Gr-III cells. The cell cytoplasm of Type-B Gr-III cell is fibrous with indistinct nucleus (Figs. 6F and 5C). The Type-C Gr-III cell (25.1 ± 8.70 µm in diameter) is larger than Type-A Gr-III and Type-B Gr-III cells. The thin fibrous cytoplasm contains very indistinct fibrous nucleus (Figs. 4G and 5D). In the circulation the population size of three subtypes (Type-A, Type-B, and Type-C) of Gr-III cells were 13 ± 2 %, 10 ± 2 %, and 6 ± 3 respectively.

The cell viability in the undiluted haemolymph is unchanged up to 45 minutes of extracted haemolymph.

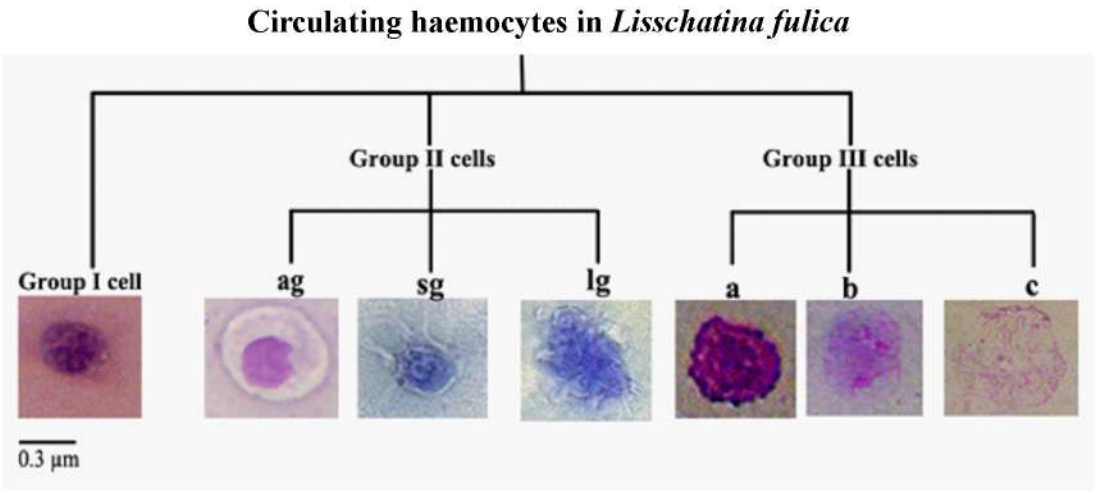


Fig. 2 An illustrative tree of different circulating haemocytes in the haemolymph of the control *Lissachatina fulica.* There are three major cell populations as Group I cells, Group II cells and Group III cells. The Group II cells include three sub-classes as the agranulocyte (ag), small granulocyte (sg), large granulocytes (lg). The Gr-III cells consist of three subclasses as Type-A Group III (a) cell, Type-B Group III (b) and Type-C Group III (c) cell.

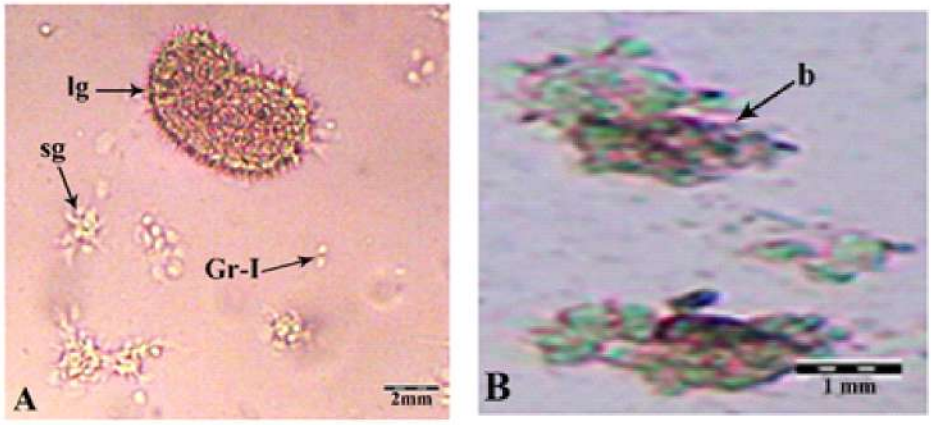


Fig. 3 Circulating haemocytes of the control *Lissachatina fulica* after single drop analysis with Jenus green stain. **A,** Group I (Gr-I) cells, small granulocyte (sg) and large granulocyte (lg). **B,** Type-B Group III cell (b).

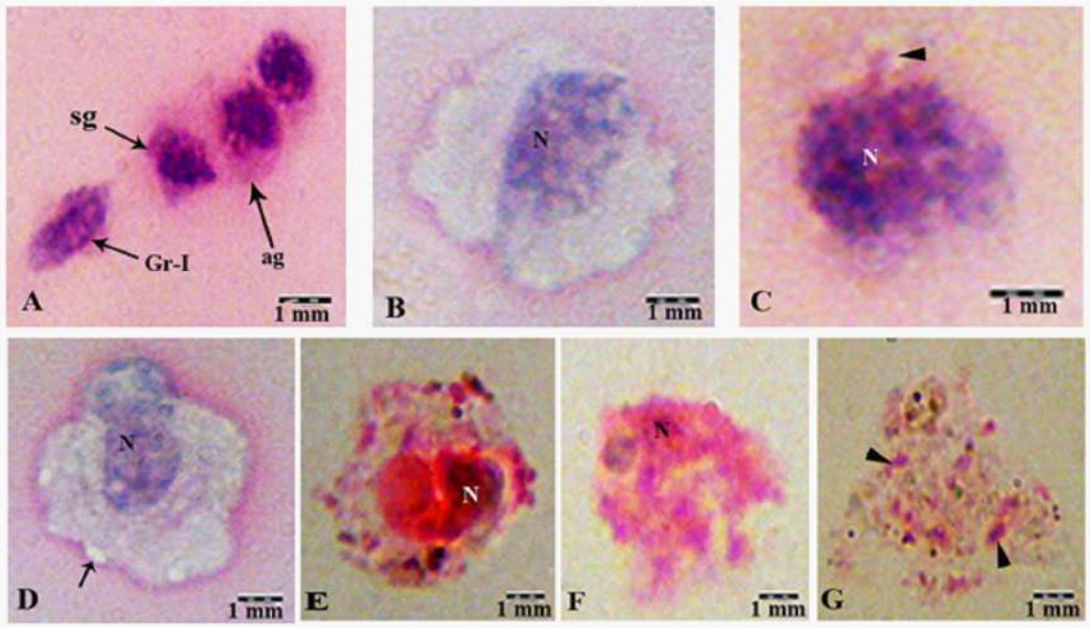
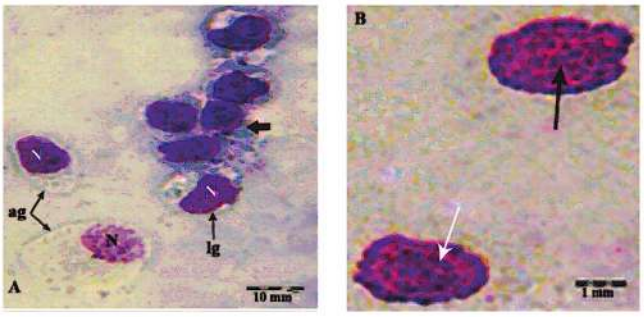


Fig. 4 Thin smear of haemolymph of the control *Lissachatina fulica* stained with haematoxylin-eosin. **A,** Gr-I cells with thin rim of cytoplasm. **B,** Agranulocyte with agranular cytoplasm. **C,** Small granulocyte with short pseudopodia (arrow head). **D,** Large granulocyte with many vacuoles (arrow) in granular cytoplasm. **E,** Type-A Gr-III cell with distinct kidney shaped deeply stained nucleus (N). **F,** Type-B Gr-III cell with indistinct nucleus in fibrous cytoplasm. **G,** Type-C Gr-III cell with fibrous nucleus and cytoplasm. Staining properties of both nucleus and cytoplasm are same. Nucleus is not clearly noticeable and indicated by deeply stained scattered granules (arrow head) in the fibrous cytoplasm. Ag = agranulocyte, Gr-I = Group I cell, sg = small granulocyte, N- nucleus.



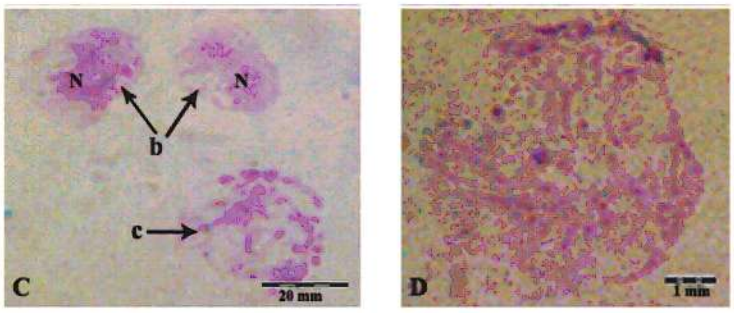


Fig. 5 Thin smear of haemolymph of the control *Lissachatina fulica* stained with Giemsa stain. **A,** Agranulocytes (ag), large granulocyte (lg) and cell nodule (bold arrow) of different haemocytes. **B,** Type-A Gr-III cell with prominent nucleus (arrows). **C,** Type-B (b) and Type-C (c) Gr-III cells with fibrous body. **D,** A typical Type-C (c) Gr-III cell with highly fibrous body. Nuclear materials are very indistinct. Staining properties of both nucleus and cytoplasm are same. lg = large granulocyte, N = nucleus.

Table 2. Morphology and morphometry comparisons of different haemocytes in the haemolymph of the control *Lissaachatina fulica*.

Cell types	Cell morphology	Cell population (%)	Nucleus morphology	Staining (Giemsa) properties		Cytoplasm	Cell diameter (µm)
				Cytoplasm	Nucleus		
1. Gr-I cell	Round with smooth cell membrane	16 ± 5	Circular, occupy most of the cytoplasm	Light pink	Blue	Small and agranular	12.92 ± 0.09
2. Gr-II cells							
ag	Semi-circular to oval with irregular cell membrane	12 ± 3	Circular or elliptical, centric or acentric	Light pink	Bluish pink	Large and granular	16.92 ± 0.09
sg	Circular with irregular cell membrane	14 ± 4	Circular, mostly occupy the cell	Light pink	Blue	Small and granular	11.35 ± 0.38
lg	Ovoid or circular with rough cell membrane	33 ± 3	Circular or elliptical, acentric or eccentric	Light pink	Blue	Large and densely granular	15.44 ± 2.55
3. Gr-III cells							
a	Mostly circular with smooth cell membrane	13 ± 2	Round, homogeneous and centric	Magenta	Deep blue	Moderate, homogeneous and densely granular	15.70 ± 2.16
b	No definite shape with lightly stained thin cell membrane	10 ± 2	Fibrous with dark coarse granules	Magenta	Magenta	Fibrous and granular	20.14 ± 4.35
c	No definite shape with very lightly stained cell membrane	6 ± 3	Fibrous with dark very small granules	Magenta	Magenta	Highly fibrous and granular	25.1 ± 8.70

Note: ag, Agranulocyte; lg, Large granulocyte; sg, Small granulocyte; a, Type A Gr-III cell; b, Type B Gr-IIII cell; c, Type C Gr-III cell.

Responses of the haemocytes against the spores of *Rhizopus*

Repeated single drop analyses of haemolymph with Janus green stain showed that after 30 minute of spore inoculation, the Gr-I cell together with other haemocytes form numerous small cell masses (Figs. 6A-E). The Gr-II cells produce a large number of pseudopods (Fig. 6A). The nodules are frequently distributed as small cell mass in the haemolymph (Figs. 6A-B). The Gr-III cells function as thin adhesive mats with very rough surface (Figs. 6C-E). The nuclear morphology, stainability, granulations of haemocytes between control and infected snails were almost same.

After 60 minute of inoculation, free Gr-I cells are not clearly seen in haemolymph. Some

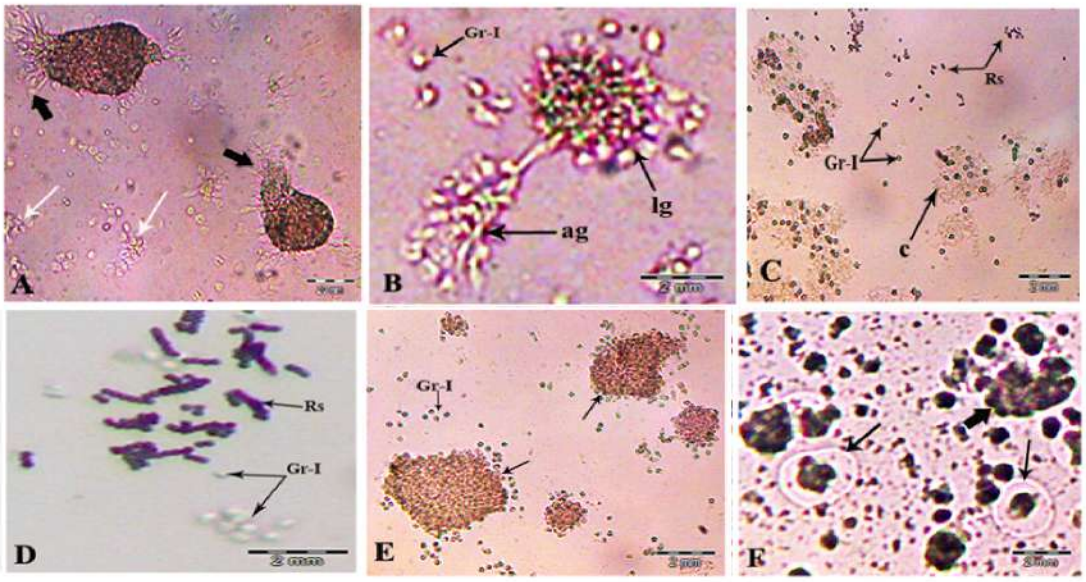


Fig. 6 Different haemocytes of the *Lissachatina fulica* after 30 minutes (A-E) and 60 minutes (F) time lag of inoculation by spores of *Rhizopus* sp (single drop analysis with Janus green stain). **A,** Large granulocytes showing numerous pseudopods (bold arrow). Aggregation of Gr-I cells and formation of small cell nodules (white arrow). **B,** Cell-cell interaction between Gr-II haemocytes. **C,** Spores are arrested on Type-C (c) Gr-III cells. Note some Gr-I cells on the Type-C cell. **D,** Mass of Gr-I cells and spores (Rs). **E,** Aggregation of numerous Gr-I cells with Type-B Gr-III cell. F, After 60 minutes phagocytic haemocytes displaying complete engulfment (thin arrows) of the spore. Note the lysis of the cell nodule (bold arrow). Ag = agranulocyte, Gr-I = Group I cell, lg = large granulocyte.

spores were completely encircled by some Gr-II cells (Fig. 6F). The changes of Gr-III cells are not found. The cell nodules are degraded into small fragments. The cell diameter of different haemocytes in the R*hizopus* challenged snails are slightly increased as Gr-I (12.95±0.02 µm in diameter), agranulocyte (16.97 ±0.01 µm in diameter), small granulocyte (11.36±0.01 µm in diameter), large granulocyte (15.45 ± 0.02 µm in diameter), Type A Gr-III cell (15.79 ± 0.1 µm in diameter), Type B Gr-III cell (20.19 ± 0.15 µm in diameter), and Type C Gr-III cell (25.17 ± 0.3 µm in diameter). These cell sizes of the various haemocytes are characteristically comparable with the haemocytes in the haemolymph of the control snail group (Table 2).

Response of haemocytes against the spores of *Aspergillus*

There are some morphological variations of the haemocytes after drop analyses with Methylene blue stain after 30 minutes of inoculation (Figs. 7A-D). The Gr-I cells form large cell mass along with other haemocytes (Fig. 7A). The Gr-II cells encircle the spores of *Aspergillus* through either tubular long pointed filopodia (Fig. 7B) or short blunt wing like lobopodia (Fig. 7C). Any significant morphological changes of the Gr-III cells are not observed.

After 60 minutes of inoculation, it is observed that some spores are completely encircled by large granulocyte cells (Fig. 7D). The cell diameter of different haemocytes in the infected snail were slightly increased as the Gr-I cell (12.94 ± 0.01 µm in diameter), agranulocyte cell (16.92 ±0.1 µm in diameter), small granulocyte (11.35 ± 0.02 µm in diameter), large granulocyte (15.44 ± 0.02 µm in diameter), Type A Gr-III cell (15.79 ± 0.2 µm in diameter), Type B Gr-III cell (20.18 ± 0.1 µm in diameter), and Type C Gr-III cell (25.17 ± 0.4 µm in diameter).

A comparative morphometrical features of the various haemocytes in the control snail group and two different fungal spore (*Rhizopus* and *Aspergillus*) challenged snail groups are graphically represented here (Fig. 8). It is

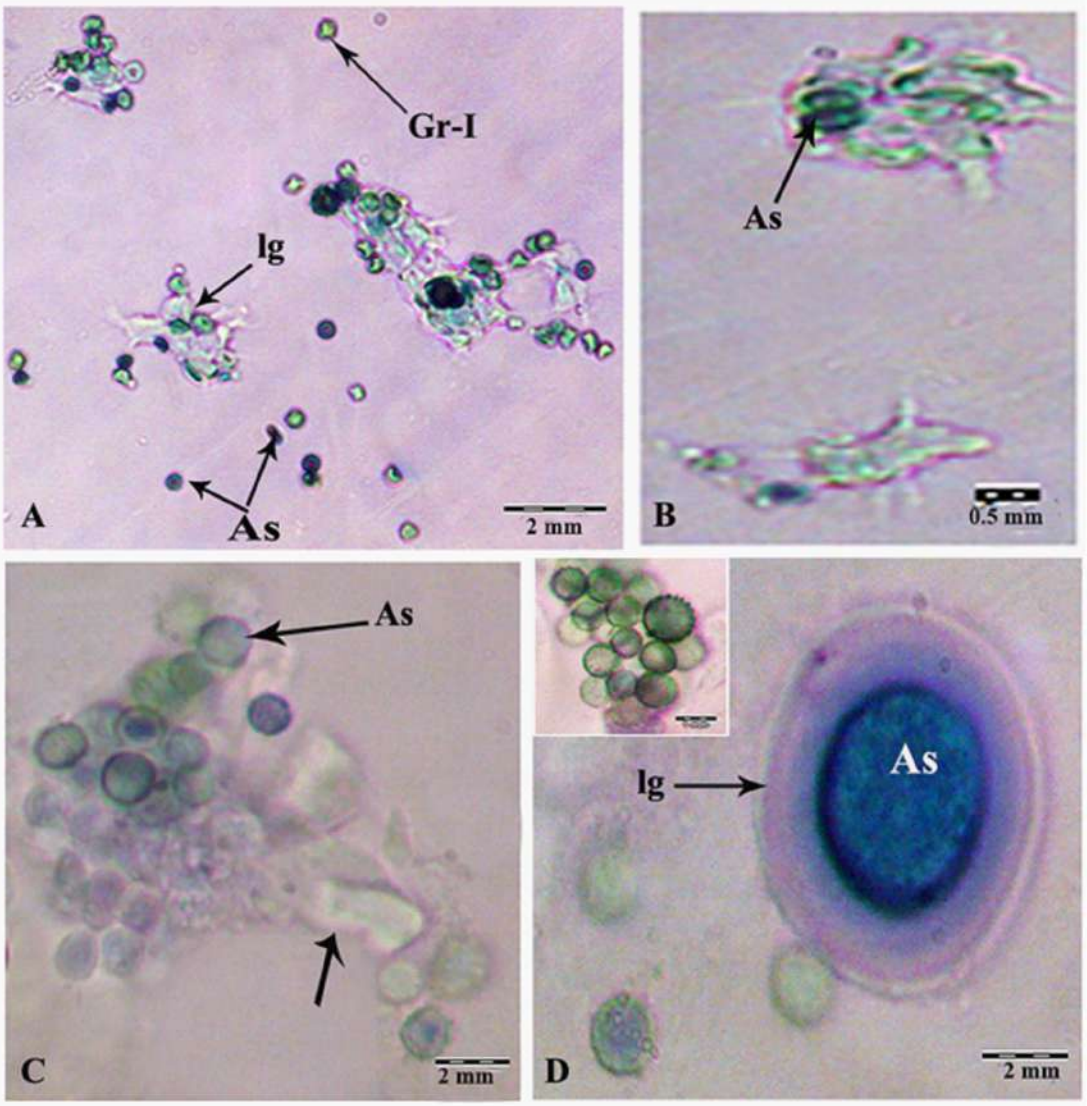


Fig. 7 Responses of haemocytes of the *Lissachatina fulica* after 30 minutes (**A-C**) and 60 minutes (**D**) time lag of inoculation by the spores of *Aspergillus* sp (Methylene blue stain). A, haemocytes involve in formation of nodule and in capturing of spores. **B,** Large granulocytes capture the spores (As). C, Wing like pseudopodial extensions (arrow) of large granulocyte. **D,** A large granulocyte (lg) completely engulfs a spore (As) of *Aspergillus*. Enlarged views of surface structure and staining property of spores are shown in shed. Gr-I = Group I cell.

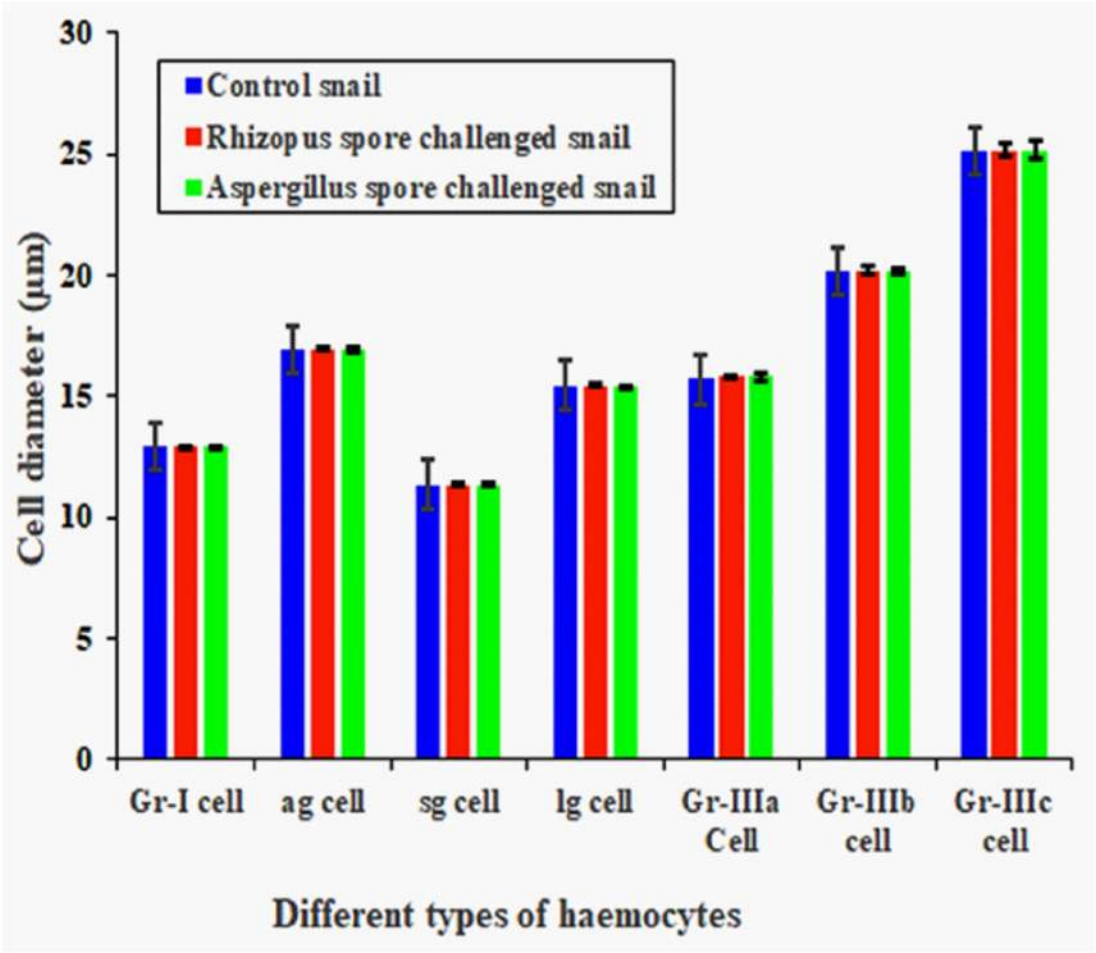


Fig. 8 Graphical comparison of the cell size (cell diameter) of the different haemocytes in the haemolymph of the control snail groups and different fungal spore challenged snail groups of *Lissachatina fulica.*

Table 3. Comparative cell diameter of different haemocytes in the haemolymph of control and two fungal spores (*Rhizopus* sp and *Aspergillus* sp) challenged *Lissaachatina fulica*.

Types of the haemocytes	Control snail	*Rhizopus* spore challenged snail	*Aspergillus* spore challenged snail
Gr-I cell	12.92 ± 0.09	12.95 ± 0.02	12.94 ± 0.01
ag cell	16.92 ± 0.09	16.97 ± 0.01	16.92 ± 0.01
sg cell	11.35 ± 0.38	11.36 ± o.o1	11.35 ± 0.02
lg cell	15.44 ± 2.55	15.45 0.02	15.44 ± 0.02
Gr-IIIa Cell	15.7 ± 2.16	15.79 ± 0.01	15.79 ± 0.2
Gr-IIIb cell	20.14 ± 4.35	20.19 ± 0.15	20.18 ± 0.1
Gr-IIIc cell	25.1 ± 8.7	25.17 ± 0.3	25.17 ± 0.4

Note: ag, Agranulocyte; lg, Large granulocyte; sg, Small granulocyte; Gr-IIIa, Type A Gr-III cell; Gr-IIIb, Type B Group-III cell; Gr-IIIc, Type C Gr-III cell.

observed that the spores of the *Rhizopus* sp have more immunogenic effects than that of the *Aspergillus* sp (Table 3).

SEM observations

The haemocytes in the control haemolymph are oval to semi-circular in shape with concavo-convex surface (Figs. 9A-F). The Gr-I cells are round to oval in shape and smaller in size than other cell groups. The Gr-II cells are with distinct concave and convex surfaces. The concave surface of Gr-II cells is rough with numerous marks of budding pseudopods (Fig. 9C). The whole concave surface of the Gr-II cells is appeared as honey combs like structure. The convex surface of the cells is almost smooth (Fig. 9B). In some cases, the Gr-II cells form short, tubular lobopodia with smooth surface (Fig. 9D). The Type-A Gr-III (Fig. 9E) and Type-B Gr-III (Fig. 9F) cells are commonly found in the smear.

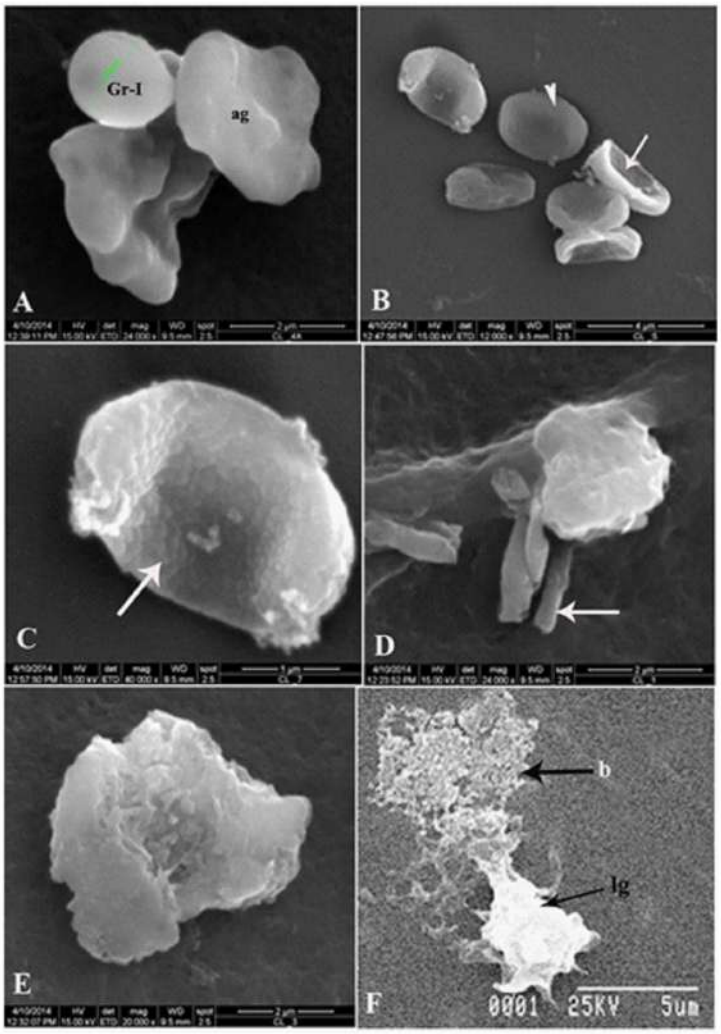


Fig. 9 Scanning electron micrographs of the haemocytes in the hemolymph of the control *Lissachatina fulica*. **A,** Cell nodule consists of different haemocytes. **B,** Large granulocyte showing smooth convex (arrow head) surface and rough concave surface (arrow). **C,** Magnified view of the concave surface (arrow) (honey comb like surface) of a large granulocyte. **D,** Lobopodia like pseudopods (arrow) of a phagocytic cell. E, Type-A Gr-III cell with uneven cell surface. F, Type-B Gr-III cell (b) adhere with a large granulocyte. ag = agranulocyte, lg = large granulocyte, sg = small granulocyte.

Responses of the haemocytes against the spores of *Rhizopus*

After 30 minute of spore inoculation, haemocytes show various structural modifications of pseudopods (Figs. 10A-K). Gr-I cells are found with no more modification. A typical whip like pseudopod is formed by Gr-II haemocytes. The cell is attached to the substratum by thick, sticky membranous structure (Fig. 10A). The filopodia (Figs. 10B, D, H, I, J), lobopodia (Figs. 10B, D, J) and axopodia (Fig. 10C) are characteristic pseudopodial modifications of Gr-II haemocytes.

After 60 minutes of inoculation, the responses of haemocytes are reduced (Figs. 11A-D). Gr-II haemocytes display very short pseudopodia (Figs. 11A-B). The Type-C Gr-III cells are very prominent in the cell nodules (Figs. 10C-D). The spores of *Rhizopus* are lightly attached on the haemocytes (Figs. 11C-D).

Responses of the haemocytes against the spores of *Aspergillus*

After 30 minute of inoculation, Gr-I cells are mostly found in nodules. Gr-II cells generate long tubular drum-stick like pseudopods from the concave surface of the cell (Figs. 12A-B). The free end of the pseudopods possesses a typical small circular head/knob (Fig. 12B). In some cases, the phagocytic haemocytes are changed with a cell surface ornamentation (Fig. 12C). The Gr-III cells displaying surface ornamentations composed of numerous small pits (Figs. 12 C-D). The marginal part of each of these membranous pits consists of short upwardly pointed spines (Fig. 12D).

After 60 minutes of inoculation, the haemocytes become solitary (Figs. 13A-D). The roughness of cell surface is increased than that of the 30 minute of lag periods (Figs. 13A-B). In haemocytes, the ability of pseudopod formation is decreased (Figs. 13A-C). The nodules are small in size but more compact in nature (Fig. 13D).

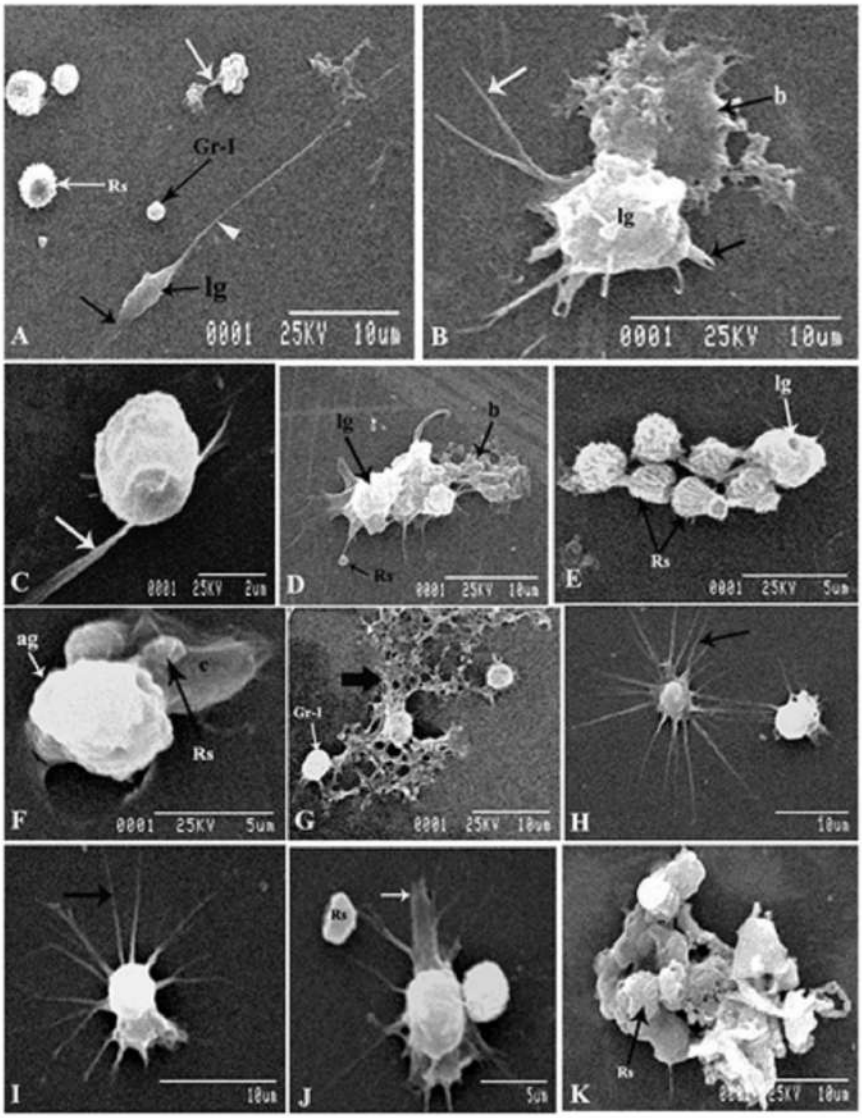


Fig. 10 Surface ultrastructure of the haemocytes of the *Lissachatina fulica* after 30 minutes inoculation of spores of *Rhizopus* sp. **A,** Whip like pseudopodial extension (white arrow head) of Gr-II cell. The cell body adheres to the substratum by a thin membranous structure (black arrow). Note the pseudopodial connection (white arrow) between cell nodules. **B,** Lobopodial (black arrow) and filopodial (white arrow) extension around the cell surface of Gr-II cell. C,

Fan like axopodia (arrow) formation of a Gr-II cell. **D,** Spore (Rs) is arrested by the filopodia of a haemocyte in a cell nodule. **E,** Phagocytic Gr-II cells arrest many spores in a file. **F,** Spores are wrapped by Gr-IIIC haemocyte. **G,** Mat or bed formation (bold arrow) by the aggregation of many Gr-IIIC cells and facilitates to hold the other haemocytes and spores. H-I, Typical tubular pointed filopodia of Gr-II phagocytic cells. **J,** Typical blunt lobopodia (white arrow) formation of Gr-II cell. **K,** Nodule formation of haemocytes and spores of *Rhizopus*. Gr-I = Group I cell, Gr-II = Group II cell, lg = large granulocyte, sg = small granulocyte, Rs- spore of *Rhizopus*.

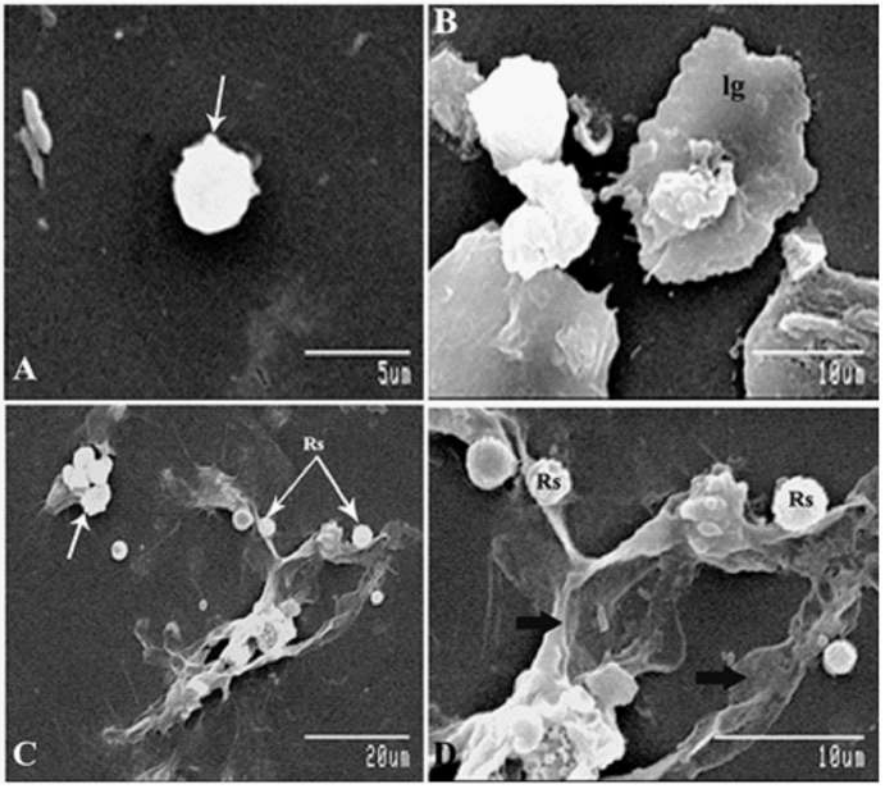


Fig. 11 The haemocytes of *Lissachatina fulica* after 60 minutes interaction with the spores of *Rhizopus* (Rs). **A,** haemocytes reduce their digitations around the cell surface (arrow). **B,** Weak cell-cell connections between haemocytes. **C,** Small nodule of haemocytes and thin cytoplasmic mat formation of Type-C Gr-III haemocytes. **D,** Magnified view of a portion of Fig. C. lg = large granulocyte.

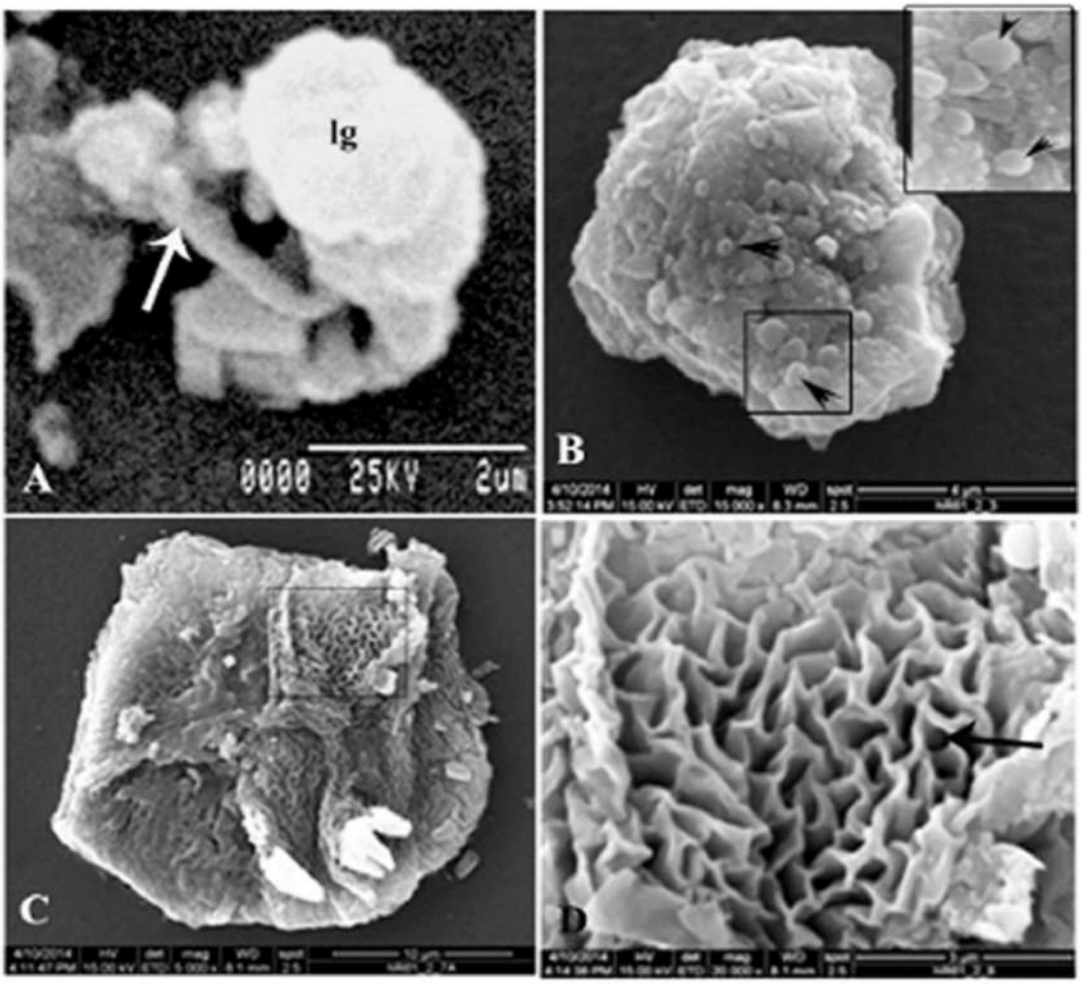


Fig. 12 Ultrastructural modifications of haemocytes of *Lissachatina fulica* after 30 minutes interaction with *Aspergillus* sp. **A,** Drum stick like pseudopodial extension (arrow) from a Gr-II phagocytic cell. **B,** Numerous drum stick like pseudopodial tips (arrow head) on the cell surface of phagocytic haemocytes (Gr-II cell). Magnified view of a marked area is shown in set. Note the knob like head on pseudopods (arrow head). **C,** Folded Gr-IIIC haemocytes displaying characteristic roughness of cell surface. **D,** Enlarged view of a marked area of Fig. C showing numerous small pits with spine like structure (arrow) on the surface of Gr-IIIC cell. lg = large granulocyte.

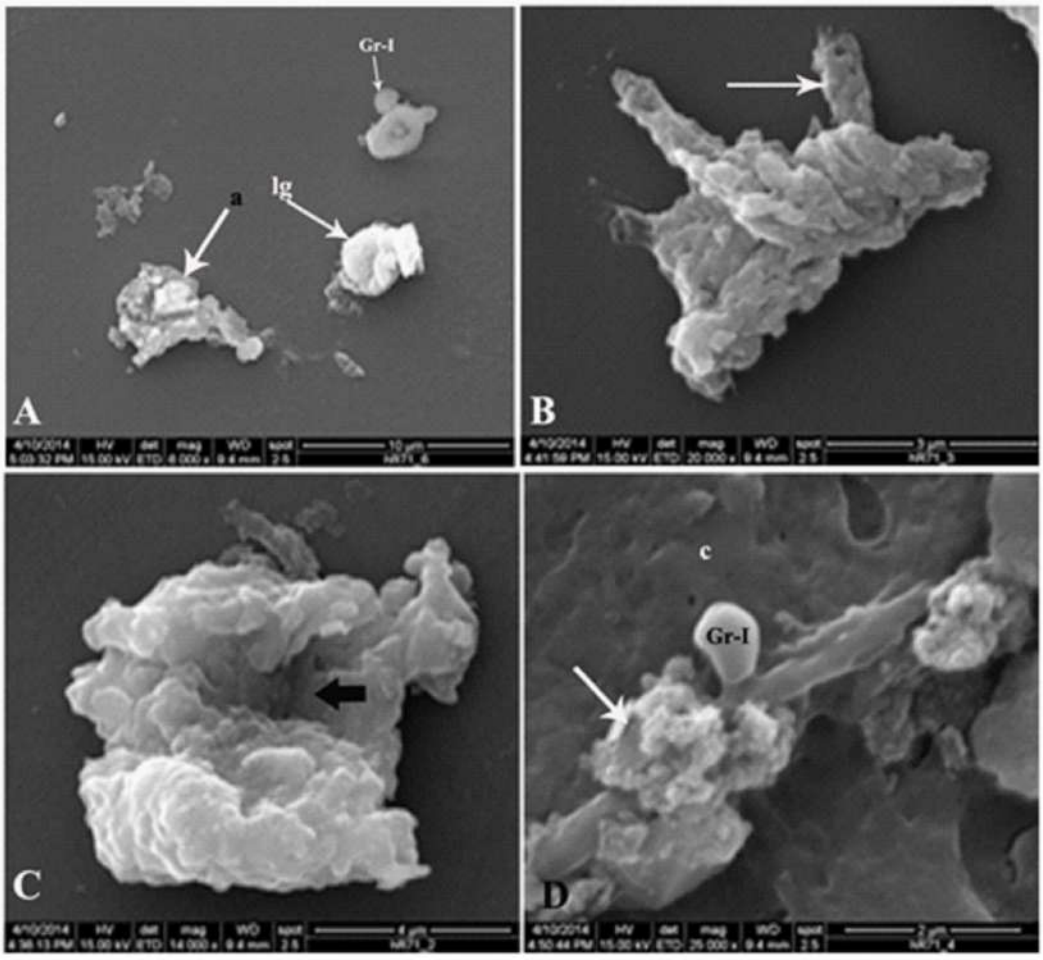


Fig. 13 Modifications of the haemocytes of of *Lissachatina fulica* after 60 minute of interaction *Aspergillus* sp. **A,** Reduction of phagocytic response of haemocytes to the *Aspergillus*. **B,** Characteristic roughness (arrow) of pseudopods of a Group II phagocytic cell. **C,** Declining of pseudopods formation from concave surface (bold arrow) of Group II cell. **D,** Formation of cell nodule (arrow) of different haemocytes on the Type-C Gr-III cell. a = Type-A Group III cell, c = Type-C Group III cell, Gr-I = Group I cell.

Discussion

Immune system differs among molluscan species by the variability of their type of haemocytes (or immunocytes) as well as their respective habitats of the individuals (Adema et al., 1992). In general, aquatic gastropods such as *Lymnaea stagnalis* (Sminia, 1972; Adema et al., 1992), *Biomphalaria glabrata, Bulinus natalensis* (Adema et al., 1992), *Megathura crenulata, Aplysia californica* (Martin et al., 2007), *Indoplanorbis exustus*, *Pila globosa* (Mahilini & Rajendran, 2008), *Bellamya bengalensis* (Ray et al., 2013), *Pomacea canaliculata* (Accorsi et al., 2013; Cueto et al., 2015) possess comparatively small haemocytes than those of terrestrial gastropods as *Helix aspersa, Achatina achatina, Achatina fulica* (Adema et al., 1992), and *Helix aspersa maxima* (Adamowicz & Bolaczek, 2003). Two types of haemocytes, large spreading cells and small round cells are reported in terrestrial *Helix aspersa* and *Achatina achatina* (Adema et al., 1992). On the other hand, the aquatic pulmolnates *I. exustus* (Mahilini & Rajendran, 2008), *L. stagnalis* (Adema et al., 1994) exhibit more than two haemocytes as agranulocytes and granulocytes with different subtypes whereas *Planorbarius corneus* exhibit only round and spreading haemocytes (Ottaviani, 1992). The present study records, for the first time, that *Lissachatina fulica* possess three distinct populations of circulating haemocytes. These cell populations exhibit morphological characters as well as their staining properties. Most of the cytological features of the haemocytes of *Lissachatina fulica* exhibit similarities with the different types of haemocytes of other gastropods.

The characteristics of the Group I cell of *Lissachatina fulica* are mostly identical with the round, non-spreading cells as described in many gastropods (Ottaviani, 1992; Adema et al., 1992; Pengsakul et al., 2013). Agranulocytes as well as small granulocytes (pro-immunocytes) show adherence activity and little ability to phagocytosis as previously reported in other molluscs (Mahilini & Rajendran, 2008; Adema et al., 1992; Ray et al., 2013; Cueto et al., 2015; Kumazawa et al., 1991; Mc Cormick-Ray & Howard, 1991). It is assumed that the large granulocytes may be the mature form of small granulocytes. The characteristics of large granulocytes corroborate to the previously described spreading cells or Granulocyte II in other gastropods (Mahilini & Rajendran, 2008; Ottaviani, 1992; Martin et al., 2007; Adema et al., 1992; Cueto et al., 2015; Pengsakul et al., 2013). Group III cells have various functional forms and are described for the first time in this gastropod species. Among the Group III cell types, Type-B is considered as intermediate form of Type-A and Type-C cells. These are all morphological variations of Group III cells and may be recognised to their response against invading antigens. The Type-

A, Type-B, and Type-C of Group III cells may be designated as normal (relax), intermediate and expanded (highly responsive) form respectively.

The defense patterns of haemocytes are considered as one of the parameters in classifying the haemocytes in the haemolymph of *Lissachatina fulica* following Accorsi et al. (2013). The cell morphometry of all cell types of three populations of haemocytes is slightly increased (but not significantly) in fungal spore challenged snails and are almost same against all tested immunogens. Population percentage of the Group I cells and small granulocytes is declined in the circulation of the fungal spore challenged snails, while it is increased for the agranulocytes and large granulocytes in the haemolymph of these experimental snails. In the present study, it is shown that not all haemocytes are reacting against immunogens. Agranulocytes, large granulocytes, Type-A and Type-B of Group III cells are major reactive haemocytes against the invading immunogens and these responses are almost same to all immunogens. Population fluctuations of the circulating haemocytes between control and infected *Lissachatina fulica* corroborate to the population dynamics of uninfected and infected *Biomphalaria alexandrina* (Bakry, 2009). The size and filamentation of Group III cells have increased on exposure of immunogens and assumed that these may promote to capture the particles of the immunogens.

An increase in cell diameter, pseudopod formation and phagocytosis are the major immune response after 30 minute of time lag since inoculation of immunogens. The formation of numerous vacuoles and pseudopodia of Group II cells might be one of the responses against antigens (Bakry, 2009). In the present study, it is observed that the intensity of the pseudopod formation on the cell surface of the various haemocytes and other immunogenic responses of *Lissachatina fulica* after 30 minute of different fungal spores administration is characteristically higher than those of the after 60 minutes of this immunogen administration. It is assumed that such changes of the immunogenic responses of the circulating haemocytes in different time-lag periods may be due to a gradual relaxation of immunogenic responses of these haemocytes after a certain time of haemocyte-immunogen interaction.

The surface ultra-structures of different haemocytes of *Lissachatina fulica* display many similarities as reported in *B. alexandrina* (Bakry, 2009) and *Oncomelania hupensis* (Pengsakul et al., 2013). The surface morphology of the Group III cells is described for the first time, which reveals numerous small pits like structure. Such structure is not reported earlier. Changes in morphology of pseudopodia of haemocytes against antigens

are reported earlier (Martin et al., 2007; Ottaviani et al., 1986; Lapointe et al., 2012). Pseudopodia of large granulocytes show different morphological change against different antigens. The pseudopodia look like long drum stick with a small head in *Aspergillus* inoculated snails, whereas it is pointed filopodia in case of *Rhizopus* inoculated snails. Thus, it is assumed that variations in pseudopod morphology might be specific against specific antigenic response. Surface of the Group III cells changed to short spine-like structure in case of antigen challenged snails. Such change in surface morphology is not specific to antigens.

The haemocytes *Lissachatina fulica* show both non-specific and specific defense responses against the two immunogens (spores of *Rhizopus* sp and *Aspergillus* sp) experimented in this study. The Group I cells, small granulocytes, and Type-B Group III cells are mainly involved in non-specific immune responses that is the reaction of haemocytes are similar against the two immunogens used in the present study. However, immunogen specific changes are distinguished in case of large granulocytes, agranulocytes, Type-A and Type-C Group III haemocytes. It is observed that the cell size of various haemocytes is slightly increased in response to the spores of *Rhizopus* sp than that of the *Aspergillus* sp. It is assumed that the spores of the *Rhizopus* sp having slightly more immunogenic ability than that of the *Aspergillus* sp.

Acknowledgement

The authors are thankful to the principal and all the staffs of Department of Zoology of City College, Calcutta, West Bengal, India. This study was supported by the fund of UGC Major Project of Dr. K. K. Chaki. Authors are grateful to the Laboratory staff of the SEM Unit, Bose Institute, Calcutta and the SEM Unit, University of Burdwan, West Bengal.

References

Accorsi, A. Bucci, L. Eguileor, M.de. Ottaviani, E. Malagoli, D. Comparative analysis of circulating immunocytes of the freshwater snail *Pomacea canaliculata*. Fish Shellfish Immunol. 2013; 34: 1260-1268.

Adamowicz, A. Bolaczek, M. Blood cells morphology of the snail *Helix aspersa maxima* (Helicidae). Zool. Pol. 2003; 48: 93"101.

Adema, C.M. Harris, R.A. Vandeutekom-Mulder, E.C. A comparative study of immunocytes from six different snails: morphology and functional aspects. J. Invertebr. Pathol. 1992; 59: 24"32.

Adema, C.M. Mohandas, A. Knap van der, W.P. Sminia, T. Separation of Lymnaea stagnalis immunocytes by density gradient centrifugation. Dev. Comp. Immunol.

1994; 18: 25"31.

Basu, D. Kulkarni, R. Overview of blood components and their preparation. Indian J. Anaesth. 2014; 58: 529"537.

Bakry, F.A. Effect of infection with *Schistosoma mansoni* on some biological parameters in *Biomphalaria alexandrina* snails. Am. Euras. J. Sci. Res. 2009; 4: 180"190.

Beckmann, N. Morse, M.P. Moore, C.M. Comparative study of phagocytosis in normal and diseased immunocytes of the bivalve mollusc *Mya arenaria*. J. Invertebr. Pathol. 1992; 59: 124"132.

Cheng, T.C. A classiûcation scheme of molluscan immunocytes based on functional evidences. In: Comparative pathology, Bullo, L.A. & Cheng, T.C. (Ed.), Vol 6, Plenum Press, New York. 1984; p111"126.

Cochennec-Laureau, N. Auffret, M. Renault, T. Langlade, A. Changes in circulating and tissue inûltrating immunocyte parameters of European ûat oysters, *Ostrea edulis*, naturally infected with *Bonamia ostreae*. J. Invertebr. Pathol. 2003; 83: 23"30.

Cueto, J.A. Rodriguez, C. Vega, I.A. Castro-Vazquez, A. Immune defenses of the invasive apple Snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae): Phagocytic Immunocytes in the Circulation and the Kidney. *PLoS ONE*. 2015: 10: e0123964.

Janeway, C.A.Jr. Medzhitov, R. Innate immune recognition. Annu. Rev. Immunol. 2002; 20: 197"216.

Kambale, N.A. Potdar, V.V. Hematological analysis of Molluscan species *Bellamya bengalensis* and *Lamiellidens marginalis*. Biol. Forum. An. Int. J. 2010; 1: 70"72.

Knaap van der, W.P.W. Sminia, T. Kroese, F.G.M. Dikkeboom, R. Elimination of bacteria from the Circulation of the pond snail *Lymnaea stagnalis*. Dev. Comp. Immunol. 1981; 5: 21"32.

Kumazawa, N.H. Tanigawa, T. Kasagi, N. Tanaka, Y. Characterization of immunocytes of an estuarine gastropod mollusc *Clithon retropictus*, based on lysosomal enzymes. J. Vet. Med. Sci. 1991; 53: 725"726.

Lapointe, J.F. Dunphy, G.B. Mandato, C.A. Immunocyte–immunocyte adhesion and nodulation reactions of the greater wax moth, *Galleria mellonella* are influenced by cholera toxin and its B-subunit. Results Immunol. 2012; 2: 54"65.

Mahilini, H.M. Rajendran, A. Categorization of immunocytes of three gastropod species *Trachea vittata* (Muller), *Pila globosa* (Swainson) and *Indoplanorbis exustus* (Dehays). J. Invertebr. Pathol. 2008; 97: 20"26.

Malham, S.K. Lacoste, F.G. Cueff, A. Polet,

S.A. Evidence for a direct link between stress and immunity in the mollusc *Haliotis tuberculata*. J. Exp. Zool. 2003; 295A: 136"144.

Martin GG, Oakes CT, Tousignant HR, Crabtree H, Yamakawa R, Structure and function of immunocytes in two marine gastropods, *Megathura crenulata* and *Aplysia californica*. J. Molluscan Stud. 2007; 73: 1"11.

Matricon-Gondran, M. Letocart, M. Internal defenses of the snail *Biomphalaria glabrata*. III. Observations on tubular helical ûlaments induced in the haemolymph by foreign material. J. Invertebr. Pathol. 1999; 74: 248"254.

Mc Cormick-Ray, M.G. Howard, T. Morphology and mobility of oyster immunocytes: evidence for seasonal variations. J. Invertebr. Pathol. 1991; 58: 219"230.

Medzhitov, R. Janeway, C.Jr. Innate immune recognition: mechanisms and pathways. Immunol. Rev. 2000; 173: 89"97.

Oakes, F.R. A non-lethal method for extracting crude hemocyanin from gastropod molluscs. European patent no. EP 1 389 123 B1, 2008.

Ottaviani, E. The blood cells of the freshwater snails Planorbarius corneus and *Biomphalaria glabrata* (Gastropoda: Pulmonata). Dev. Comp. Immunol. 1983; 7: 209"216.

Ottaviani, E. Aggazzotti, G. Tricoli, S. Kinetics of bacterial clearance and selected enzyme activities in serum and immunocytes of the freshwater snail *Planorbarius corneus* (L.) (Gastropoda, Pulmonata) during the primary and secondary response to *Staphylococcus aureus*. Comp. Biochem. Physiol. 1986; 85A: 91"95.

Ottaviani, E. Franchini, A. Ultrastructural study of immunocytes of the fresh water snail *Planorbis corneus* (L) (Gastropoda, Pulmonata). Acta Zool. 1988; 69: 157"162.

Ottaviani, E. Immunorecognition in gastropod molluscs with particular reference to the freshwater snail *Planorbarius corneus* (L.) (Gastropoda, Pulmonata). Boll. Zool. 1992; 59:129"139.

Ottaviani, E. Immunocyte: the invertebrate counterpart of the vertebrate macrophage. Inf. Syst. J. 2011; 8: 1"4.

Pengsakul, T. Suleiman, Y.A. Cheng, Z. Morphological and structural characterization of immunocytes of *Oncomelania hupensis* (Gastropoda: Pomatiopsidae). Ital. J. Zool. 2013; 80: 494"502.

Plows, L.D. Cook, R.T. Davies, A.J. Walker, A.J. Carbohydrates that mimic schistosome surface coat components affect ERK and PKC signalling in

Lymnaea stagnalis immunocytes. Int. J. Parasitol. 2005; 35: 293"302.

Ray, M. Bhunia, N.S. Bhunia, A.S. Ray, S. A comparative analyses of morphological variations, phagocytosis and generation of cytotoxic agents in ûow cytometrically isolated immunocytes of Indian molluscs. Fish Shellûsh Immunol. 2013; 34: 244"253.

Serpentini, A. Ghayor, C. Poncet, J.M. Hebert, V. Galera, P. Pujol, J.P. Boucaud-Camou, E. Lebel, J.M. Collagen study and regulation of the de novo synthesis by IGF-1 immunocytes from the gastropod mollusc, *Haliotis tuberculata*. J. Exp. Zool. 2000; 287: 275"284.

Sminia, T. Structure and function of blood and connective tissue cells of the fresh water pulmonate *Lymnaea stagnalis* studied by electron microscopy and enzyme histochemistry. Zeitschrift Zellforschung. 1972; 130; 497"526.

Sminia, T. *Gastropods: Invertebrate blood cells*. In: Ratcliffe NA, Rowley AE, (Ed.), Vol. I, Academic Press, London, 1981; p191"232.

Voltzow, J. Gastopoda: Prosobranchia. In: Microscopic Anatomy of Invertebrates, Harrison FW, Kohn AJ, (Eds.), Vol 5. Wiley-Liss, Inc., New York, 1994; p111"252.

Yonow, Y.N. Renwrantz, L. Studies on the immunocytes of *Acteontornatilis* (L) (Opisthobranchia: Acteonidae). J. Molluscan Stud. 1986; 52: 150"155.

Yoshino, T.P. Granath, W.O.J.R. Characterization of molluscan phagocyte subpopulations based on lysosomal enzyme markers. J. Exp. Zool. 1985; 226: 205"210.

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Solving with Fuzzy Reasoning for a Green Production–Transportation Supply Chain Model

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ARTICLE INFO
Received: 10.07.2021;
Revised: 15.08.2021;
Accepted: 18.08.2021

Key words:
Crude steel production; Pollution; Transportation; Fuzzy reasoning; Modeling; Optimization.

ABSTRACT

In this article we solve a global crude steel production transportation model under the effect of air pollution using fuzzy reasoning technique. First of all, we formulate a cost minimization crisp supply chain model under some specific assumptions which is basically the extension of the article of Bhattacharya et *al.* (2020). Then considering fuzzy system we have shown that, fuzzy reasoning can ultimately optimize the model with respect to some other existing model. Numerical study, graphical illustrations are done to validate the model.

Introduction

Production of crude steel is a noteworthy issue for economic growth of any country in the world. Because of its versatile uses in different sectors, it is now becoming a product of 'all time' uses in our day-to-day activities. But its production process includes several phases requiring major components such as iron ore as raw material, coals for burning and heat generation, water for cooling the system and the uses of high technology for controlling pollution. Karmakar et *al* (2017, 2018) studied extensively to explore the production and production remanufacturing process of a Sponge Iron industry in which they were able to construct functional dependencies of production reproduction quantities with respect to the amount of pollution through the cumulative effect of air, soil and water contamination by the harmful particles containing Sulphur Dioxide (SO_2), Sulphur Trioxide (SO_3), Nitrogen Dioxide (NO_2), Led (Pb), Carbon Di oxide (CO_2), Carbon Monoxide (CO) etc. However, the problem of transporting goods is another critical issue of a production process because it includes fuel costs as well as carbon emission cost. Researchers are basically involved in controlling carbon emission in supply chain (SC) networks (Hernandez-Pellon and Fernandez-Olmo (2018), Ebadi et *al.* (2016), Mancini et *al.* (2016) etc.) in recent times. But the concept of need based production and its corresponding pollution, pollution due to transportation is not studied yet.

At the early stage, most of the models are solved by deterministic and stochastic environment. But they do not obey the realistic

models of real-world problems because of the versatile nature of the parameters associated with the models. To overcome the gaps of the deterministic models, researchers were involved to develop the stochastic models where the parameters involved in a model are assumed to be a random number each or every at a time. At the early stage, the operation research (OR) practitioner's/ decision maker's (DM's) problem was how to capture the uncertainty of the several components of any kind of management problems. After the invention of Zadeh (1965)'s fuzzy set theory the situations become quite favorable to all the DM. Since then, numerous research articles have been made to explain and control the complexities of the competitive real-world phenomenon. Some notable works over the EOQ models on fuzzy environments may be pointed out over here. Researchers like De et al. (2014), De and Sana (2015) discussed a deteriorating EOQ model with natural idle time for imprecise demand and in a backlogging model by employing hesitant fuzzy set. The concepts of dense fuzzy set studied by De and Beg (2016) to discuss the frequent learning effect of the fuzzy parameters and contemporarily, the concepts of cloudy fuzzy set were coined by De and Mahata (2017) to enhance the same for continuous time dependent learning effect. Analyzing the behavior of human thinking process De (2017) developed a new fuzzy set namely Triangular dense fuzzy lock set along with its new defuzzification method. After this invention many articles have been made by eminent researchers [De and Mahata (2019a, 2019b), De and Sana (2013a, 2013b, 2018) etc.] to control the individual or group decision making problems on pollution sensitive inventory modelling. The study of fuzzy linguistic dense fuzzy lock set has been discussed by De and Mahata (2019c) recent times to capture the cost effectiveness in trade credit policy. Moreover, researchers like Zadeh (1975), Pal and Mandal (1991) etc. worked over linguistic fuzzy system incorporating the fuzzy approximates reasoning. Fuzzy solutions in fuzzy linear programming problems and preference based on relational fuzzy system were analyzed by Zimmermann (1985), Ramik and Rimanek (1985), Tanaka and Asai (1984) etc.

However, for any kind of optimization problem with some constraints the concept of duality plays an important role to characterize the bounds of the objective values. Ovchinnikov (1991) discussed the duality principle in fuzzy set theory. Wu (2003, 2007) worked extensively over duality theory in fuzzy linear programming problems with fuzzy coefficients, necessity theory and saddle point optimality conditions in fuzzy optimization problems. Zhang et *al.* (2005) studied duality theory in fuzzy mathematical programming problems with fuzzy coefficients. Convex fuzzy mapping with differentiability and its

application in fuzzy optimization was discussed by Panigrahi et *al.* (2008). Song and Zhao (2010) developed solution procedures of fuzzy multi-objective optimization of passive suspension parameters. Beyond these concepts, a new approach to duality in fuzzy LPP has been analysed by Nasseri and Ebrahimnejad (2012). To deal with nonlinear fuzzy optimization problems an extension on duality theory was minutely discussed by Zou (2015). Very recent, De et al. (2021a) studied a pollution sensitive model using fuzzy approximate reasoning . Subsequently De et al. (2021b) developed another model and solved it under volumetric fuzzy system.

From the above study it is seen that not a single article has been studied on fuzzy approximate reasoning to design the inventory control problems. Thus, in this article we solve a production transportation model with pollution index under the effect of fuzzy reasoning on the cost parameters of the model. The equivalent crisp problem is constructed by the help of fuzzy alpha cuts and dual fuzzy *k* cuts for the specific feasible region posed by an approximation index. This article organizes as follows: section 1 includes introduction; Section 2 includes materials and methods describing the concept approximate fuzzy reasoning, dual space, data collection, curve fitting and problem definition. Section 3 discusses the model formulation and solution. Section 4 develops results; section 5 indicates discussion by graphical illustration. Section 6 describes conclusion.

2. Materials and Methods [De (2020)]

This section is designed by some basic definitions of fuzzy membership functions and concepts of fuzzy reasoning. We note that we are going to discuss the minimization problem of the application of OR problems, so we take left fuzzy numbers (L-fuzzy numbers) throughout the article.

2.1 Defining fuzzy membership function

Definition 1: L- fuzzy number: Let us consider the L fuzzy number of the form where and x_0 as initial value of the fuzzy number $\tilde{A}$ and δ is the maximum tolerance

$$\widetilde{A} =< x, \mu(x) > \text{where } \mu(x) = \begin{cases} 1 & if \quad x \geq x_0 \\ 1 - \frac{x_0 - x}{\delta} if & x_0 - \delta \leq x \leq x_0 \\ 0 & if x \leq x_0 - \delta \end{cases}$$

level along with its graphical representations (shown in Fig.-1)

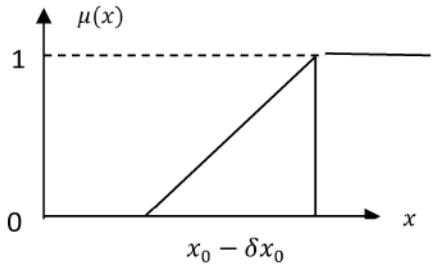

Fig.-1: Membership function of

Definition 2: Let $\tilde{A}$ be the fuzzy number defined as above then its approximated form is denoted as $\mu_A(x)$ (shown in Fig.2) to be obtained from the following possibility formula (obtained from Fig.-3)

$$\mu_A(x) = Poss\big(\mu_1(x) \cup \mu_2(x)\big) = \mu_1(x) + \mu_2(x) - \mu_1(x)\mu_2(x)$$

where $\mu_1(x) = \begin{cases} 1 & if x \geq 2y \\ \frac{x-y}{y} & if y \leq x \leq 2y \\ 0 & if x \leq y \end{cases}$ and $\mu_2(x) = \mu_1(x = x_0 - \delta) = \begin{cases} 0 & if x \geq 2y \\ \frac{x_0-\delta}{y} - 1 & if y \leq x \leq 2y \\ 1 & if x \leq y \end{cases}$

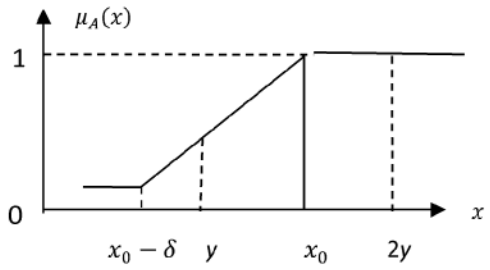

Fig.-2: Membership function of fuzzy reasoning $\tilde{A}$

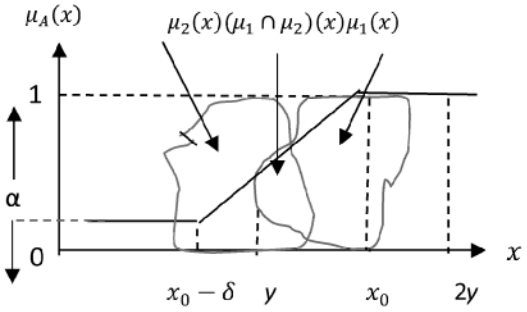

Fig.-3: Approximating membership of $\tilde{A}$

Now from above we get

$$\mu_A(x) = \begin{cases} 1 & if\ x \geq 2y \\ lx - m & if\ y \leq x \leq 2y \\ \frac{x_0-\delta}{y} - 1 & if\ x \leq y \end{cases} \quad \text{where} \quad \begin{cases} l = \left(\frac{2}{y} - \frac{x_0-\delta}{y^2}\right) \\ m = 3 - \frac{2(x_0-\delta)}{y} \end{cases}$$

2.2 Defining the Probability density function of the random variable *Y*

Let the probability density function of the random variable *Y* is given by

$p(y) = \frac{2}{\delta}\begin{cases} 1 - \frac{x_0-y}{\delta} & if\ x_0 - \delta \leq y \leq x_0 \\ 0 & otherwise \end{cases}$ such that we always have $\int_{-\infty}^{+\infty} p(y)\,dy = 1$.

2.3 Approximating $\alpha - cut\ of\ \mu_A(x)$

From the fuzzy set $\mu_A(x)$ we construct the $\alpha - cut$ sets as follows: $lx - m \geq \alpha \Rightarrow x \geq \frac{m+\alpha}{l} =$

$\frac{\alpha+3-\frac{2(x_0-\delta)}{y}}{\left(\frac{2}{y}-\frac{x_0-\delta}{y^2}\right)} = \frac{y^2(\alpha+3)-2(x_0-\delta)y}{2y-2(x_0-\delta)}$. The expected value of the $\alpha - cuts\ of\ \mu_A(x)$ is obtained as $E(x) = \frac{(\alpha+3)E(y^2)-2(x_0-\delta)E(y)}{2E(y)-2(x_0-\delta)}$ where, $E(y) = \int_{-\infty}^{+\infty} yp(y)\,dy = \frac{2}{\delta^2}\int_{x_0-\delta}^{x_0} y\{y-(x_0-\delta)\}\,dy = \left(x_0 - \frac{\delta}{3}\right)$

And similarly, $E(y^2) = \frac{2}{\delta^2}\int_{x_0-\delta}^{x_0} y^2\{y-(x_0-\delta)\}\,dy = \left(x_0^2 - \frac{2}{3}\delta x_0 + \frac{\delta^2}{6}\right)$. On substitution we have

$E(x) = \frac{\alpha\left(x_0^2-\frac{2}{3}\delta x_0+\frac{\delta^2}{6}\right)+\left(x_0^2+\frac{2}{3}\delta x_0-\frac{\delta^2}{6}\right)}{x_0+\frac{\delta}{3}}$. The rest part of $\mu_A(x)$ gives $\frac{x_0-\delta}{y} - 1 \leq \alpha \Rightarrow x_0 \leq \delta + (\alpha+1)y$

and giving the expectations $x_0 \leq \delta + (\alpha+1)E(y) \Rightarrow x_0 \leq \delta + (\alpha+1)\left(x_0 - \frac{\delta}{3}\right)$

2.4 Dual β cuts and dual space

Definition 3: Let $\mu_A(x) \geq \alpha$ *with* $0 < \alpha < 1$ be the α–cut of the fuzzy set $\mu_A(x)$ defined on the subset of the universal set X. Let us consider another cut k corresponding to α such that $0 < \alpha\beta < 1$. Then β is said to be dual cut of $\mu_A(x)$ over the convex set $\alpha < 1$.

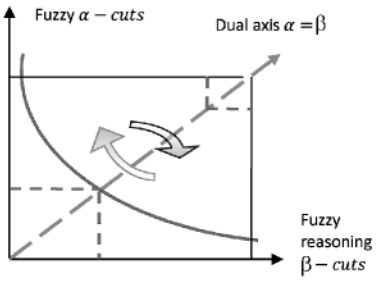

Fig.4: Exchange of dual feasible space

2.5 Approximating feasible space [De(2020)]

Introducing k as approximation parameter (commonly known as approximation index) in such a way that we could be able to get $x_0 \geq \delta$ always and this can be done by approximating x_0 such that $x_0 \geq \delta\left(\frac{2+\alpha\beta}{3\alpha\beta}\right)$ *with* $\alpha\beta < 1$ *and* $\beta < 1$ *where,* α–β together constitute a dual feasible space.

2.6 Problem Definition under Production –Consumption- Pollution scenario

From the above discussion and motivation over the Global Crude Steel production plant model it is seen that Pollution depends upon Production and its transportation. Thus, we may define the research problem in the following way:

i) What will be the actual amount of Global Crude Steel Production such that the aggregate pollution level/ index become minimum?
ii) What should be the actual Consumption level such that the World remains greenery?
iii) How much beneficial the approximate fuzzy reasoning method over traditional optimization method?

2.7 Data collection and curve fitting

Let us consider the secondary data of major 61 crude steel producing countries of the world for

the year 2017. We have gathered the data of crude steel production (in terms 10^5 MT), consumption (10^5 MT) (demand), pollution index for 61 countries each. The curve under study is three dimensional, so using production rate (K), demand (consumption) rate (D) and pollution index (P) as three-dimensional variables and taking the help of MATLAB software we fit plane curve in such a way that the R^2 (a statistic) value gets maximum value and the errors assume minimum value. Thus, we obtain equation-1 with its corresponding graphical representation as follows.

$$K - 1.083D = 33.78 - 0.7318\,P \qquad (1)$$

For setting a model we have considered the following inputs (shown in Table 1) which are generally used in several crude steel production sectors in the world.

Table-1: Costs of the proposed model

Set-up cost per cycle $20000	Holding cost per cycle in production plant per MT $5	Deterioration cost per cycle in production plant per unit item $10	Preservation technology cost per cycle $1000
Pollution cost due to production per MT $43.89	Holding cost per cycle in retailer plant per MT $10	Production cost per MT $327.56	Deterioration fraction 0.002
Social cost of carbon per MT $417	Transportation cost per gallon fuel $3.5	Length covered by freight train 600 Miles	Scale parameter for preservation cost (A) 0.00001

3. Model formulation and solution

In this section we formulated our proposed model by considering some notations and assumptions.

3.1. Notations and Assumptions

We may consider the following notations and assumptions for developing the model.

Notations

K Manufacturing rate per cycle (decision variable)

D Retailer's demand rate per cycle

θ Deterioration fraction

ξ Technology uses cost per cycle ($) ($M(\xi) = \theta(1-e^{-\alpha\xi})$, $0< \alpha<1$)

T_1 Production time (decision variable)

T_2 Inventory opening time of Retailer

T_3 Time at zero inventory in retailer's plant
S_1 Set up cost ($) for supplier's production plant
S_2 Set up cost ($) for retailer's plant
h_1 Holding cost for supplier's plant
h_2 Holding cost for retailer's plant
C_S Global social cost of carbon ($) incurs due to transportation
C_T Transportation cost per unit gallon fuel ($)
C_{Po} Pollution cost per unit item production ($)
C_{Pr} Production cost of unit item ($)
d_C Deterioration cost of unit item ($)
L Transported distance of the produced items
P Pollution index for each country
Q Produced items after time

Assumptions

1. Excessive use of raw materials might destroy the environment.
2. Deteriorated items cannot be recoverable.
3. The time requires for the travel (up and down) of rail freight train equals the manufacturing time per cycle.
4. No deterioration is viewed in the final product during transportation and retailer's inventory.

3.2. Construction of crisp and fuzzy mathematical model [Extension of Bhattacharya et al. (2021)]

a) Supplier's problem

Let the production process starts at supplier's plant with constant production rate K and it continues up to time T_1. During the production run time T_1, some deterioration is viewed which is checked by using modern technology. The inventory level gradually increases and it reaches its maximum value at the end of time T_1. Then finished product gets ready to transport to the retailer at time span $(T_2 - T_1)$. During transportation, the production again starts at zero stock with manufacturing rate K. Due to application of preservation technology, the reduced deterioration is $\delta = \theta - m\ (\xi)$ (say). Therefore, the governing differential equation of supplier's problem is $\frac{dq_1(t)}{dt} = K - \delta q_1(t), \mathrm{q}_1\ (0) = 0$ (2)

Solving (2) we get, $q_1(t) = \frac{\kappa}{\delta}\left(1 - e^{-\delta t}\right), \quad 0 \leq t \leq T_1$ (3)

The inventory holding cost (HCS) for supplier's problem is given by

$HCS = h_1 \int_0^{T_1} q_1(t)dt = \frac{h_1 K}{\delta}\left[T_1 + \frac{e^{-\delta T_1}-1}{\delta}\right]$ (4)

Then the production cost (PC) is given by $PC = C_{Pr}\left[\int_0^{T_1} q_1(t)dt\right] = \frac{C_{Pr}K}{\delta}\left[T_1 + \frac{e^{-\delta T_1}-1}{\delta}\right]$ (5)

Also, the deterioration cost (DC) is given by $DC = d_c(KT_1 - Q) = d_c K\left[T_1 - \frac{1-e^{-\delta T_1}}{\delta}\right]$ (6)

Where $Q = \frac{K}{\delta}\left(1 - e^{-\delta T_1}\right) = D(T_3 - T_2)$ (7)

The transportation cost (TC) is given by $TC = C_T \times \frac{2LQ}{471} = 0.00424628LQC_T$ (8)

Pollution cost due to transportation (TPC) is given by

$TPC = C_S \times \frac{2LQ}{471} \times 22.38 \times 0.000454 = 0.0000431445LQC_S$ (9)

Pollution cost due to production (PPC) is given by $PPC = C_{Po} \times KT_1$ (10)

The set-up cost (SCS) for supplier's problem is given by $SCS = S_1$ (11)

b) Retailer's problem

At the time T_2, the items are received and began to sale at the retailer's counter and the inventory will get at time T_3 because of demand D. During the interval $[T_2, T_3]$, the variation in the inventory depletes for demand only. Therefore, the governing differential equation of retailer's problem is

$\frac{dq_2(t)}{dt} = -D, \quad T_2 \leq t \leq T_3, \quad q_2(T_3) = 0$ (12)

Which gives $q_2(t) = D(T_3 - t), \quad T_2 \leq t \leq T_3$ (13)

The inventory holding cost (HCR) for retailer's problem is given by

$HCR = h_2 \int_{T_2}^{T_3} q_2(t)dt = \frac{h_2 D}{2}(T_3 - T_2)^2$ (14)

The set-up cost (SCR) for retailer's problem is given by $SCR = S_2$ (15)

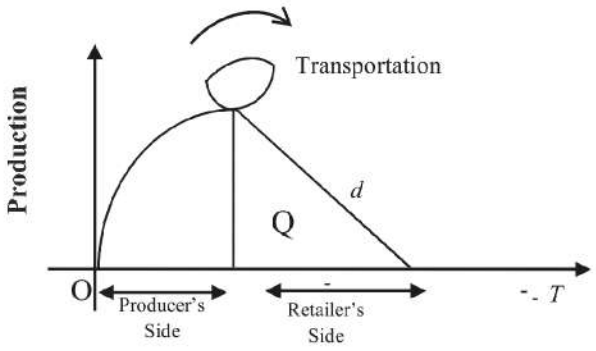

Fig. 5: Production-Transportation Model

Combining supplier's problem and retailer's problem, the total average joint inventory system cost (z) is given by

$$z = \frac{1}{T_1}\left\{\frac{h_1 K}{\delta}\left(T_1 + \frac{e^{-\delta T_1}-1}{\delta}\right) + d_c K\left(T_1 - \frac{1-e^{-\delta T_1}}{\delta}\right) + \frac{C_{Prod} K}{\delta}\left(T_1 + \frac{e^{-\delta T_1}-1}{\delta}\right) + C_{Pol} \times K T_1 + S_1\right\} + \frac{1}{2(T_2-T_1)}\{C_T \times 0.00424628LQ + C_S \times 0.0000431445LQ\} + \frac{1}{(T_3-T_2)}\left\{\frac{h_2 D(T_3-T_2)^2}{2} + S_2\right\} \quad (16)$$

Therefore, the basic mathematical problem of the model is obtained as

$$\begin{cases} Minimize\ z(K, T_1, Q, P, D) \\ subject\ to, T_2 = \frac{3}{2}T_1, T_3 = \frac{5}{2}T_1 \\ Q = \frac{K}{\delta}\left(1 - e^{-\delta T_1}\right) = DT_1 \\ K - 1.083D = 33.78 - 0.7318P \end{cases} \quad (17)$$

This can be defined in another form as

$$\begin{cases} Minimize\ z = F_1 + F_2 \\ subject\ to \\ Q = \frac{K}{\delta}\left(1 - e^{-\delta T_1}\right) = DT_1 \\ K - 1.083D = 33.78 - 0.7318P \end{cases} \quad (18)$$

With the condition:

$$\begin{cases} F_1 = c_1 f_1 + c_2 f_2 + c_3 f_3 + c_4 f_4 + c_5 f_5 + c_6 f_6 + c_7 f_7 \\ F_2 = c_8 f_8 + c_9 f_9 \\ (c_1, c_2, c_3, c_4, c_5, c_6, c_7, c_8, c_9) \equiv (h_1, d_c, c_{pd}, c_{po}, s_1, c_T, c_s, h_2, s_2) \\ f_1 = \frac{K}{\delta T_1}\left(T_1 - \frac{1-e^{-\delta T_1}}{\delta}\right), f_2 = \frac{K\left(T_1 - \frac{1-e^{-\delta T_1}}{\delta}\right)}{T_1} \\ f_3 = \frac{K}{\delta T_1}\left(T_1 - \frac{1-e^{-\delta T_1}}{\delta}\right), f_4 = K, f_5 = \frac{1}{T_1} \\ f_6 = \frac{0.00424628LK(1-e^{-\delta T_1})}{T_1 \delta}, f_7 = \frac{0.0000431445LK(1-e^{-\delta T_1})}{T_1 \delta} \\ f_8 = \frac{DT_1}{2}, f_9 = \frac{1}{T_1} \end{cases} \quad (19)$$

Now, we consider all the cost parameters associated with the model might follow triangular fuzzy number as defined in the section 2 then the equivalent fuzzy problem of the proposed model is as below

$$\begin{cases} Minimize\ \widetilde{z} = \widetilde{F_1} + \widetilde{F_2} \\ Subject\ to,\ Q = \frac{K}{\delta}\left(1 - e^{-\delta T_1}\right) = DT_1 \\ K - 1.083D = 33.78 - 0.7318P \end{cases} \tag{20}$$

$$\text{where } \begin{cases} \widetilde{F_1} = \widetilde{c_1}\, f_1 + \widetilde{c_2}\, f_2 + \widetilde{c_3}\ f_3 + \widetilde{c_4}\ f_4 + \widetilde{c_5}\ f_5 + \widetilde{c_6}\ f_6 + \widetilde{c_7}\ f_7 \\ \widetilde{F_2} = \widetilde{c_8}\ f_8 + \widetilde{c_9}\ f_9 \end{cases} \tag{21}$$

For defuzzification of the problem (20) using the concepts of approximate fuzzy reasoning studied at subsections (2.3- 2.5) and α – cuts of fuzzy numbers the equivalent crisp problem is given by

$$\begin{cases} Maximize\ \alpha\ , 0 < \alpha < 1 \\ Subject\ to \quad E(Z_\alpha) \le Z_0 + (1-\alpha)\delta_0 \\ c_{i0} \ge \delta_{1i}\left(\frac{2+\alpha\beta}{3\alpha\beta}\right), \quad i = 1,2,\ldots,9 \\ \alpha\beta < 1\ , \beta < 1 \\ Q = \frac{K}{\delta}\left(1 - e^{-\delta T_1}\right) = DT_1, K - 1.083D = 33.78 - 0.7318\mathrm{P} \end{cases} \tag{22}$$

$$\begin{cases} E(Z_\alpha) = c_{1\alpha}\, f_1 + c_{2\alpha}\, f_2 + \ c_{3\alpha}\, f_3 + c_{4\alpha}\, f_4 + c_{5\alpha}\, f_5 + \ c_{6\alpha}\, f_6 + c_{7\alpha}\, f_7 + c_{8\alpha}\, f_8 + c_{9\alpha}\, f_9 \\ c_{i\alpha} = \frac{\alpha\left(c_{i0}^2 - \frac{2}{3}\delta_{1i}c_{i0} + \frac{{\delta_{1i}}^2}{6}\right) + \left(c_{i0}^2 + \frac{2}{3}\delta_{1i}c_{i0} - \frac{{\delta_{1i}}^2}{6}\right)}{c_{i0} + \frac{\delta_{1i}}{3}}, i = 1,2,\ldots,9 \\ f_1 = \frac{K}{\delta T_1}\left(T_1 - \frac{1-e^{-\delta T_1}}{\delta}\right), f_2 = \frac{K\left(T_1 - \frac{1-e^{-\delta T_1}}{\delta}\right)}{T_1}, f_3 = \frac{K}{\delta T_1}\left(T_1 - \frac{1-e^{-\delta T_1}}{\delta}\right), f_4 = K, f_5 = \frac{1}{T_1} \\ f_6 = \frac{0.00424628LK\left(1-e^{-\delta T_1}\right)}{T_1\delta}, f_7 = \frac{0.0000431445LK\left(1-e^{-\delta T_1}\right)}{T_1\delta}, f_8 = \frac{DT_1}{2}, f_9 = \frac{1}{T_1} \end{cases} \tag{23}$$

4. Results

In this section we illustrate the novelty of the methodology by using numerical data collected from a crude steel industry.

4.1 Crisp optimal solution

Using this data set stated in Table 1 and utilizing the pollution function (1) in the main problem (18) with the condition (19) we get the optimal results by taking the help of LINGO software and they are put in Table 2.

Table-2: Crisp solution of the proposed model

P^*	K^*	D^*	T_1^*	T_2^*	T_3^*	Q^*	Z^*
26	280.57	245.44	1.45	2.18	3.625	355.89	48342.68

Table 2 shows the optimal average inventory cost $ 48342.68 with the optimal order quantity 355.89 MT, production time 1.45 months. The optimal production rate and demand rate is 280.57 MT and 245.44 MT. In this case the pollution index takes the value explicitly.

4.2 Optimal solution using fuzzy approximate reasoning method

For finding fuzzy optimal solution under the effect of fuzzy approximate reasoning we consider the values of the fuzzy deviation parameters δ_0=9000, δ_{10}=1, δ_{20}=2,δ_{30}=60,δ_{40}= 9,δ_{50}=2500,δ_{60} = 0.7,δ_{70}= 80,δ_{80} =2,δ_{90}= 2500, z_0 = 44018 along with P=26,K=280.57, D= 245.44, T_1=1.45. The fuzzy optimal solutions are tabulated in table 3.

Table 3: Optimal solution using approximate fuzzy reasoning method

α^*	β^*	Q^*	T_1^*	Z^*
0.21	0.99	302.201	1.23	46621.37
0.22	0.95	245.44	1.00	46523.54
0.23	0.90	308.215	1.26	46412.74
0.25	0.85	311.790	1.27	46288.90
0.26	0.80	245.44	1.00	46149.58
0.28	0.75	245.44	1.00	45991.68
0.30	0.70	325.608	1.33	45811.23
0.32	0.65	331.642	1.35	45603.02
0.35	0.60	338.684	1.38	45360.11
0.38	0.55	347.01	1.41	45073.02
0.42	0.50	356.99	1.45	44728.53
0.47	0.45	302.953	1.23	44307.47
0.36	0.581	341.649	1.39	45257.85
0.34	0.611	245.44	1.00	45419.27

Table 3 expresses the optimal average inventory cost, optimum order quantity and optimum

production run time with respect to different α cuts and its dual cuts β. We see that the average inventory cost takes minimum value $ 44307.47 with respect to order quantity 302.953 MT and production cycle 1.23 months for the primal-dual cut value (0.47, 0.45) and the average inventory cost takes its maximum value $ 46621.37 with respect to order quantity 302.201 MT and same production cycle 1.23 months for the primal-dual cut value (0.21, 0.99).

4.3. Sensitivity Analysis

To investigate the sensitivity of the parameters associated with the problem we take the changes of the cost parameters as well as fuzzy deviation parameters from -20% to +20% each. The optimal production run time, order quantity, average inventory cost and the primal-dual cut value are furnished in Table 4A and 4B.

Table 4A: Sensitivity analysis of fuzzy cost parameters (optimum α)

Parameter	% Change	α*	β*	Q*	T1*	Z*	$\frac{z-z^*}{z^*}\times 100\%$
C_{10}	+20	0.309	0.588	245.44	1.00	50233.55	3.91
	+10	0.519	0.350	401.798	1.64	48342.68	0
	-10	0.320	0.569	346.861	1.41	50140.26	3.72
	-20	0.519	0.350	355.896	1.45	48342.68	0
C_{20}	+20	0.309	0.588	245.44	1.00	50233.55	3.91
	+10	0.519	0.350	355.896	1.45	48342.68	0
	-10	0.519	0.350	401.798	1.64	48342.68	0
	-20	0.519	0.350	355.896	1.45	48342.68	0
C_{30}	+20	0.519	0.35	355.896	1.45	48342.68	0
	+10	0.309	0.588	245.44	1.00	50233.55	3.91
	-10	0.519	0.35	401.798	1.64	48342.68	0
	-20	0.519	0.35	401.798	1.64	48342.68	0
C_{40}	+20	0.272	0.668	263.876	1.08	50568.67	4.6
	+10	0.288	0.631	253.262	1.03	50425.48	4.31
	-10	0.519	0.35	389.782	1.59	48342.68	0
	-20	0.396	0.471	245.44	1.00	49449.71	2.29
C_{50}	+20	0.519	0.35	450.651	1.84	48342.68	0
	+10	0.519	0.35	421.457	1.72	48342.68	0
	-10	0.583	0.35	381.736	1.56	47770.19	-1.18
	-20	0.664	0.35	548.024	2.23	47037.93	-2.7

C_{60}	+20	0.299	0.609	246.852	1.01	50331.41	4.11
	+10	0.519	0.35	362.747	1.48	48342.68	0
	-10	0.519	0.35	399.642	1.63	48342.68	0
	-20	0.519	0.35	343.042	1.40	48342.68	0
C_{70}	+20	0.519	0.35	373.027	1.52	48342.68	0
	+10	0.301	0.604	245.501	1.00	50310.74	4.07
	-10	0.519	0.35	347.988	1.42	48342.68	0
	-20	0.519	0.35	340.470	1.39	48342.68	0
C_{80}	+20	0.301	0.603	245.44	1.00	50306.36	4.06
	+10	0.519	0.35	361.532	1.47	48342.68	0
	-10	0.519	0.35	401.799	1.64	48342.68	0
	-20	0.318	0.35	245.44	1.00	50159.75	3.76
C_{90}	+20	0.519	0.35	450.651	1.84	48342.68	0
	+10	0.519	0.35	421.457	1.72	50141.64	3.72
	-10	0.343	0.594	245.44	1.00	48342.68	0
	-20	0.664	0.35	548.023	2.23	50142.47	3.72
δ_0	+20	0.519	0.350	339.393	1.38	49207.61	1.79
	+10	0.325	0.560	245.44	1.00	50703.14	4.88
	-10	0.302	0.602	248.79	1.01	49673.19	2.75
	-20	0.519	0.35	374.37	1.53	47477.74	-1.79

Table 4A shows the sensitivity of the cost parameters associated with the model. We see that the average inventory cost reaches its upper bound $ 50703.14 with 4.88 % increment and takes its lower bound $ 47037.93 with 2.7 % decrement. The order quantity and the production run time lie between the respective intervals [245.44, 548.02] MT and [1.00, 2.23] months. Also, the primal α cut lies in the interval [0.272, 0.664] whereas the dual β cut lies in the interval [0.350, 0.668] throughout the table.

Table 4B: Sensitivity analysis of fuzzy deviation parameters (optimum α)

Parameter	% Change	α*	β*	Q*	T^*_1	Z*	$\frac{z-z^*}{z^*}\times 100\%$
C_{10}	+20	0.519	0.35	355.896	1.45	48342.68	0
	+10	0.320	0.569	346.819	1.41	50141.64	3.72
	-10	0.519	0.35	401.798	1.64	48342.68	0
	-20	0.319	0.569	346.794	1.41	50142.47	3.72
C_{20}	+20	0.320	0.569	346.811	1.41	50141.91	3.72
	+10	0.320	0.569	346.811	1.41	50141.91	3.72
	-10	0.320	0.569	346.811	1.41	50141.91	3.72
	-20	0.519	0.35	355.896	1.45	48342.68	0
C_{30}	+20	0.519	0.35	401.798	1.64	48342.68	0
	+10	0.519	0.35	401.798	1.64	48342.68	0
	-10	0.318	0.572	346.295	1.41	50158.73	3.76
	-20	0.519	0.350	355.896	1.45	48342.68	0
C_{40}	+20	0.519	0.35	401.164	1.63	48342.68	0
	+10	0.519	0.35	401.482	1.64	48342.68	0
	-10	0.519	0.35	402.114	1.64	48342.68	0
	-20	0.309	0.588	245.44	1.00	50235.55	3.92
C_{50}	+20	0.635	0.35	457.689	1.86	47303.71	-2.15
	+10	0.577	0.35	400.204	1.63	47827.96	-1.06
	-10	0.519	0.35	402.183	1.64	48342.68	0
	-20	0.519	0.35	402.565	1.64	48342.68	0
C_{60}	+20	0.519	0.35	401.688	1.64	48342.68	0
	+10	0.519	0.35	401.744	1.64	48342.68	0
	-10	0.519	0.35	356.065	1.45	48342.68	0
	-20	0.519	0.35	356.235	1.45	48342.68	0
C_{70}	+20	0.519	0.35	355.501	1.45	48342.68	0
	+10	0.519	0.35	355.698	1.45	48342.68	0
	-10	0.519	0.35	356.093	1.45	48342.68	0
	-20	0.519	0.35	401.925	1.64	48342.68	0
C_{80}	+20	0.519	0.35	355.619	1.45	48342.68	0
	+10	0.519	0.35	355.758	1.45	48342.68	0
	-10	0.519	0.35	356.034	1.45	48342.68	0
	-20	0.569	0.319	346.781	1.41	50142.90	3.72
C_{90}	+20	0.635	0.35	457.689	1.86	47303.71	-2.15
	+10	0.317	0.637	248.363	1.01	50165.41	3.77
	-10	0.519	0.35	402.183	1.64	48342.68	0
	-20	0.519	0.35	402.565	1.64	48342.68	0
δ_0	+20	0.519	0.350	245.44	1.00	57146.28	18.21
	+10	0.420	0.432	245.44	1.00	53634.74	10.95
	-10	0.268	0.679	277.55	1.13	46207.85	-4.42
	-20	0.228	0.797	324.62	1.32	42162.54	-12.78

Table 4B shows the sensitivity of the deviation parameters associated with the model. We see that the average inventory cost reaches its upper bound $ 57146.28 with 18.21 % increment and takes its lower bound $ 42162.54 with 12.78 % decrement. The order quantity and the production run time lie between the respective intervals [245.44, 457.69] MT and [1.00, 1.86] months. Also, the primal α cut lies in the interval [0.228, 0.635] whereas the dual β cut lies in the interval [0.319, 0.797] throughout the table.

Table 5: Variation of supply chain cost with rail distance in fuzzy approximate reasoning

L	α^*	β^*	Q^*	T_1^*	Z^*
500	0.519	0.35	394.092	1.606	48342.68
600	0.519	0.35	355.896	1.45	48342.68
700	0.319	0.569	353.479	1.44	50138.80

Table 5 shows the variation of average inventory cost, order quantity, production run time and the primal-dual cuts with respect to the variation of distance covered by the transportation vehicles. We see that the average inventory cost is increasing and both the order quantity and production run time are decreasing with respect to the increasing transportation distances.

5. Discussion

In this section we shall draw some graphs using numerical outcomes of the model stated in tables 2 to 5.

Fig. 6: Change of order quantity due to variation of z_0, δ_0 and δ_{20}

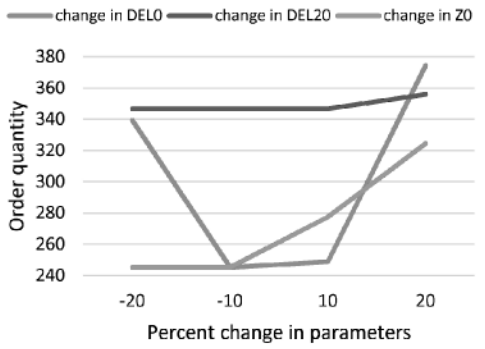

Fig. 7: Change of production run time due to variation of z_0, δ_0 and δ_{20}

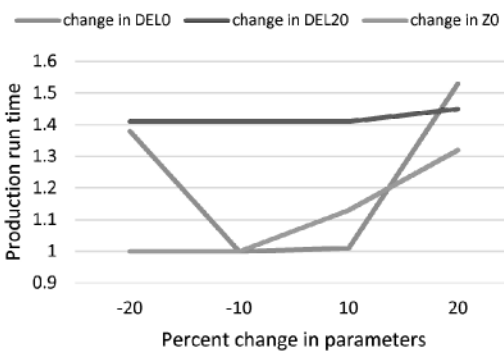

Fig. 6 and Fig. 7 show the variation of the order quantity and production run time due to the % change of the parameters z_0, δ_0 and δ_{20}. We see that δ_{20} is less sensitive and z_0, δ_0 are much sensitive according to the order quantity curve and production run time curve. The order quantity

takes maximum value at 375 MT for 20 % hike of δ_0 and takes minimum value at 245 MT for 10% decrease of both z_0 and δ_0. The production run time reaches its maximum at 1.55 months for 20 % hike of δ_0 and takes minimum value at 1.00 months for 10% decrease of both z_0 and δ_0.

Fig.8: Change of average supply chain cost due to variation of z_0, δ_0 and δ_{20}

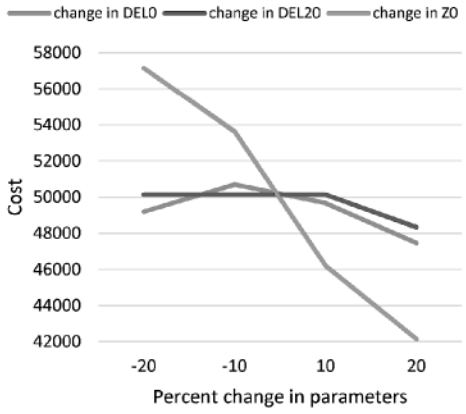

Fig. 9: Variation of average SC cost with production run time in approximate fuzzy reasoning method

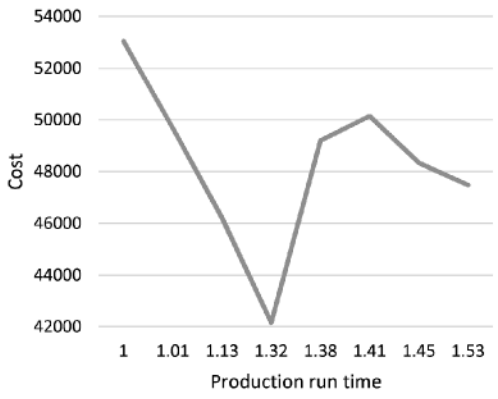

Fig. 8 shows the variation of the average supply chain cost due to the % change of the parameters z_0, δ_0 and δ_{20}. We see that z_0 is much sensitive rather than δ_0 and δ_{20} according to the cost curve. The average supply chain cost becomes maximum (\$ 57000) with 20% reduction of z_0 and becomes minimum (\$ 42000) with 20 % hike of z_0. Fig. 9 gives the variation of average SC cost with the variation of production run time. The cost curve takes V-shape with valley \$42000 corresponding to 1.32 months production time and takes two peaks with \$ 53000 and \$ 50000 corresponding to 1.00 months and 1.41 months respectively.

Fig. 10:Variation of Supply Chain cost under α-dual β–cuts solutions

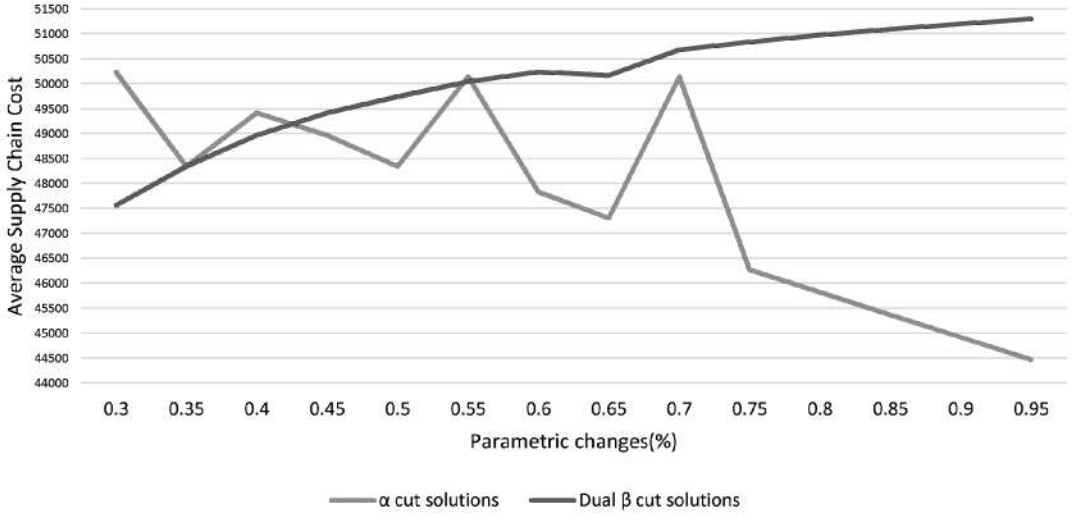

Fig. 10 represents the variation of the average supply chain cost under primal α-dual β–cuts solutions. We see that the average SC cost curve takes a parabolic view with respect to the dual cut whereas it takes a zigzag view with respect to the primal cut. When the primal cut becomes maximum with 0.95 then the average SC cost becomes minimum with $ 44500 and when the dual cut becomes maximum with 0.95 then the average SC cost becomes maximum with $ 51000.

Fig. 11: Variation of cost function due to α and β

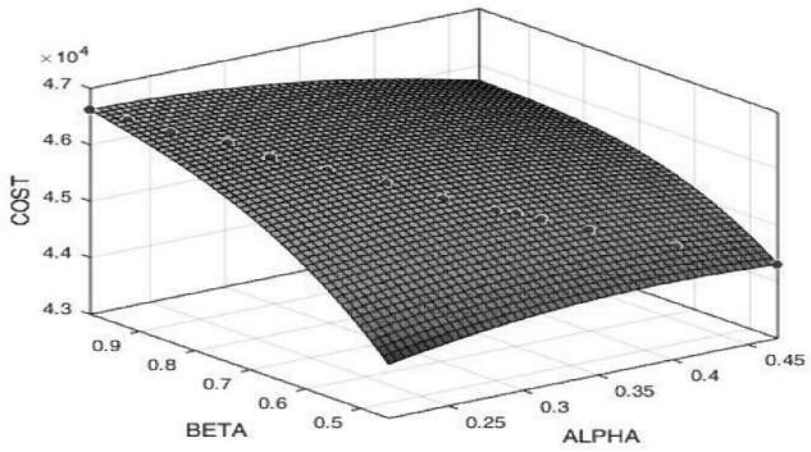

Fig. 11 expresses a three-dimensional view of average SC cost under the effect of primal and dual cuts. It is a surface like curve with concave nature. We see that the average SC cost becomes minimum (\$ 43500) with respect to the value of primal-dual cut (α, β) = (0.26, 0.60) and it becomes maximum (\$ 46500) with respect to the value of primal-dual cut (α, β) = (0.2, 0.95) respectively.

6. Conclusion

In this study we have discussed a noble application of fuzzy approximate reasoning in the field of inventory management problems. This article explores a new horizon on managerial decision making utilizing the approximating fuzzy mathematical modelling. To do this, the concept of possibility theory in approximating fuzzy membership function is incorporated. The concept of negation (non-membership degree) is applied within approximated fuzzy membership degree. Boosting the traditional approach of α-cuts of fuzzy sets, duality of α-cuts has been introduced over a rectangular hyperbolic type convex set. The basic managerial insights include as follows:

a) More choices have come to get decision at any time because of the presence of dual solution of the original objective function side by side.
b) Fuzzy reasoning solution approach gives nearly 8.5 % cost reduction on average with respect to crisp optimal solution.
c) Reducing length of cycle time, the DMs will have enough time to think further for any kind of strategic change.
d) Applying fuzzy reasoning it is possible to reduce individual differences of selecting particular fuzzy number and hence its global acceptance even less qualified DM also.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgements

The authors are grateful to the anonymous reviewers, honorable Editor-in-Chief for their valuable suggestions and to improve the quality of the article.

References

1. Bhattacharya K, De SK, Khan A, Nayak PK, Pollution sensitive global crude steel production–transportation model under the effect of corruption perception index, OPSEARCH, 2021, 58, 636-660.
2. De SK, Solving an EOQ model under fuzzy Reasoning, Applied Soft Computing, 2020, https://doi.org/10.1016/j.asoc.2020.106892.
3. De SK, Triangular dense fuzzy lock sets, Soft Computing, 2017, https://doi.org/10.1007/s00500-017-2726-0.
4. De SK, Beg I, Triangular dense fuzzy sets and new defuzzification methods, Journal of Intelligent and Fuzzy Systems, 2016, 31(1), 469-477.
5. De SK, Mahata GC, Decision of a Fuzzy Inventory with Fuzzy Backorder Model under Cloudy Fuzzy Demand Rate, International Journal of Applied and Computational Mathematics, 2017, 3(3), 2593-2609.
6. De SK, Mahata GC, An EPQ model for three-layer supply chain with partial backordering and disruption: Triangular dense fuzzy lock set approach, Sadhana, 2019a, https://doi.org/10.1007/s12046-019-1160-7.
7. De SK, Mahata GC, A production inventory supply chain model with partial backordering and disruption under triangular linguistic dense fuzzy lock set approach, Soft Computing, 2019b, https://doi.org/10.1007/s00500-019-04254-2.
8. De SK, Mahata GC, A comprehensive study of an economic order quantity model under fuzzy monsoon demand, Sadhana, 2019c, https://doi.org/10.1007/s12046-019-1059-3.
9. De SK, Bhattacharya K , Roy B, Solution of a Pollution Sensitive Supply Chain Model under fuzzy approximate reasoning, Int.J. of Intelligent Systems, 2021a DOI: 10.1002/int.22522
10. De SK, Mahata GC , Maity S, Carbon emission sensitive deteriorating inventory model with trade credit under volumetric fuzzy system, Int.J. of Intelligent Systems, 2021b DOI: 10.1002/int.22599
11. De SK, Sana SS, Fuzzy order quantity inventory model with fuzzy shortage quantity and fuzzy promotional index, Economic Modelling, 2013a, 31, 351-358.
12. De SK, Sana SS, Backlogging EOQ model for promotional effort and selling price sensitive demand- an intuitionistic fuzzy approach, Annals of Operations Research, 2013b, https://doi.org/10.1007/s10479-013-1476-3.
13. De SK, Sana SS, Multi-criteria multi-attribute decision making for EOQ model under hesitant fuzzy environment, Pacific Science Review A: Natural Science and Engineering, 2015, 17(2), 61-68.
14. De SK, Sana SS, The (p,q,r,l) Model for

Stochastic Demand under Intuitionistic Fuzzy Aggregation with Bonferroni Mean, Journal of Intelligent Manufacturing, 2018, 29(8), 1753-1771.

15. De SK, Goswami A, Sana SS, An interpolating by pass to Pareto optimality in intuitionistic fuzzy technique for a EOQ model with time sensitive backlogging. Applied Mathematics and Computation, 2014, 230, 664-674.
16. Ebadi A, Moharram NN, Rahnamaee MT, Motesadi ZS, Determining the ecological footprint of vehicles in Tehran, Iran. Applied Ecology and Environmental Research, 2016, 14(3), 439-450.
17. Hernandez-Pellon A, Fernandez-Olmo I, Airborne concentration and deposition of trace metals and metalloids in an urban area downwind of a manganese alloy plant, Atmospheric Pollution Research, 2018, https://doi.org/10.1016/j.apr.2018.11.009.
18. Karmakar S, De SK, Goswami A, A pollution sensitive dense fuzzy economic production quantity model with cycle time dependent production rate, J. Clean. Prod., 2017, 154, 139-150.
19. Karmakar S, De SK, Goswami A, A pollution sensitive remanufacturing model with waste items: Triangular dense fuzzy lock set approach, J. Clean. Prod., 2018, 187, 789-803.
20. Mancini MS, Galli A, Niccolucci V, Lin D, Bastianoni S, Wackernagel M, Marchettini N, Ecological footprint: Refining the carbon footprint calculation, Ecological Indicators, 2016, 61, 390-403.
21. Nasseri SH, Ebrahimnejad A, A New Approach to Duality in Fuzzy Linear Programming, 2012, https://doi.org/10.1007/978-3-642-28592-9_2.
22. Ovchinnikov S, The duality principle in fuzzy set theory, Fuzzy sets and Systems, 1991, 42, 133-144.
23. Pal SK, Mandal DP, Fuzzy Logic and Approximate Reasoning: An Overview, IEEE Journal of Research, 1991, 37(5-6), 548-560.
24. Panigrahi M, Panda G, Nanda S, Convex fuzzy mapping with differentiability and its application in fuzzy optimization, European Journal of Operational Research, 2008, 185, 47–62.
25. Ramik J, Rimanek J, Inequality relation between fuzzy numbers and its use in fuzzy optimization, Fuzzy Sets and Systems, 1985, 16, 123-138.
26. Song CZ, Zhao YQ, Fuzzy multi-objective optimization of passive suspension parameters, Fuzzy Information and Engineering, 2010, 1, 87-100.
27. Tanaka H, Asai K, Fuzzy solutions in fuzzy linear programming problems, IEEE Transactions on Systems, Man and Cybernetics, 1984, SMC-14, 325-328.
28. Wu HC, Duality theory in fuzzy linear

programming problems with fuzzy coefficients. Fuzzy Optimization and Decision Making, 2003, 2 (1), 61-73.
29. Wu HC, Duality theorems and saddle point optimality conditions in fuzzy nonlinear programming problems based on different solution concepts, Fuzzy Sets and Systems, 2007, 158, 1588 – 1607.
30. Zadeh LA, Fuzzy sets, Information Control, 1965, 8(3), 338-356.
31. Zadeh LA, The concept of Linguistic variable and its application to approximate reasoning, Parts I, II, III, Information Sciences. 1975, 8, 199-251;8, 301-357; 9, 43-80.
32. Zhang C, Yung XH, Lee ES, Duality theory in fuzzy mathematical programming problems with fuzzy coefficients, Comput. Math. Appl., 2005, 49, 1709-1730.
33. Zimmermann HJ, Application of fuzzy set theory to Mathematical Programming, Information Sciences, 1985, 36, 29-58.
34. Zou T, Extended Duality in Fuzzy Optimization Problems, Mathematical Problems in Engineering, 2015, https://doi.org/10.1155/2015/826752.

Diversity of Fishes in the Rasulpur River of Purba Medinipur District, West Bengal

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ARTICLE INFO
Received: 7.7.2021
Revised: 6.8.2021
Accepted: 9.8.2021

Key words:
Fish diversity, Rasulpur River, Abundance of finfish and shell fish

ABSTRACT

The present study envisaged with the fish diversity of Rasulpur River in the Purba Medinipur district of West Bengal. The experimental study was conducted during the period of March - June, 2018 to record the different types of important fish species available in this river. The river filled with the varying salinities of water and enriched with varieties of brackish water fishes. The results of the present investigation showed that, the total 21 fin fish species belonging to 10 Orders, 18 Families & 21 Genera; 12 shell fish species belonging to 4 Order,7 Family 11 Genera were observed during study period. Among the collected fish species, Order-Perciformes showed most dominant constituting 38% followed by the Order- Rajiformes 10%, Order- Siluriformes, Pleuronectiformes Clupeiformes 9% each and Order- Anguilliformes& Beloniformes constitute 5%. The Crustacean species includes the Order Decapoda, Xiphosura, Sessikia, Stomatopoda Order- Xiphosura was found 9%, Order Sessikia & Stomatopoda was also found 8% each. The species of Order- Gobiidae, Aulopiformes and clupiformes showed very rich abundance, i.e, almost 75%. Fishing operations were done throughout the year but fish production fluctuates in the Rasulpur river. Therefore, recently fish production abruptly decreases day by day owing to severe man made river pollution.

Introduction:

Fishes are one of the important commodities in the economy of many nations as they have been a stable item in the diet of many peoples. India is one of the 17 mega biodiversity countries of the world. With only 2.5% of the land area, India accounts for 7.8% of the recorded species of the world. In India, 2118 species of fish belonging to 711 genera, 123 sub family, 209 families and 54 orders with

119 species of coldwater, 34 species of cold-warm water, 372 species of warm water, 72 species of warm-brackish-marine water, 18 species brackishwater, 68 species warm-brackishwater, 87 species of brackish-marine water and 1,348 species of marine water fishes (NFDB report 2017-2018). According to National Bureau of Fish Genetic Resources(NBFGR), Lucknow, India., database, now contains information on 2662 native finfishes (Freshwater-877, Brackishwater-113 and Marinewater-1672) reported from Indian waters belonging to 1019 genera under 246 families and 42 orders; and 291 exotic fishes. As per the FAO, a sustainable fisheries development envisages an eco-friendly, equitable mode of development that can sustain livelihood over generations. Among the different states of the country, West Bengal holds the high diversity of fish resources (Sanyal et al., 2012).

FishBase (Froese and Pauly, 2016) has listed 917 freshwater fish species (out of 2465 total fish species) as occurring in our country.

Biodiversity loss in freshwater ecosystem is an increasing phenomenon, mainly due to human activities (Abell, 2002). Dubey et al., (2017) opine that climate resilient aquaculture have been possible in Indian Sundarban areas. Mandal et. al., (2012) stated that indigenous knowledge associated with conservation of chocolate Mahseer (*Neolissocheilus hexagonolepis*) by the War-jaintia community practiced in Meghalaya state. The principal causes are the habitat destruction and defragmentation, exotic species introduction and global climatic change impacts (Saunders et al., 2002). Payra et.al, (2014) stated that different crafts and gears operated in brackish water fed canal for harvesting fishes in different seasons to maintain livelihood of the fishermen communities inhabited in the South Bengal coastal area. Mandal et. al., 2016 observed on parasitic occurrence in indigenous climbing perch, *Anabas testudineus* (Bloch, 1972) from West Bengal State of India. The Midnapore costal tract (longitudinal extension 87°20'E to 88°.5'E & latitudinal extension 21°30'N to 22°.2'N) in between Hooghly& Subarnarekha estuarine confluence with the Bay of Bengal, although very short in length, it display unique habitat diversities in respect to vegetation composition, occurrence of dunes, mudflats, sandflat & other ecological parameters like variation of salinity, temperature, texture of sediments etc. Purba Medinipur district is part of the lower Gangetic Plain & Eastern coastal plains. Topographically, the district can be divided into two parts: a) almost entirely flat plains on the West, East& North and b) The coastal plains on the South. The vast expanse of land is forms of alluvium & is composed of younger & costal alluvial. The elevation of the

district is within 10 metres above main sea level (Bandyopadhyay. et al., 2009). The district has a long coastline of 65.5 km along its southern & south eastern boundary. Tidal floods are quite regular in the five Blocks. Rasulpur River is one of the important river in the Eastern coastal parts of Purba Medinipur district of West Bengal. It is the last tributaries of the Hooghly River. The total river length is 19 km and this river connected into the Bay of Bengal with Odisha canal & other small canals and main distributaries of this river is Itaberia and Mugberia canals.

Materials and Methods:

Study area:

The study area is divided into three sampling stations, i.e. Petua-ghat (Station- 1:Marine Zone), Rasulpur-ghat (Station-2: Brackish Water Zone) and Kalinagar-ghat (Station- 3: Fresh Water Zone) where fin fish & shellfish information were collected from the fishermen. Data was collected from March-June, 2018 following lunar periodicity (full moon and New moon) as during these periods higher abundance of finfishes & shell fishes were reported by the fishermen communities. Thus

by conducting eight samplings per month total 32 samples were collected during this study period form three stations.

Identification of Fish samples:

Fish samples are collected from the local landing centers from the fishermen. Generally fishermen sort non target fishes after catching in the river. Generally, the fishermen used set bag net, Set Gill Net, drift gill net and Moshari bar jal, cast net, dragnet etc. for fishing operation. The 10% of the total catches were collected by ice box (frozen) from each station for laboratory study. In the laboratory, the fish samples were sorted and identified to through different methods as developed by Fischer and Whitehead, 1974; Shafi and Quddus, 1982; Talwar and Jhingran, 1991, Talwar and Kakkar, 1984 & De Bruin et al., 1995, Datta Munshi and Srivastava,1988 and Hossain et al., 2007.

Results:

During the study period different fin fishes and shell fishes have been observed in the Rasulpur river of Purba Medinipur district. The result showed that the river rich in fin fish and shell fish diversity; the finfish belong to 10 orders & 18 families and shell fish belong to 4 order & 7 families were recorded. In the present study 21 finfish species from 21 different genera and 18 families were recorded and 12 shellfish species from 11 genera and 7 families also recorded from the Rasulpur River during March to June 2018. The members of the order Perciformes were dominated by 8 species. But order Siluriformes, Pleuronectiformes, Clupeiformes, Rajiformes represent only 2 species and order Aulopiformes, Tetradontiformes, Anguiliformes, Beloniformes represent only single species. The order Decapoda represents 9 species and order Xiphosuran, Sessikia, Stomatopoda also represents single species. The dominated fish species are *Scatophagus argus, Sillago sihama, Terapon jarbua, Mugil cephalus, Lates calcarifer, Trichiurus lepturu Polynemus paradiseus, Johnius soldad* and less abundant fishes includes followed by order Siluriformes which includes *Sperata aor, Hemibagrus gracilis*. The major species from order Pleuronectiformes represent *Synapturs panoides, Brachirus panoides*; the order Clupeiformes include *species are Coilia dussumieri, Setipinna phas*. The order Rajiformes include *Glaucostegus granulatus, Trygon sephen* species. Therefore, 7 shell fish family represented 12 species of which order Decapoda was dominant, the dominant species from this order includes *Dardanus megistos, Cllinectes sapidus, Scylla serrata, Portunus sanguinolentus, Thalamita prymna, Uca demani, Penaeus indicus, Penaeus monodon, Metapenaeus monoceros* and followed by order Xiphosura which represents *Limulus* sp. Among all these order Perciformes was most dominant constitute 38% followed by order

Rajiformes which constitutes 10%,order Siluriformes, Pleuronecitiformes and Clupeiformes which comprises 9% & order Gobiiformes, Aulopiformes, Tetradontiformes, Anguilliformes, Beloniformes constitute only 5%. Therefore, Order Decapoda constitute 75% among all shell fishes and order Xiphosuran constitute 9%, Order Sessikia & Stomatopoda represent 8%.Among all the fishes few shell fishes like *Penaeus monodon*, *Scylla serrata*and *P. indicus* are having good market demand due to their palatable nature.

Table1: Finfish diversity in Rasulpur river & their abundant status during March to June 2018

Order	Family	Scientific name	Common name	Abundant Status
Decapoda	Portunidae	*Dardanus megistos*	Hermit crab	–
		Cllinectes sapidus	Blue crab	–
		Scylla serrata	Mud crab	–
		Portunussanguinolentus	Swimming crab	–
		Thalamita prymna	Swimming crab	
		Ucademani	Fidddler crab	++
		Penaeusindicus	Indian prawn	
	Grapsidae			–
	Ocypodoidea	*Penaeusmonodon*	Tiger prawn	–
	Penaeidae	*Metapenaeusmonoceros*	Indian prawn	
				++
				+++
Xiphosura	Limulidae	*Limulus polyphemus*	Atlantic horseshoe crab	+
Sessikia		*Balanusglandula*	Balanus	++
Stomatopoda	Squillidae	Odontodactylus scyllarus	Mantis shrimp	++

NB: Status: +++ Most abundant : ++ Abundant : + Less abundant and - Rare

Table.2: Shellfish diversity in Rasulpur River & their abundant status during March to June 2018

Order	**Family**	**Scientific name**	**Common name**	**Abundant Status**
Decapoda	Portunidae	*Dardanus megistos*	Hermit crab	–
		Cllinectes sapidus	Blue crab	–
		Scylla serrata	Mud crab	–
		Portunussanguinolentus	Swimming crab	–
		Thalamita prymna	Swimming crab	++
		Ucademani	Fidddler crab	–
		Penaeusindicus	Indian prawn	–
	Grapsidae			
	Ocypodoidea	*Penaeusmonodon*	Tiger prawn	++
	Penaeidae	*Metapenaeusmonoceros*	Indian prawn	+++
Xiphosura	Limulidae	*Limulus polyphemus*	Atlantic horseshoe crab	+
Sessikia		*Balanusglandula*	Balanus	++
Stomatopoda	Squillidae	Odontodactylus scyllarus	Mantis shrimp	++

NB: Status: +++ Most abundant : ++ Abundant : + Less abundant and - Rare

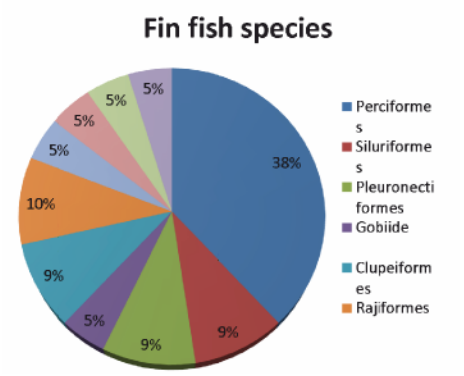

Fig -1: Finfish diversity according to the percentage of fishes available in different order

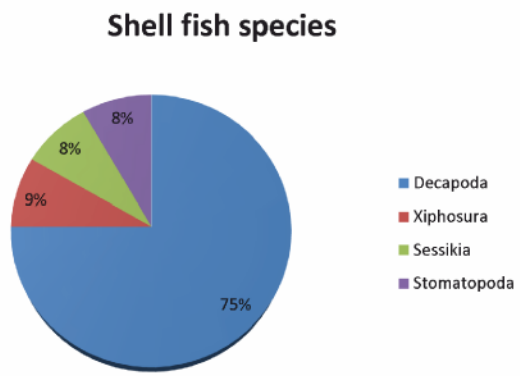

Fig-2.: Shell fish diversity according to the percentage of fishes available in different order

Discussion:

Discussion of the present work is completely based on the outcome result of different experiment. Similar types of work has been done by different scientist, like Payra et al., 2018 counted the ichthyfauna diversity in Negua diversion canal of Purba Medinipur district, Maity et al., 2017 documented the different major group of fishes available in the Haldi river and Mandal et al. in 2015 recorded the crab diversity in Digha coast. Mandal et al. 2015 also observed that different types of parasitic occurrence in the giant freshwater prawn *Macrobrachium rosenbergii* collected from coastal West Bengal. Through the survey it has been observed that 21 fin fish species and 12 shell fish species found most predominant species belong to different order. The order Perciformes was most dominant constituting 38% followed by the order-Rajiformes 10%, order-Siluriformes and Clupeiformes 9%, order-Anguilliformes & Beloniformes constituting 5% which reflected in the Fig. 1. The shellfish group includes Crustacean species order Decapoda, comprising 75%, order- Xiphosura 9%, order-Sessikia & Stomatopoda ware found 8% which showed in the Fig.-2. Overall studies on the diversity of fishes suggest that continuous documentation is very essential for determining the present status of fishery potential in the experimental river. This will help to take fruitful strategies to conserve vulnerable fishes in future also. Further it also observed that availability of different group of fish species depends on tidal fluctuation of the river which directly influences the physico-chemical characteristics of riverine water.

Conclusion:

Present study indicates that habitat loss due to environmental degradation as well as manmade pollution has seriously affected the fish faunal

population in the tidal-fed Rasulpur River. Therefore, the protection of these habitats is a matter of major concern since they contribute significantly to the total biodiversity of the entire estuarine ecosystem of said river.

Plates: Few important fishes in Rasulpur river

Scylla serrata

Penaeus indicus

Portunus sanguinolentus

References:

Abell R. (2002). Conservation biology for biodiversity crisis: Freshwater follow-up. *Conserve. Biol*. 16:1435-1437.

Basudev Mandal, PijushPayra, Ratan Samanta (2015). Seasonal Availability of Crabs & their Distribution in Digha Coast, *International Research Journal of Basic & Applied Science*,pp:27-30.

Basudev Mandal, P. L. D. Hia, B. C. Patra and D. Ghosh (2012). Indigenous knowledge associated with conservation of chocolate Mahseer (*Neolissocheilus hexagonolepis*) by the War-jaintia community practiced in Meghalaya, *Indian Journal of Biological Science*; 18: 41-47.

Basudev Mandal, S.K. Dubey, A.K. Ghosh and G.Dash (2015). Parasitic occurrence in the giant freshwater prawn Macrobrachium rosenbergii from coastal West Bengal, *India. Journal of Parasitology and Vector Biology:* 7 (6): 115-119. **DOI**: 10.5897/JPVB2015.0197.

Basudev Mandal, Sourabh Dubey, Puja Dasgupta, Bijay Kali Mahapatra and Bimal Kinkar Chand (2016). A preliminary survey on parasitic occurrence in indigenous climbing perch, *Anabas testudineus* (Bloch, 1972) from West Bengal State of India, *Journal of Coastal Life Medicine*; 4 (8): 592-596.

Datta Munshi J. S. and Srivastava M. P. (1988). Natural history of Indian fish and systematics of freshwater fishes of India. Narendra Publishing House, New Delhi.

Froese R. and Pauly D. (eds.). (2016). FishBase. www.fishbase.org. Electronic version accessed 12/05/2016.

G.H.P. DeBruin, B.C. Russell, A. Bogusch (1995). FAO Species Identification Field Guide for Fishery Purposes, The Marine Fishery Resources of Sri Lanka, Food and Agricultural Organisation, Rome 400 pp.

M. Shafi, and M.M.A. Quddus (1982). Fisheries resources of Bay of Bengal

(BonggoposagarerMatshayaSampad, In Bengali) Bangla Academy, Dhaka.

M.S. Hossain, N.G. Das, M.S.N. Chowdhury (2007). Fisheries Management of the Naaf RiverChittagong, Coastal and Ocean Research Group of Bangladesh 257 pp.

Pijush Payra, Basudev Mandal and Ganesh Chandra Rana (2014). Crafts and gears operated in brackish water fed canal for harvesting fishes in different seasons to maintain livelihood of the fishermen communities, *International Research Journal of Biological Sciences*, 3(9): 1-7.

Pijush payra, Basudev Mandal and Ganesh Chandra Rana (2018). Study of Ichthyofaunal diversity of Negua Diversion Canal of Purba Medinipur District, *Trends in Fisheries Research*:7(3): 24-39.

Riyanka Maity, Basudev Mandal and Asish Gupta (2017). Impact of heavy metals on thread fin catfish (*Ariusarius*) in the mouth of Haldi river of West Bengal, India *International Journal of Current Research*:9(10): 58442-58445.

Sanyal A. K., Alfred J. R. B., Venkatraman K., Tiwari S. K. and Mitra S. (2012). Status of biodiversity of West Bengal. Zoological Survey of India, Kolkata, 969pp.

Saunders D. L., Meeuwing J. J. and Vincent A. C. J. (2002). Freshwater protected areas: strategies for conservation,*Conserve. Biol.* 16:30-41.

Sourabh Kumar Dubey, Raman Kumar Trivedi, Bimal Kinkar Chand, Basudev Mandal, Sangram Keshari Routa (2017). Farmers' perceptions of climate change, impacts on freshwater aquaculture and adaptation strategies in climatic change hotspots: A case of the Indian Sundarban delta. *Journal of Environmental Development*. 21 (2017): 38-51 (Elsevier). DOI http://dx.doi.org / 10.1016/j.envdev. 2016.12.002.

Talwar P. K. and Jhingran A. (1991). Inland fishes of India and adjacent countries. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 2(19): 1158.

Talwar P. K. and Kakkar R. K. (1984). Commercial sea fishes of India, Handbook (Zoological Survey of India, Kolkata), pp 997.

W. Fischer and P.J.P. Whitehead (1974). FAO Species Identification Sheets for Fishery Purposes. Eastern Indian Ocean (fishing area 57) and Western Central Pacific (fishing area 71), vol. 14., FAO, Rome .

Bandyopadhyay, Sunando & Mukherjee, Dipanwita & Parhari, Debprakash. (2009). Coastal Erosion and its Management at Digha, Medinipur, West Bengal.

Impact of COVID-19 on Environmental Health - A review

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ARTICLE INFO

Received: 15.10.2021

Revised: 16.11.2021

Accepted: 17.11.2021

Key words:

SARS-CoV-2, greenhouse gas, pandemic, environmental health

ABSTRACT

At a time when health systems throughout the world are grappling with the coronavirus disease 2019 (COVID-19) pandemic, its effect on global environment is also a very important factor for consideration. It is a two-way process where the pre-COVID climate factors influenced the landscape in which the disease proliferates around the world andconsequences of the pandemic on our surroundings. The environmental health disparities will also have a long-lasting effect on public health response. The ongoing research on the novel coronavirus SARS-CoV-2 and COVID-19 must also include the role of environmental factors in the process of infection and differential severity of the disease.Studies have shown that the virus has created both positive and negative ramifications on the world environment, especially in countries that are the most critically affected by the pandemic. Contingency measures to slow down the virus like self-distancing and total lockdowns have shown improvements in air, water and noise quality with a concomitant decrease in greenhouse gas (GHG) emissions. On the other hand, waste management is a cause for concern that can result in negative effects on planetary health. At the peak of the infection, most attention has been diverted towards the medical aspects of the pandemic. Gradually, the policy makers have to shift their focus on social and economic avenues, environmental development and sustainability. The objective of this review will be to analyze the impact of the coronavirus on environment and water resources. The study shall update the readers on the various facets of the interaction between this pandemic and environmental health with a model development for long-term sustainability.

Introduction

On late December 2019 an unusual pneumonia was noticed in a hospital in Wuhan city, in China with a link to an animal market that sells poultry, fish and other animals to the public (Xu et al., 2020). According to WHO, 2020, the new strain of coronavirus (SARS-CoV-2) has affected almost the entire world including 216 countries infecting 28040853 people with 906092 confirmed deaths till date and created an unprecedented and irreversible impact. However, the new dynamics of the outbreaks seem highly variable amongst countries (Dong, Du, & Gardner, 2019). COVID-19 is an acute viral illness, instigated by coronavirus called the SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). The symptoms of COVID-19 range from mild to severe, and include mainly fever, cough, and respiratory distress. Severe cases with pneumonia and hypoxemia result in considerable mortality (Rothan, 2020).Currently, most countries have tried to fight the spread of the virus with massive COVID-19 screening tests and establishing public policies of social distancing. It is clear that the priority revolves around people's health (Manuel, 2020).

The course of an epidemic is defined by several factors, including demographic and environmental, many of which have an unknown correlation for COVID-19 (Anderson et al., 2020). Manuel, 2020 has also mentioned that initial studies have reported a positive indirect impact on the environment. Due to the social distancing policies taken up by the governments, climate experts predict that greenhouse gas (GHG) emissions could drop to proportions never before seen since World War II according to the reports of the Global Carbon Project, 2020. Among the extra-human factors, climatic factors can play an important role in the spread of the coronavirus, which makes it crucial to understand the drivers of the disease spread, as they are vital to guide the imposition of restrictive measures (Rosario et al. 2020). Air pollution is also one of the greatest challenges of our millennium, and some early studies have highlighted a positive correlation between air pollution and the spread of the virus. Therefore, it is crucial to define which role the atmospheric particulate plays in the spread, morbidity, and mortality of the virus (Comunian et al. 2020). Covid-19, similarly to other viruses, could also have an airborne transmission, and particulate matter (PM) could act as a carrier through the aerosol, conveying the virus and increasing its spread (Qing et al. 2019).Tamerius et al., 2013 and Yuan et al., 2006 had already mentioned that the transmission and survival 50 of viruses responsible for respiratory diseases such as influenza and SARS viruses are known to be influenced by environmental conditions. With references to difference in Covid-19 cases in

Brazil Rosario et al. 2020 suggested that changes in the seasons towards more temperate weather, it is of pivotal importance to know the role of environmental conditions in the transmission of the virus to raise awareness on the prevention of disease spread. It has been documented that the temperature and its variations might have affected the SARS outbreak (Tan, 2005). Temperature (Pinheiro et al., 2014) and diurnal temperature range (DTR) (Luo et al., 2013) have been linked to the death from respiratory diseases. A study demonstrated that absolute humidity had significant correlations with influenza viral survival and transmission rates (Metz and Finn, 2015). Few studies reported that the COVID-19 was related to the meteorological factors, which decreased with the temperature increasing (Oliveiros et al., 2020; C. Wang et al., 2020; M. Wang et al., 2020), but their effects on the mortality have not been reported (Ma et al. 2020). As for the anthropogenic interventions, Calma, 2020 has reported that in USA, there has been an increase in garbage from personal protective equipment such as masks and gloves. Patients and health care workers are quickly going through medical supplies and disposable personal protective equipment, like masks. Eventually all that used gear piles up as medical waste that needs to be safely discarded.

In this review article we are going to discuss about the different perspectives of the environmental factors, including climatic and anthropological parameters that have direct or indirect effects on the spreading and transmission of the COVID-19 viral disease.

Relationship between air quality and COVID- 19

Microbial agent as components of bioaerosol, move around the environment making the circulating air as their vehicle (Zhou et al. 2020). Particulate matter (PM) in the surrounding atmosphere can acts as a carrier of a transport vector, thus increasing the effectiveness of the persistent spread of viruses in the aerosol as it creates a suitable microenvironment (Setti et al. 2020). Different epidemiological and experimental studies for the evaluation of the relationship between the effect of air pollutants and viral respiratory infections were carried out by Ciencewicki and Jaspers in 2007. Chen et al. 2010 has reported a positive correlation between the spread of measles virus and PM concentration in China. PM can induce increase in infected cases by several mechanisms one of which is binding of viruses to PM particles favoured by ambient climatic conditions (Chen et al. 2017). Concerning the effect of PM pollution and the spread of viruses in the population, several recent studies have analyzed whether the different areas of the world with a high and rapid increase in Covid-19's contagion were

correlated to a greater level of air pollution. The investigation of this possible correlation should be analyzed at two levels: (a) the high level of air pollution over the last years, which has made the population more sensitive to Covid-19 (longterm exposition); or (b) the sensitivity to the virus, which is linked to the high level of air pollution in the period when the virus appeared (short-term exposition) (Comunian et al., 2020). Pansini and Fornacca, 2020, associating several annual satellite and ground indexes of air quality in China, Iran, Italy, Spain, France, Germany, United Kindom, and USA with the Covid-19 infection, found statistically significant positive correlations between the high level of air pollution and Covid-19 infections. Wu et al., 2020, has shown that 1 ug/m3 increase in long-term exposure to PM2.5 is associated with a 15% increase in Covid-19 mortality rate. The results of this article suggest that long-term exposure to air pollution increases vulnerability to the occurrence of more severe Covid-19 results. These findings are in line with the known relationship between PM2.5 exposure and many of the cardiovascular and respiratory comorbidities that significantly increase the risk of death in Covid-19 patients. Concerning the effect of the short-term PM exposition and the spread of viruses in the population, the position paper proposed by the Italian Society of Environmental Medicine (SIMA) considers PM as an important carrier that has contributed to the spread of Covid-19 (Setti et al., 2020).

Relationship with temperature and humidity

SARS (severe acute respiratory syndrome) outbreaks have always been associated with the temperature and its variations (Tan et al., 2005). Apart from providing helpful conditions for virus survival and spreading, low temperature and humidity also hinders the innate immune response (Sun et al., 2020, Kudo et al., 2019). Cold conditions reduce the supply of blood immune cells to the nasal mucosa. Low relative humidity can reduce the capacity of cilia cells in the airway to remove virus particles. In addition, low-humidity environments impair the innate immune defense system, making it vulnerable to virus invasion. For the above-mentioned reasons, several studies are being dedicated to delineate the effect of temperature and humidity on the spread of COVID-19 with contradictory conclusions. Recently, Wu et al. employed log-linear generalized additive model (GAM) to analyze the effects of temperature and humidity on COVID-19 infection and deaths in 166 countries (Wu et al., 2020). Considering both the lag and cumulative effects, a negative correlation has been discovered between temperature and relative humidity to that of daily new cases and deaths caused by SARS-CoV-2. Diurnal temperature range (DTR) is also having a

positive association with daily mortality of COVID-19 (Ma et al., 2020). In general, tropical climate delays the onset of confirmed COVID-19 positive cases in comparison to tempered climates (Méndez, 2020). On the contrary, using wavelet transform coherence, partial and multiple wavelet coherence, Iqbal et al. observed that the average daily temperature between 3 °C to 21 °C does not have any significant effect on the containment of COVID-19 in Wuhan (Iqbal et al., 2020). Meteorological data from 122 cities in China showed no evidence that COVID-19 cases would reduce in warmer weather (Xie and Zhu, 2020). At < 3 °C, 1 °C rise in the mean temperature was associated with a 4.861% increase in the daily-confirmed cases.

Another study conducted in China, does not support the hypothesis that rise in temperature or ultraviolet radiation can reduce the transmissibility of the coronavirus (Yao et al., 2020). Outside of China, in USA, Bashir et al. analyzed the effect of different climate indicators on the COVID-19 pandemic (Bashir et al., 2020).

Although they found a significant association between average temperature, minimum temperature and air quality with the spread of the disease, no evidence was obtained to suggest that warm weather suppresses the virus. One probable reason for these contradictions could be that different studies were conducted with distinct data sets. Moreover, the population size and density were also different. As a result of which the probability of infection also varies between different regions. Research conducted at a global scale with larger temperature and humidity range may provide an accurate correlation between these climatic factors and the spread of SARS-CoV-2. Another challenge will be the normalization of the complex epidemiological data generated from different parts of the world. Nonetheless, there seems to be a collective effect of temperature and humidity on the spread as well as the mortality caused by COVID-19.

COVID-19 and waste management

Increase in waste

The generation of organic and inorganicwaste is indirectly accompanied by a wide range of environmental issues, such as soil erosion, deforestation, air, and water pollution (Mourad, 2016; Schanes et al., 2018). The quarantine policies in most of the countries has led to the increased number of consumers for online shopping and home delivery leading to an increased organic waste generation by households. Also, ship packed food purchased online has led to the increase of inorganic waste (Manuel et al., 2020). Medical waste is also in its rise. In Wuhan, China, hospitals produce an average of 240 metric tons of medical waste per day during the outbreak, compared to their previous average of fewer than 50 tons (Calma,

2020).

Waste recycling reduction

Waste recycling has always been a major environmental problem of interest to all countries (Liu et al., 2020) which is a common and effective way to prevent pollution, save energy, and conserve natural resources (Varotto and Spagnolli, 2017; Ma et al., 2019). Due to the pandemic, countries like USA has stopped recycling programs in some of their cities, as authorities have been concerned about the risk of COVID-19 spreading in recycling centers. In affected European countries, waste management has been restricted. For example, Italy has prohibited infected residents from sorting their waste. Also, the industry has seized the opportunity to repeal disposable bag bans, even though single-use plastic can still harbor viruses and bacteria **(Bir, 2020)**.

Conclusions

The analyses on the PM and Covid-19 correlation are the foundations to start wider research. The correlation between these factors is positive, but it is important to understand the mechanism that explains it. All mentioned studies indicate that both long-term exposure and short-term exposure to high levels of pollutants are correlated to an increase in Covid-19 contagion worldwide. Thus, it may be interesting to perform a systematic study, placing PM collection units at strategic points all over the world(Comunian, 2020).Among the indirect effects, the increase in domestic and medical waste were mentioned. The restriction to recycle waste in countries like the USA and Italy has been another negative indirect effect of SARS-CoV2. The safe management of domestic waste could be critical during the COVID-19 emergency. Medical waste such as contaminated masks, gloves, used or expired medications, and other items can easily be mixed with domestic waste. However, they should be treated as hazardous waste and disposed of separately (Manuel, 2020).

References

1. Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., Liu, S., Zhao, P., Liu, H., & Zhu,L. (2020). "Pathological findings of COVID-19 associated with acute respiratory distresssyndrome." *The Lancet respiratory Medicine*, 8(4), p. 420-422.
2. WHO. (2020). https://www.who.int/emergencies/diseases/novel-coronavirus-2019. Accessed date: 12th September 2020.
3. Dong, E., Du, H. and Gardner, L. (2020). "An interactive web-based dashboard to track COVID-19 in real time." *Lancet Infect. Dis*, p. 19. doi.org/10.1016/S1473-3099(20)30120-1.
4. Rothan, H.A. and Byrareddy, S. N. (2020). "The epidemiology and pathogenesis of coronavirus disease (COVID-19)

outbreak." *J Autoimmun*, p. 102433.

5. Manuel, A., Monserrate, Z., Ruano, M. A., Alcalde L. S. (2020). "Indirect effects of COVID-19 on the environment." *Science of the Total Environment*, 728, p. 138813.
6. Anderson, R. M., Heesterbeek, H., Klinkenberg, D. and Hollingsworth, T.D. (2020). "How will country-based mitigation measures influence the course of the COVID-19 epidemic?" *The Lancet*. 395, p. 931–934. doi.org/10.1016/S0140-6736(20)30567-5.
7. Rosario, D. K. A., Mutz, Y. S., Bernardes, P. C. and Conte-Junior, C. A. (2020). Relationship between COVID-19 and weather: Case study in a tropical country. *International Journal of Hygiene and Environmental Health*, p. 113587. doi: 10.1016/j.ijheh.2020.113587.
8. Comunian, S., Dongo, D., Milani, C. and Palestini, P. (2020). Air Pollution and Covid-19: The Role of Particulate Matter in the Spread and Increase of Covid-19's Morbidity and Mortality. *International Journal of Environmental Research and Public Health*, 17(12), p. 4487. doi:10.3390/ijerph17124487.
9. Qing, H., Wang, X., Zhang, N., Zheng, K., Du, K., Zheng, M., Li, Y., Chang, Y., Zhang, L. and Bachert, C. (2019). "The Effect of Fine Particulate Matter on the Inflammatory Responses in Human Upper Airway Mucosa." *Am. J. Respir Crit Care Med*, 10, p. 1315–1318.
10. Tamerius, J.D., Shaman, J., Alonso, W.J., Bloom-Feshbach, K., Uejio, C.K., Comrie, A. and Viboud, C. (2013). "Environmental Predictors of Seasonal Influenza Epidemics across Temperate and Tropical Climates." *PLoSPathog*, 9, p. e1003194. doi.org/10.1371/journal.ppat.1003194.
11. Yuan, J., Yun, H., Lan, W., Wang, W., Sullivan, S.G., Jia, S., Bittles, A.H. (2006). "A climatologic investigation of the SARS-CoV outbreak in Beijing, China." *Am. J. Infect. Control*, 34, p. 234–236. doi.org/10.1016/j.ajic.2005.12.006.
12. Tan, J. (2005). "An initial investigation of the association between the SARS outbreak and weather: with the view of the environmental temperature and its variation." *J. Epidemiol. Community Health*, 59 (3), p. 186–192. doi.org/10.1136/ jech.2004.020180.
13. Pinheiro, S. D. L. L., Saldiva, P. H. N., Schwartz, J. and Zanobetti, A. (2014). "Isolated and synergistic effects of PM10 and average temperature on cardiovascular and respiratory mortality." *Rev. Saude Publica*, 48 (6), p. 881–888. doi.org/10.1590/S0034- 8910.2014048005218.
14. Luo, Y., Zhang, Y., Liu, T., Rutherford, S., Xu, Y., Xu, X., Wu, W., Xiao, J., Zeng, W., Chu, C. and Ma, W. (2013). "Lagged

effect of diurnal temperature range on mortality in a subtropical megacity of China." *PLoS One*, 8 (2), p. e55280. doi.org/10.1371/journal. pone.0055280.

15. Metz, J.A. and Finn, A. (2015). "Influenza and humidity – why a bit more damp may be good for you!" *J. Inf. Secur*, 71, p. S54–S58. doi.org/10.1016/j.jinf.2015.04.013.
16. Oliveiros, B., Caramelo, L., Ferreira, N. C. and Caramelo, F. (2020). "Role of temperature and humidity in the modulation of the doubling time of COVID-19 cases." *medRxiv*. doi.org/10.1101/2020.03.05.20031872.
17. Wang, C., Horby, P. W., Hayden, F. G. and Gao, G. F. (2020). "A novel coronavirus outbreak of global health concern." *Lancet*, 395 (10223), p. 470–473. doi.org/10.1016/S0140-6736(20)30185-9.
18. Wang, M., Jiang, A., Gong, L., Luo, L., Guo, W., Li, C., Zheng, J., Li, C., Yang, B. and Zeng, J. (2020). "Temperature significant change COVID-19 transmission in 429 cities." *medRxiv*. doi.org/10.1101/2020.02.22.20025791.
19. Ma, Y., Zhao, Y., Liu, J., He, X., Wang, B., Fu, S., Yan, J., Niu, J., Zhou, J. and Luo, B. (2020). "Effects of temperature variation and humidity on the death of COVID-19 in Wuhan, China." *Science of The Total Environment*, p. 138226. doi: 10.1016/j.scitotenv.2020.138226.
20. Calma, J. (2020). https://www.theverge.com/2020/3/26/21194647/the-covid-19 pandemic- is-generating-tons-of-medical-waste Accessed date: 5 April 2020.
21. Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H. and Cao, B. (2020). "Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study." *Lancet*, 395, p. 1054–1062.
22. Setti, L., Passarini, F., de Gennaro, G., Di Gilio, A., Palmisani, J., Buono, P., Fornari, G., Perrone, M.G., Piazzalunga, A., Barbieri, P., Rizzo, E. and Miani, A. (2020). "Evaluation of the potential relationship between Particulate Matter (PM) pollution and COVID-19 infection spread in Italy." Available online: http://www.simaonlus.it/wpsima/wp-content/uploads/2020/03/COVID_19_position-paper_ENG.pdf (accessed on 22 May 2020).
23. Ciencewicki, J. and Jaspers, I. (2007). "Air pollution and respiratory viral infection." *InhalToxicol*, 19 (14), p. 1135-46.
24. Chen, P. S., Tsai, F. T., Lin, C. K.; Yang, C. Y., Chan, C. C., Young, C. Y. and Lee, C. H. (2010). "Ambient influenza and avian influenza virus during dust storm days and background days." *Environ. Health*

Perspect, 118, p. 1211–1216.

25. Chen, G., Zhang, W., Li, S., Williams, G., Liu, C., Morgan, G. G., Jaakkola, J. J. K. and Guo, Y. (2017). "Is short-term exposure to ambient fine particles associated with measles incidence in China? A multi-city study." *Environ. Res*, 156, p. 306–311.
26. Pansini, R. and Fornacca, D. (2020). "Higher virulence of COVID-19 in the air-polluted regions of eight severely affected countries." *medRxiv*, Available online: https://www.medrxiv.org/content/10.1101/2020.04.30.20086496v1 (accessed on 20 May 2020).
27. Wu, X., Nethery, R. C., Sabath, M. B., Braun, D. and Dominici, F. (2020). "Exposure to air pollution and COVID-19 mortality in the United States." https://projects.iq.harvard.edu/covid-pm (accessed on 22 May 2020).
28. Tan, J., Mu. L., Huang, J., Yu, S., Chen, B. and Yin, J. (2005). "An initial investigation of the association between the SARS outbreak and weather: with the view of the environmental temperature and its variation." *J Epidemiol Community Health*, 59(3), p. 186-92. doi: 10.1136/jech.2004.020180.
29. Sun, Z., Thilakavathy, K., Kumar, S. S., He, G. and Liu, S. V. (2020). "Potential Factors Influencing Repeated SARS Outbreaks in China." *Int J Environ Res Public Health*, 17(5), p. 1633. doi: 10.3390/ijerph17051633.
30. Kudo, E., Song, E., Yockey, L. J., Rakib, T., Wong, P. W., Homer, R. J. and Iwasaki, A. (2019). "Low ambient humidity impairs barrier function and innate resistance against influenza infection." *Proc Natl Acad Sci*, 116(22), p. 10905-10910. doi: 10.1073/pnas.1902840116.
31. Wu, Y., Jing, W., Liu, J., Ma, Q., Yuan, J., Wang, Y., Du, M. and Liu, M. (2020). "Effects of temperature and humidity on the daily new cases and new deaths of COVID-19 in 166 countries." *Sci Total Environ*, 729: p. 139051. doi: 10.1016/j.scitotenv.2020.139051.
32. Ma, Y., Zhao, Y., Liu, J., He, X., Wang, B., Fu, S., Yan, J., Niu, J., Zhou, J. and Luo, B. (2020). "Effects of temperature variation and humidity on the death of COVID-19 in Wuhan, China." *Sci Total Environ*, 724, p. 138226. doi: 10.1016/j.scitotenv.2020.138226.
33. Méndez-Arriaga, F. (2020). "The temperature and regional climate effects on communitarian COVID-19 contagion in Mexico throughout phase 1." *Sci Total Environ*, 735, p. 139560. doi: 10.1016/j.scitotenv.2020.139560.
34. Iqbal, N., Fareed, Z., Shahzad, F., He, X., Shahzad, U. and Lina, M. (2020). "The

nexus between COVID-19, temperature and exchange rate in Wuhan city: New findings from partial and multiple wavelet coherence." *Sci Total Environ*, 729, p. 138916. doi: 10.1016/j.scitotenv.2020.138916.

35. Xie, J. and Zhu, Y. (2020). "Association between ambient temperature and COVID-19 infection in 122 cities from China." *Sci Total Environ*, 724, p. 138201. doi: 10.1016/j.scitotenv.2020.138201.
36. Yao, Y., Pan, J., Liu, Z., Meng, X., Wang, W., Kan, H. and Wang, W. (2020). "No association of COVID-19 transmission with temperature or UV radiation in Chinese cities." *Eur Respir J*, 55(5), p. 2000517. doi: 10.1183/13993003.00517-2020.
37. Bashir, M. F., Ma B, Bilal, Komal, B., Bashir, M.A., Tan, D. and Bashir, M. (2020). "Correlation between climate indicators and COVID-19 pandemic in New York, USA." *Sci Total Environ*, 728, p. 138835. doi: 10.1016/j.scitotenv.2020.138835.
38. Liu, M., Tan, S., Zhang, M., He, G., Chen, Z., Fu, Z. and Luan, C. (2020). "Waste paper recycling decision system based on material flow analysis and life cycle assessment: a case study of waste paper recycling from China." *J. Environ. Manag*, 255, p. 109859.
39. Varotto, A. and Spagnolli, A. (2017). "Psychological strategies to promote household recycling. A systematic review with meta-analysis of validated field interventions." *J. Environ. Psychol*, 51, p. 168–188.
40. Ma, B., Li, X., Jiang, Z. and Jiang, J. (2019). "Recycle more, waste more? When recycling efforts increase resource consumption." *J. Clean. Prod*, 206, p. 870–877.
41. Bir, B. (2020). https://www.aa.com.tr/en/health/single-use-items-not-safest-option-amidcovid-19/1787067 Accessed date: 5 April 2020.

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Shantijal-Its Bio Perspective And Present Scenario –A Preliminary Survey Report

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ARTICLE INFO
Received: 15.10.2021
Revised: 16.11.2021
Accepted: 17.11.2021

Key words:
Shantijal,
Bioperspective,
Present scenario,
Potability

ABSTRACT

In Hindu Religion, Shantijal is regarded as pure and sacred water which remains within the Mangal –ghat (Auspicious vessels) which is made up of Brass or Silver.

In this study, attempts have been made to know about the purity or potability of Shantijal and its bio perspective. During study, it has been studied that the plant parts or their products which are used in Mangal –ghat show antibacterial activity and also other medicinal importance. In the Mangal –ghat , different plant parts like Panchapallav [Am (*Mangifera indica* L. , Aswatha (*Ficus elastica* L.), bat (*Ficus benghalensis* L.), pakur (*Ficus rumphi* Bl.) , etc are kept inside within it. These components differ from place to place or by different opinions. As these above mentioned plants or plant parts are used for worshiped purpose, so by this way these plants will be conserved and bio-diversity conservation strategy will be fulfilled. So far, such type of study has yet been done. This type of study will be helpful for making the people aware about the purity of Shantijal; any adulterant of plant parts or products which are used in the auspicious ghat; or the process of using the original plant parts or products in definite proportion which will be helpful for purification of source water.

Aims and Objectives:

The main aims and objectives of this work are–

- To evaluate qualitative and quantitative information on the physical, chemical and biological characteristics of source and worshipped water(after treatment)
- To characterise present condition and trends of those water bodies from where source water is collected
- To inform people about the uses of original plant material and purity of "Shantijal"
- To make people aware about any adulterated plant parts or products which are being used in the "Auspicious pot" or

"Mangal ghat"

- To inform about the process of using the original plant parts or products in definite proportion which will be helpful for purification of source water
- To evaluate the presence of coliform in source water
- To know the source of coliform contamination
- To study other pathogenic bacteria present in water
- To compare potability between the source water and worshipped water
- As the above mentioned plants are used for worshipping purposes, so by this way the plants will be conserved and thus bio-diversity conservation strategy will be fulfilled.

Introduction:

In Indian subcontinent the Hindu are dominant and the oldest religion in the World is 'Hinduism". Since ancient periods, traditionally the Hindu people use a large number of plant species for worshipping different Gods and Goddesses. The important of plants which are used for religious purpose, has been described in different Vedas and also it could be seen in the hymns. In India , the Hindu are used to use many plant species which are associated with religious function, rituals and also in the celebration of festival. Such useful information has been recorded in the religious books and this knowledge has been transmitted from generation to generation.

During "Kalasha pooja"(Ghat pooja) Some of these useful plants and their parts are used. Kalasha is a metal pot and usually made of copper, gold or brass. It has a large base and small mouth, large enough to hold a coconut. The kalasha is usually filled with copper coins, grains, gems gold or a combination of these. According to (Kabyabhusan B.,1973; Bhattacharya S.M.,1904; Bhattacharya S.,1948) the coronet of 5 mango leaves is placed on the mouth of the Kalash in such a way that the tips of the leaves touch water in the kalasha. Also some other plants like Aswatha (*Ficus religiosa* L.), Bot (*Ficus benghalensis* L.), Pakar/Pakur (*Fucus virens* Aiton), Jagya dumur(*Ficus racemosa* L.), Haritaki (*Terminalia chebula* Reg.), Supari (*Areca catechu* L.), Elaichi (*Elettaria cardamomum* L. Maton), Labanga/Laung (*Syzygium aromaticum* L. Merrill & Pery), Haldi/Turmeric (*Curcuma longa* L.), Paan leaves (*Piper betle* L.) etc. are added to the kalasha or also known as "Mangal ghat". The above components may differ from place to place or by different opinion .The "kalasha" is also viewed as an "Auspicious pot" which brings good fortune and success. The coconut is sometimes wrapped with a red cloth and red thread is tied around the metal pot or Mangal Ghat. These entire arrangement of "kalasha"

is called as "Purna-kalasha".

There are Other interpretation of the "Purna kalasha" associate with the five elements or the chakras. The wide base of the metal pot represents the element Prithvi (Earth), the expanded centre – Ap (Water), neck of the pot – Agni(Fire), the opening of the mouth –Vayu (Air) and the coconut and the mango leaves – Akasha(Aether). The "Purna kalasha" is also worshipped at Hindu ceremonies like Griha pravesh(house warming), child naming, havan(fire sacrifice), vaastu(dosha) rectification and daily worship.

After obtains are offered to different deities, the purified water from the "kalasha" or "Mangal ghat" which is also known as "Shantijal" or "Water of peace" is taken and sprinkled on all devotees assembled around and the objects by chanting the Shanti mantra. During sprinkling Peace is recited three times: "Om Shanti, Om Shanti, Om Shanti"

In this study, attempts have been made to know about the purity or potability of Shantijal and also of the source water from where it has been collected and kept in "Mangal ghat" and after its worship which is known as "Shantijal". From literature survey, it has been studied that the plant parts or their products which are used in the "Mangal ghat" show some antimicrobial properties (Table No.- 3) and also show other medicinal importance (Table No.-4).

Area of Study:

Sample water for testing physical, biochemical and bacteriological parameters were collected from different puja mandaps and water sources of Midnapore ,Kharagpur town in paschim midnapore district and Howrah district, West Bengal. Kharagpur town and Midnapore town belongs to Paschim Medinipur district. The district Paschim Medinipur is located at longitude 87.318188 and latitude 22.431568. The area Kharagpur is located at longitude 87.231972 and latitude 22.346010 and the area Howrah is located at longitude 88.263641 and latitude 22.595770. The studied areas are situated 23m, 57m and 12m above Sea level respectively. (wikipedia, mapsofindia.com)

Materials and Methodology:

For the present study 10 numbers of water samples were collected mainly from ponds, wells and puja mandaps between the time periods of July 2019 to February 2020. Photographs of different plants and plant parts used in Mangal Ghat have been taken and identified by taxonomic literatures (Prain D., 1963; Bennet S.S.R., 1987,) . Common names have been confirmed by Local priests. Habit, Habitat and ecological also have been recorded during field collection which has been given in the Table No.-5.

Sample collection:

The water Sample was collected randomly

from puja mandaps, ponds, wells and river Ganga. Following equipments and materials were used for these purposes;

a) Gloves, b) Sterilized bottles with screw caps, c) Bottle carrying box, d) Water bucket

Samples were collected in sterilized sampling bottle (500ml),with proper precautions.

Methodology:

Detection of Total Coliform Bacteria:

Conventional MTF (Multiple Tube Fermentation) technique was used to determine the Most Probable Number (MPN) of coliform bacteria present in samples of both water from Mangal ghat (Treated- 'T') and source water (Source- 'S'). This technique involve s three main steps: Presumptive test, confirmed test and then complete test.

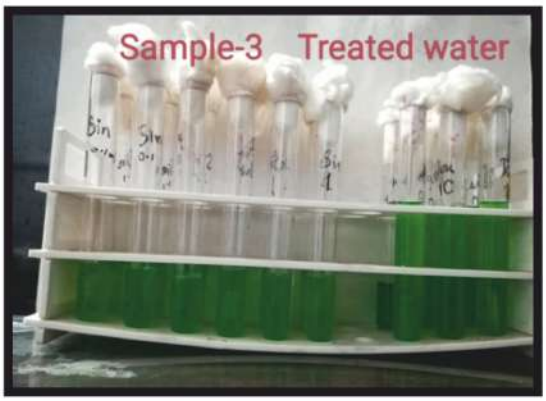
Results:

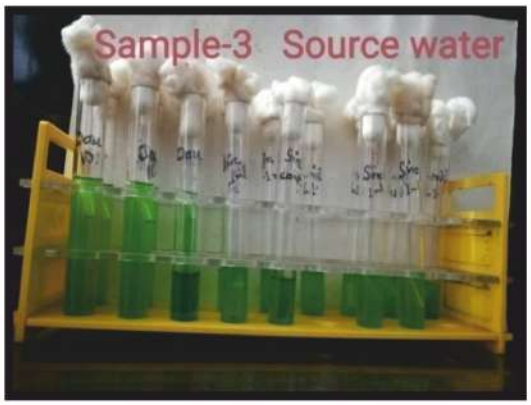
Table 1 (a) : TDS, pH , Temperature and MPN of different sources of water

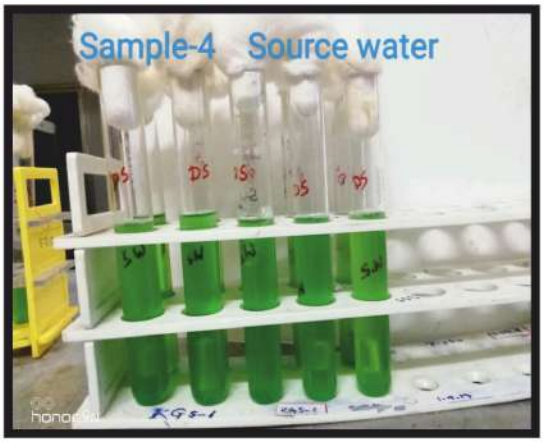
WATER TYPE		TDS (mg/l)	pH	TEMPERATURE (℃)	MPN
S1	S	358	9	20.4	350
	T	389	8	20	175
S2	S	113	7	20	31
	T	208	7	19.8	2
S3	S	156	7	22.6	110
	T	315	7	22.6	29
S4	S	135	9	20	910
	T	280	8	20	210
S5	S	289	8	22.7	750
	T	299	8	22.7	170
S6	S	363	7	21.3	345
	T	389	7	21.3	210
S7	S	120	8	20	38
	T	195	8	20	11
S8	S	140	7	22.2	175
	T	340	7	22.2	41

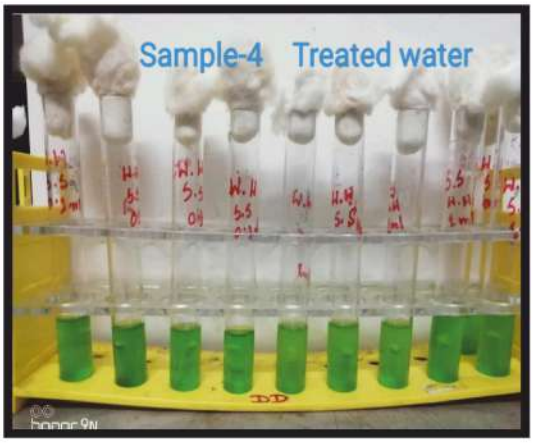
Table 1 (b) : MPN Index for coliform bacteria of different sources of water

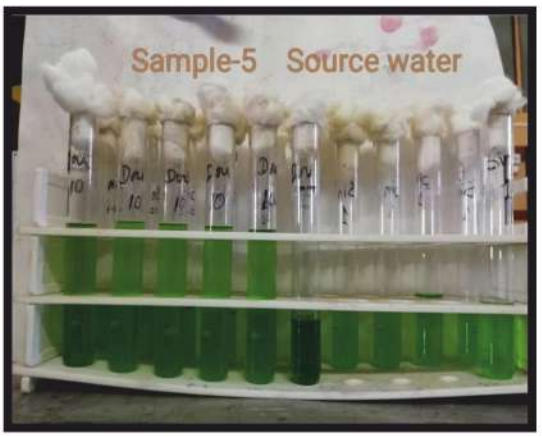
W Q I (W ater Q uality Index)	W ater Q uality
<50	Excellent
50-100	Good W ater
100-200	Poor W ater
200-300	Very poor w ater
>300	W ater unsuitable for drinking


Sample-3 Treated water


Sample-3 Source water


Sample-4 Source water


Sample-4 Treated water


Sample-5 Source water

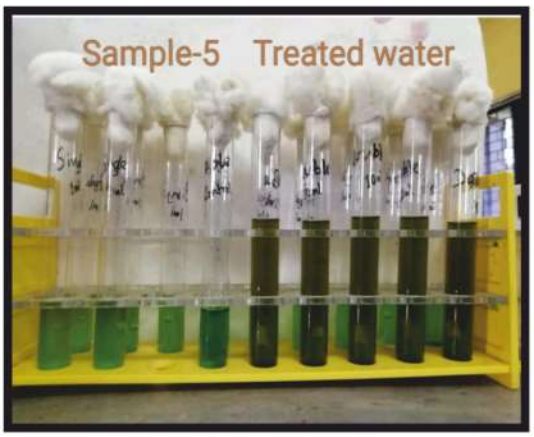

Sample-5 Treated water

Figure- MPN test using Brilliant Green Blue

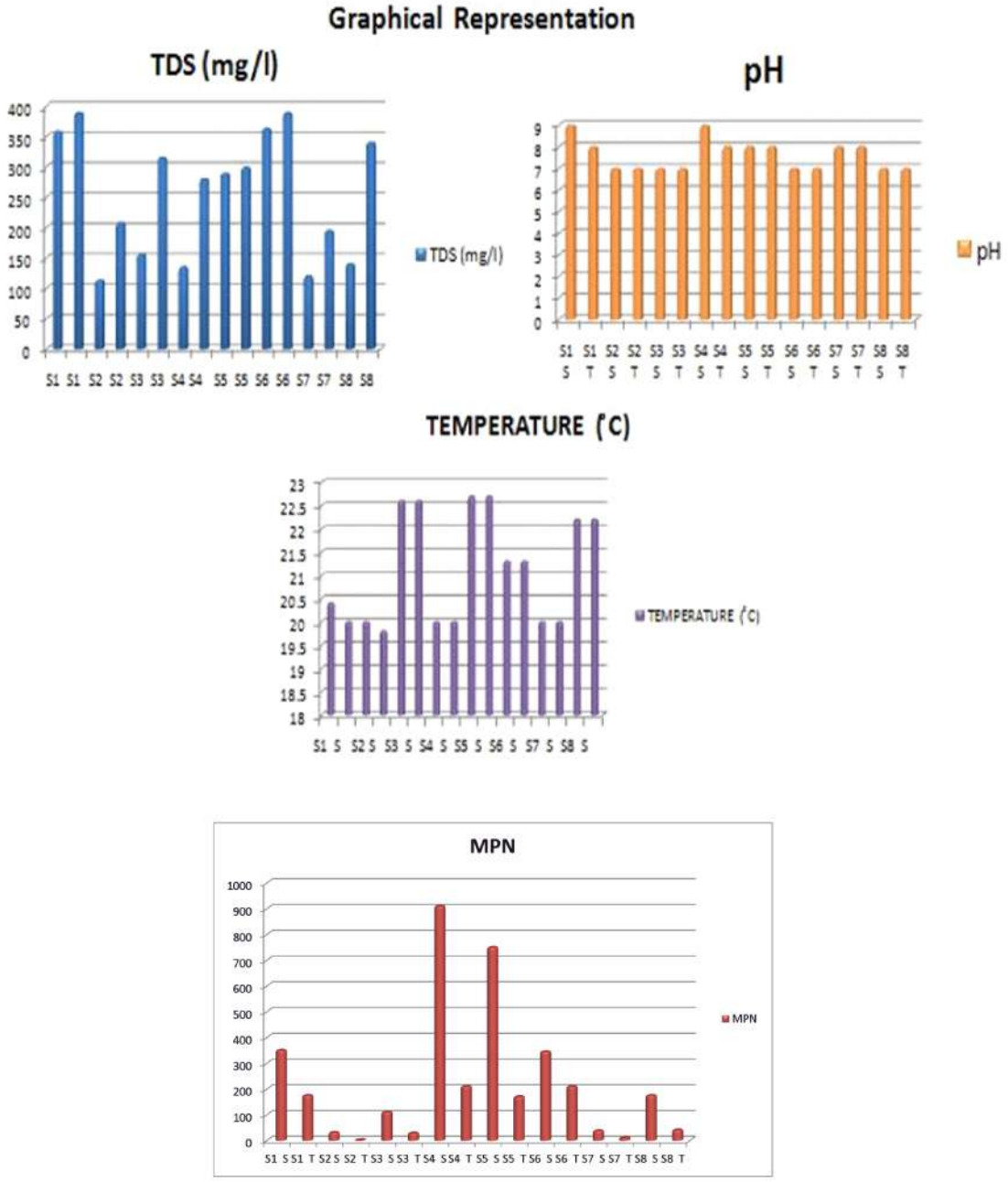


Fig: Graph showing values of MPN for different water samples

Table- 2: Chemical Ingredients of Plant Parts Used in Mangal Ghat :

Sl. No.	Common Name	Scientific Name	Family	Active Chemical Ingredient	Reference
1.	Supari	*Areca catechu L.*	Arecaceae	Phenolic compounds such as flavonoids, tannins, and alkaloids. Saponin, Glycoside, Coumarin,Trimer procyanidin, Dimer procyanidin, Catechin, Quercetin, Reducing sugar	Sari M.L. *et al.* (2020); Rajamani R.K. *et al.* (2016)
2.	Elichi	*Elettaria cardamomum* L.	Zingiberaceae	Alkaloid,Flavonoid, Saponin,Tanins, Phenolic compounds, Catechin,Quercetin, Gallic acid, Luteolin,Myrecetin	Ahmed H. *et al.* (2019); Moulai-Hacene Faiza *et al.* (2020)
3.	Bat	*Ficus benghalensis* L.	Moraceae	Alkaloids,sterols, ß flavanoids, phenols, tannins,saponins,ketones, flavonols , terpenoids, coumarins, esters, carbohydrates, serine protease.	Ahmad S. *et al.* (2011) ; Chaudhary S. *et al.* (2016)
4.	Dumur	*Ficus racemosa* L.	Moraceae	Alkaloids, Steroids, Flavonoids, Tannins, Saponin, Gum Reducing sugars, β-sitosterol, glauanol, hentriacontane,glauanolacetate, glucose, aspartic protease, racemosic acid	Mohiuddin A.K. & Lia S. (2020) ; Deep P. *et al.* (2013)
5.	Aswatha	*Ficus religiosa* L.	Moraceae	Carbohydrates saponins, phenols, flavonoids, tanins and terpenoid, n-octacosanol, methyl oleanolate, lanosterol, β-sitosteryl-Dglucoside, stigmasterol, lupen-3-one, quercetin, myricetin, kaempeferol, , aliphatic alcohols, amino acids and minerals.	Devanesan E.B. *et al.* (2018) ; Prakash V. *et al.* (2017)
6.	Pakar	*Ficus rumphii* Blume.	Moraceae	β-amyrin, β-sitosterol, flavonol glycoside, erol and flavonol glycoside.[26] The leaves contained stigmast-5-en3-yl acetate, 1-isopentyl3,4-dioxomethylene-2-phenol, 3-acetyl-2H-chromen-2- one and 3-(2-hydroxyphenyl)-1-(piperidin-1-yl) propan1-one	Ali M. *et al.* (2020)
7.	Aam	*Mangifera indica* L.	Anacardiacea e	Polyphenols, terpenoids, sugars, saponins,leucoanthocyanins, catechic , gallic acid, tannins, mangiferin, kaempferol and quercitin, Isoflavones, β-relemens, aromandrene, α-guaiene, β-endesmol, β-sitrosterol and β-campester	Okwu D. E. and Ezenagu V. (2008) ; Kabir Y. (2017)
8.	Labanga	*Syzygium aromaticum* L.	Myrtaceae	Tannins, saponins, phlobatanins, phenolics, reducing sugar, trepenoid, steroid, glycosides, alkanoids, flavonoids, eugenol, β-caryophyllene, eugenyl acetate, chavicol, eugenol, eugenyl acetate	Gaylor R. *et al.* (2014) ; Jimoh S. *et al.* (2017)
9.	Pan	*Piper betel* L	Piperaceae	The important antioxidants present in leaves are: flavonoids,tannis,saponins, alkaloids, terpenoids ets.	Aishwarya et al (2016)
10.	Halud	*Curcuma longa* L.	Zingiberaceae	Rhizome contains Curcuminoids which is a mixture of Curcumin, demethoxy curcumin and bis demethoxycurcumin	Niranjan A et al.,2008

Table- 3: Anti-Microbial Activity Of Plant Parts Used In Mangal-Ghat :

Sl.No.	Scientific Name	Used Parts	Anti-Microbial Activity Against	Reference
1.	*Areca catechu L.*	Nut	*Staphylococcus aureus, Streptococcus pyogenes., E. coli, S.mutans, S.salivarius, S. mitis, L. acidophilus, C.albicans, P.intermedia. Candida albicans.*	Cyriac M. *et al.* (2012) ; Nisreen J M Al-Bayati. (2016).
2.	*Elettaria cardamomum* L.	Fruit	*S. aureus, L. monocytogenes, Bacillus cereus, Pseudomonas aeruginosa , E.Coli, S. pyogenes, S. typhi*	Jazila El Malti *et al.* (2007) ; Kaushik Purshotam *et al.* (2010)
3.	*Ficus benghalensis* L.	Leaf, Stem Bark, Fruit	*S. aureus, B. cereus, A. viscosus, P. aeruginosa, E. coli,, S. typhi, L. acidophilus T. rubrum, C. albicans*	Hafiz Abdul Khaliq.(2017); Gaherwal S.(2013).
4.	*Ficus racemosa* L.	Fruit, Leaf	*E.coli, S. aureus, S. typhi, K. pnem, Bacillus subtilis, Pseudomonas aeruginosa. Aspergillus niger and C. albicans, Rhizopus Trichoderma.*	Thilagavath T. and Kathiravan G.,(2017); Pingale T. *et al.* (2019); Kingsley, J. (2014).
5.	*Ficus religiosa* L.	Bark, Leaf	*B.subtilis, E.coli, P.vulgaris, S.aureus, S.typhi, S.typhimurium, K.pnemoniae, P.aeroginosa. A.niger, P.chrysogenum, Penicillium gluacum. Paramecium.*	Parasharami Varsha A. *et al.* (2014); Manimozhi D.M. *et al.* (2012);
6.	*Ficus rumphii* Blume.	Bark, Leaves, Fruit	*Bacillus subtilis, Sarcina lutea,E. Coli, Pseudomonus sp.*	Haque Md. Islamul and Azad Md. Mohsin Uddin. (2018).
7.	*Mangifera indica* L.	Leaf, Seed,Pulp	*S aureus, S pyogenes, Streptococcus sp, Pseudomonas aeruginosa, Salmonella typhi, Enterobacter aeroginosa, E.Coli. Candida albicans, Aspergillus niger, Aspergillus fumigates, Aspergillus flavus* Herpes simplex virus (HSV) type 2, HIV and hepatitis B virus.	Awad A. *et al.* (2012); Disegha G. and Akani N. (2019). Parvez, G M Masud. (2016)
8.	*Syzygium aromaticum* L.	Flower Bud	*S. aureus, E. Coli, S. benthamianum, Salmonella typhimurium. Aspergillus flavus , Penicillium citrinum, P. viridicatum ,F. oxysproum, F.solani, F. monliforme, T. paradoxa, B. theobromae, R.solani Feline calicivirus* (FCV)	Okmen Gulten *et al.* (2018); Hamini-Kadar N. *et al.* (2014); Aboubakr Hamada A. *et al.* (2016)
9.	Pan	*Piper betel* L	*Staphylococcus aureus, Streptococcus pyogenes*	Deans,S.G and M.T. Baratta,1998; Balaji.K et al., 2011
10.	Halud	***Curcuma longa* L.**	*Escherechia coli, Staphylococcus aureus, Klebsiella pneumonia, Staphylococcus epidermidis*	Niamsa.N et al,2009

Table- 4: Medicinal Importance Of Plant Parts Used In Mangal-Ghat :

Sl.No.	Scientific Name	Used Parts	Medicinal Importance	Reference
1.	*Areca catechu L.*	Nut	1. Useful in the treatment of AIDS (HIV), Gastro Intestinal (GI) disorders, diabetes, Cardiac Disorders, High blood pressure. 2. Areca nut possess anti allergic activity. 3. Areca nut possess anti-helminthic/anti-parasitic activity. 4. Areca nut possess anti- inflammatory activity and Wound healing effect.	Tiwari, Dr and Talreja, Shreya. (2020); ICAR-Central Plantation Crops Research Institute.
2.	*Elettaria cardamomum* L.	Fruits	1.It is used as a powerful pleasant aromatic stimulant, carminative, stomachic and diuretic. 2.Seeds of E. cardamomum possess anti-inflammatory, analgesic and antispasmodic properties. 3. Powdered E. Cardamomum posses antihypertensive activity. 4. It can also be used to ease cigarette addiction.	Sharma S. *et al.* (2018); Korikanthimathm Vs *et al.* (2001).
3.	*Ficus benghalensis* L.	Leaf, Fruit , Bark, Root, Latex	1.It is very effective in various treatments such as dysentery, diarrhoea,diabetes,leucorrhoea, menorrhagia,nervous disorders, tonic and astringent. 2. The water extract of F. benghalensis bark has been reported to possess hypocholesterolaemic and hypolipidaemic effects. 3. Milky juice is used for pains, rheumatism, lumbago and bruises 4. It has antistress and antiallergic activity	Tripathi, Ruchita *et al.* (2015); Patel, Ramesh and Gautam, Piyush. (2014).
4.	*Ficus racemosa* L.	Leaf , Fruit , Seed, Bark, Latex, Root sap	1. The fruits are astringent, stomachic, refrigerent, dry cough, loss of voice, disease of kidney and spleen, astringent to bowel, styptic, tonic, useful in the treatment of leucorrhoea, blood disorder, burning sensation, fatigue, urinary discharges, leprosy, intestinal worms and carminative. 2. Latex is aphrodisiac and administered in haemorrhoids, diarrhoea, diabetes, boils, traumatic swelling, toothache and vaginal disorder. 3.Bark is highly efficacious in threatened abortion and also recommended in urological disorders, diabetes, hiccough, leprosy, dysentery and piles. 4. The leaves are good wash for wounds and ulcers. They are useful in dysentery and diarrhoea. The infusion of bark and leaves is also employed as mouth wash to spongy gums and internally in dysentery, menorrhagia, effective remedy in glandular swelling, abscess, chronic wounds, cervical adenitis and haemoptysis	Deep P. *et al.* (2013); Shah S.K. *et al.* (2016).

Sl.No.	Scientific Name	Used Parts	Medicinal Importance	Reference
5.	*Ficus religiosa* L.	Leaves, Bark	1. Leaves tied on bleeding wound are reported to immediately stop the flow of blood, it can cure fever and flu. 2. Paste of bark powder mixed with honey gives freshness to face by applied on skin 3. Putting of a few drops of leaves sap in nostrils have been reported to stop nose bleeding. 4. It contain highest amount of Serotonin which is responsible for its anticonvulsant effect	Sandeep *et al.* (2018); Singh Shailja and Jaiswal Shalini. (2014).
6.	*Ficus rumphii* Blume.	Bark,Latex, Fruit	1. It's fruit juice after mixing with turmeric, pepper and butter-fat considered efficacious against asthema. 2. The latex and fruits are emetic and anthelmintic, and used to treat itch. 3. The latex is given internally as a vermifuge and for the relief of asthma 4. Bark is used for snakebite.	Wikipedia ; Dogra Kuldip S. *et al.* (2015).
7.	*Mangifera indica* L.	Leaf,Stem,Seed,Plup	1. The aqueous and ethanol extract of leaves and stems of manghas activity against bacteria; Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Pseudomonas aeruginosa, Candida albicans, Enterococcus faecalis. 2. It has Anti-hemorrhagic, Anti-tetanus, Anti-inflammator, Antidiabetic effects. 3. It has e cardio protective effect. 4. Mangiferin significantly accelerated gastro intestinal tract	Parvez G M Masud. (2016); Khan Nazma *et al.* (2017).
8.	*Syzygium aromaticum* L.	Flower Bud	1. Clove is used extensively in dental care for relieving toothache, sore gums and oral ulcers. 2. Clove oil stimulates the circulatory system, clearing the mind and reducing mental exhaustion and fatigue. It has also been used to aid insomnia, memory loss, anxiety and depression. 3. Not only purifies the blood, but also aids in stabilizing blood sugar levels, and may have benefits for diabetic individuals. 4. he active essential oil in clove, eugenol, has been shown to act as a an effective platelet inhibitor, preventing blood clot.	Batiha G. E. *et al.* (2020); Kumar K *et al.* (2012).
9.	*Piper betel* L	leaves	Leaves are used for curing diseases like diabetes, hypertensions, boils and abscesses, obesity, wound healing, voice problems, conjunctivitis, constipations, headache, hysteria, itches, Leucorrhoea, swelling of Gum, rheumatism, abrasion, cuts and injuries, halitosis(Bad breath) etc.	Aishwarya et al (2016)
10.	***Curcuma longa* L.**	Rhizome	Rhizome is used to treat several diseases like Leucoderma, Scabies, Urinary discharges, inflammation, Ozonea, biliousness, dyspepsia, elephantiasis, small pox, swellins, boils, catarrh, diarrhoea, intermittent fever, dropsy, bronchitis, skin diseases etc. and leaf juice for the treatment of dysentery .	Paria N.D.(Ed),2005

Table- 5: Habit, habitat and ecological status

Sl No	Common name	Habit	Habitat	Ecological status
1.	Supari	Tree	Terrestrial	Frequent ,Cultivated
2	Elichi	Shrub	Terrestrial	Frequent, Cultivated
3	Bat	Thorny climber	Semi aquatic	Common, wild and Planted
4	Dumur	Tree	Terrestrial	Common, wild and Planted
5	Aswatha	Tree	Terrestrial	Common, wild and Planted
6	Pakar	Tree	Terrestrial	Common, wild and Planted
7	Aam	Tree	Terrestrial	Common, Cultivated
8	Labanga	Shrub	Terrestrial	Cultivated, not grown in this district
9	Pan	Herb, climber	Terrestrial	Cultivated
10	Halud		Terrestrial	Cultivated

Some plant extracts used in Mangal Ghat

Fig: Bat (*Ficus benghalensis* L.)

Fig: Aam (*Mangifera indica* L.)

Fig: Dumur (Ficus racemosa L.)
Syn.name- Ficus glomerata Roxb.

Fig:Aswatha (*Ficus religiosa* L.)

Fig:Pakar (*Ficus rumphii* Bl.)

Fig: Supari (*Areca catechu* L.)

Fig: Haritaki (*Terminalia chebula* Retz.)

Fig: Labanga (*Syzygium aromaticum* L.)

Fig: Elaichi (*Elettaria cardamomum* L.)

A PREPARED MANGAL GHAT

Pan-*Piper betel* L

Halud- ***Curcuma longa* L.**

DISCUSSION :

From the above table it is clear that the TDS (Total dissolve solute) value when compared among the source water and treated water, the value remains slightly higher in case of the treated water. Also after the use of different plant parts, their extracts or products etc. During worshipping of the water as "Shantijal" or "Water of peace" it is observed that the MPN (Most Probable Number) value is somehow reduced on treating the source water or so called "Shantijal" with different plant parts or their extracts which contain varieties of chemicals(Table no.-2). This can be seen clearly in the 'Sample 4' and 'Sample 5' water, where the source shows an MPN value of 910 and treated shows a vast decrease of 210. Also in 'Sample 5' the source shows an MPN value of 750 and the treated shows a value of 170 which means that the treating effect on the

source water shows antimicrobial properties which are able to reduce the effect of the coliforms present in almost polluted source water. Thus proper use of the plant materials, products or any kind of adulterants if used in a proper optimum amount may reduce the effect and action of microorganisms, the reduction process may not be completed but still effective to some extent.

CONCLUSION:

So, during the project study in the dept. of Botany ,funded by the Institution, Midnapore College (Autonomous) attempts have been made to know about the purity and potability of "Water of peace" or called "Shantijal" and also of the source water and its original source from where it has been collected.

In respect of present scenario, the source of water which is kept within Mangal ghat, is generally the pond or river water. Now-a-days this pond water is being polluted for using untreated poultry litter, Cow dung as fish feed by Farmers. So the source water is being polluted by different anthropogenic activities(De D. *et al*., 2021).

Though these plant products and other components used in Mangal Ghat have antimicrobial activities, but cannot disinfect this so called Peace water or pure water totally because of their less amount and presence of adulterant.

Therefore, we should keep in mind about the purity or potability of Shanti Jal, otherwise it will create health hazards. During survey it has also been noted that some are using tap water in exchange of Pond or River water as a source of water used in Mangal Ghat and there no such pollution are found. So, time has come to think about it. Further study is needed for anti bacterial assay by these plant extracts which can be used as water purifier. Besides, these plants also have other medicinal values (Table No.- 4). So in this perspective, these plants can be conserved as these are used for worshiping.

Acknowlwdgements: The Authors are grateful to Midnapore Collrge (Autonomous) for institutional funding. They are also grateful to Dr. Gopal Chandra Bera, Principal, Midnapore College (autonomous) for his constant help and inspiration.

We are also grateful to Sri Debasish Chakraborty,Purohit for giving some information in this regard.

References:

• Aboubakr Hamada A., Nauertz Andrew, Luong Nhungoc T., Agrawal Shivani, El-Sohaimy Sobhy A. A., Youssef Mohammed M. and Goyal Sagar M. In Vitro Antiviral Activity of Clove and Ginger Aqueous Extracts against Feline Calicivirus, a Surrogate for Human Norovirus. Journal of Food Protection, 2016, Vol. 79, No. 6, Pages 1001–1012

- Ahmad, S., Rao, H., Akhtar, M., Ahmad, I., & Iqbal, Z. Phytochemical composition and pharmacological prospectus of *Ficus bengalensis* Linn. (Moraceae)- A review. Journal of Medicinal Plants Research, 2011, 5.
- Ahmed, Hamza & Ramadhani, Ashraf & Erwa, Ibrahim. Phytochemical screening, chemical composition and antibacterial activity of essential oil of Cardamom. World Journal of Pharmaceutical Research.2019, 8. 1166-1175. 10.20959/wjpr20199-15504.
- Aishwarya et al. A review: Nutraceuticals properties of Piper betel(Paan). AJPCT. 2016. (4)(02), 028-041
- Ali Mohammad, Sultana Shahnaz, Mir Showkat Rasool and Rangari Vinod Damoji. Chemical constituents from the roots of *Elephantopus scaber*, and leaves of *Ficus rumphii* and *Iphiona scabra*. European Journal of Pharmaceutical and Medical Research. 2020,7(12), 493-500
- Awad, Amgad & MRP, Joseph & Mahmoud, Ismail & Abdelkareem, Abdelkareem & Al-Hakami, Ahmed & Hamid, Mohamed.Antimicrobial Activities of Seed Extracts of Mango (*Mangifera indica* L.). Advances in Microbiology. 2012. 2. 571-576.. 10.4236/aim.2012.24074.
- Batiha, G. E., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. *Syzygium aromaticum* L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. *Biomolecules*, 2020, *10*(2), 202. https:// doi.org/ 10.3390/biom10020202
- Bennet S.S.R. Name Changes in flowering plants of India and Adjacent Region, Triseas Publishers, Dehra dun, 1987
- Bhattacharya Shyamacharan, Bhaabaadev paddhati, 1948,1st Ed.
- Bhattacharya Surendra Mohan, Purohit Darpan, 1904, 1st Ed.
- Chaudhary S, Alok S and Verma. A: Phytochemical Screening and Chromatographic Evaluation of *Ficus Benghalensis* Leaves. Int J Pharm Sci Res; 2016, 7(8): 3522-32.doi: 10.13040/ IJPSR.0975-8232.7(8).3522-32
- Cyriac, Maria & Vidya, Pai & Varghese, Ipe & Shantaram, Manjula & Jose, Maji. Antimicrobial properties of *Areca catechu* (Areca nut) husk extracts against common oral pathogens. International journal of research in Ayurveda and Pharmacy. 2012. 3. 81-84.
- De Dulal, Mandal Debojyoti and Giri Sreyashri. Effect Of Anthropogenic Activities On The Water Quality Of Community Pond In Keshpur Block Of Paschim Medinipur - A Survey Report. IJEP ,2021,41 (4) : 403-411.
- Deans,S.G. and M.T. Baratta, Antimicrobial

and antioxidant properties of some essential oils, Flau, Fragrance, 1998.13.235-244

- Deep, Prakash & Singh, Amrit & Ansari, Mohammed Tahir & Raghav, Prashant. Pharmacological Potentials of *Ficus racemosa* - A Review. International Journal of Pharmaceutical Sciences Review and Research. 2013. 22. 29-34.
- Devanesan Enit Beena, Anand Arumugam Vijaya , Kumar Palanisamy Sampath , Vinayagamoorthy Puthamohan and Basavaraju Preethi. Phytochemistry and Pharmacology of *Ficus religiosa* . Systematic Reviews in Pharmacy, 2018, 9(1):45-48
- Disegha, Gabriel & Akani, Nedie. Antifungal activity of *Mangifera indica* leaf extracts on selected fungi. 2019. 4. 46-58.
- Dogra Kuldip S., Chauhan Sandeep and Jalal Jeewan S. Assessment of Indian medicinal plants for the treatment of asthma. Journal of Medicinal Plants Research. 2015. Vol. 9(32), pp. 851-862.
- Gaherwal S. Anti-Bacterial Activity of *Ficus benghalensis* (Banyan) Fruit Extract Against Difffrent Bacteria. International Journal of Microbiological Research, 2013, 4 (2): 177-179.
- Gaylor, Razafimamonjison & Jahiel, Michel & Thierry, Duclos & Ramanoelina, Panja & Fawbush, Fanja & Danthu, Pascal. Bud, leaf and stem essential oil composition of *Syzygium aromaticum* from Madagascar, Indonesia and Zanzibar. International Journal of Basic and Applied Sciences. 2014. 3. 10.14419/ijbas.v3i3.2473.
- Hafiz Abdul Khaliq.A review of pharmacognostic, physicochemical, phytochemical and pharmacological studies on *Ficus bengalensis* L.. Journal of Scientific and Innovative Research ; 2017: 6(4): 151-163
- Hamini-Kadar N , Hamdane F, Boutoutaou R, Kihal M and Henni J E. Antifungal activity of clove (*Syzygium aromaticum* L.) Essential oil against phytopathogenic fungi of tomato (*Solanum lycopersicum* L) in algeria. Journal of Experimental Biology and Agricultural Sciences; 2014; Volume – 2(5).
- Haque Md. Islamul and Azad Md. Mohsin Uddin. A Study on the Chemical Separation of *Ficus Rumphii* Blume Extract. Global Journal of Science Frontier Research: B Chemistry. 2018. Volume 18 Issue 1 Version 1.0.
- http://en.m.wikipedia.org (received on 16.08.08.21)
- https://www.mapsofindia.com/maps/westbengal/districts/medinipur.html (received on 16.08.08.21)
- http://www.thehindu.com (received on 16.08.08.21)
- ICAR-Central Plantation Crops Research Institute. Kasaragod- 671124, Kerala. Health

benefits of Arecanut.

- Jazila El Malti, Driss Mountassif and Hamid Amarouch. Antimicrobial activity of *Elettaria cardamomum*: Toxicity, biochemical and histological studies,Food Chemistry, 2007, Volume 104, Issue 4,Pages 1560-1568.
- Jimoh, Simiat & Arowolo, Lateefah & Alabi, Kazeem. Phytochemical Screening and Antimicrobial Evaluation of *Syzygium aromaticum* Extract and Essential oil. International Journal of Current Microbiology and Applied Sciences. 2017. 6. 4557-4567. 10.20546/ijcmas.2017.607.476.
- Kabir, Yearul & Shekhar, Hossain & Sidhu, Jiwan. Phytochemical Compounds in Functional Properties of Mangoes. 2017. 10.1002/9781119014362.ch12.
- Kabyabhusan Bhagabaticharan , Hindu kriya kalpadrum, 1937,1st Ed.
- Kaushik Purshotam, Goyal Pankaj, Chauhan Abhishek and Chauhan Garima. In Vitro Evaluation of Antibacterial Potential of Dry Fruit Extracts of *Elettaria cardamomum* Maton (Chhoti Elaichi). Iranian Journal of Pharmaceutical Research, 2010, 9 (3): 287-292.
- Kaveti Balaji , Lisa Tan, Sarania, Tan Sin Kaun, Mirza Baig. Antibacterial activity of *Piper betel* leaves.IJPTP. 2011.2(3), 129-132
- Khan, Nazma & Khushtar, Mohd & Ahmad, Nesar & Hasan, Noorul & Khan, Zafar & Idris, Sahar & Ahmad, Aijaz & Zishan, Mohd. Nutritional importance and pharmacological activity of *Mangifera indica*. World journal of pharmacy and pharmaceutical sciences. 2017. 6. 258-273. 10.20959/wjpps20175-8974.
- Kingsley, J. & Prashant, J & Tirkey, Manik & Chauhan, Ritika & Abraham, Jayanthi. Evaluation of antimicrobial activity of *Ficus Racemosa* Linn leaves extract. Intenational Journal of Pharmacy and Technology. 2014. 6. 5-7.
- Korikanthimathm, Vs & Prasath, D. & Rao, Govardhana. Medicinal properties of Elettaria *cardamomum*. J Med Aromat Plant Sci. 2001. 22/23.
- Kumar, K. & Yadav, Akhilesh & Srivastava, Shweta & Paswan, Shravan. Recent Trends in Indian Traditional Herbs *Syzygium Aromaticum* and its Health Benefits. J Pharmacogn Phytochem. 2012. 1.
- Manimozhi D.M., Sankaranarayanan S. and Kumar GS. Effect of different extracts of stem bark of *Ficus* sp. on multidrug resistant pathogenic bacteria. *Int J Pharm Sci Res*; 2012;Vol. 3(7): 2122-2129
- Mohiuddin, Abdul Kader & Lia, Sayra. PHYTOCHEMICAL SCREENING & BIOLOGICAL INVESTIGATIONS OF *Ficus racemosa*. 2020. 10.5281/zenodo.3970120.

- Moulai-Hacene, Faiza & Boufadi, Mokhtaria Yasmina & Keddari, Soumia & Homrani, Abdelkader. Chemical Composition and Antimicrobial Properties of *Elettaria cardamomum* Extract. Pharmacognosy Journal. 2020. 12. 1058-1063. 10.5530/pj.2020.12.149.
- Niansa.N and C. Sittiwet. Antimicrobial activity of Curcuma longa aquas extracts. J.Pharmacol.Toxicol.. 2009. 4(4),173-177
- Niranjan A.and Dhan Prakash. Chemical constituents and biological activities of turmeric(*Curcuma longa* L.) –A review- J. Food Sci Technol. 2008. 42(2),109-116.
- Nisreen J M Al-Bayati. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016. 7(6).282.
- Okmen Gulten , Mammadhkanli Mahabbat and Vurkun Mustafa. The antibacterial activities of *Syzygium aromaticum* (l.) Merr. & perry against oral bacteria and its antioxidant and antimutagenic activities. International Journal of Pharmaceutical Sciences and Research. ; 2018. Vol. 9(11): 4634-4641.
- Okwu Donatus Ebere and Ezenagu Vitus. Evaluation of the phytochemical composition of mango (*Mangifera indica* linn) stem bark and leaves. Int. J. Chem. Sci.: 2008, 6(2)
- Parasharami Varsha A., Vati Vindya, Rabade Bhakti and Mehta Urmil J. Recent Antimicrobial and Pharmacological studies in *Ficus religiosa* Linn. Int.J.Curr.Microbiol.App.Sci , 2014: 3(1): 461-475.
- Parvez, G M Masud. Pharmacological Activities of Mango (*Mangifera indica*): A Review . Journal of Pharmacognosy and Phytochemistry , 2016: 5(3): 01-07.
- Paschim Medinipur, West Bengal-Wikipedia (received on 16.08.08.21)
- Patel, Ramesh & Gautam, Piyush. Medicinal potency of *Ficus bengalensis*: a review International Journal of Medicinal Chemistry & Analysis. 2014. 4. 53-58.
- Pingale T, Duse P, Ogale S. Antibacterial and Antifungal Approaches of *Ficus racemosa*. Pharmacog J. 2019. 11(2):355-7
- Prain D. Bengal plants, Botanical Survey of India, Kolkata, 1963 ,Vol. I & II , Reprinted Ed.
- Prakash, Ved & Gandotra, Shivali & Kumar, Prateek & Singh, Nishtha. Phytochemical Screening and Antimicrobial Activity of *Ficus religiosa*. Journal of Pharmaceutical Sciences and Research. 2017. 9. 100-101.
- Rajamani, Ranjithkumar & Kuppusamy, K.Selvam & M, Shanmugavadivu & Devadass, Dr. Rajmohan. Preliminary Phytochemical Screening of Aqueous Extract of Betel Nut and Betel Leaves. International Journal of Biosciences and Nanosciences. 2016. 3. 14-18.

- Sandeep, & Kumar, Ashwani & Sepla, Dimple & Tomer, Vidisha & Gat, Yogesh & Kumar, Vikas. *Ficus religiosa*: A wholesome medicinal tree. Journal of Pharmacognosy and Phytochemistry , 2018: 7(4): 32-37
- Sari, L. M., Hakim, R. F., Mubarak, Z., & Andriyanto, A. Analysis of phenolic compounds and immunomodulatory activity of areca nut extract from Aceh, Indonesia, against *Staphylococcus aureus* infection in Sprague-Dawley rats. *Veterinary world*, 2020, 13(1), 134–140. https://doi.org/10.14202/vetworld.2020.134-140
- Shah Sunil Kumar, Garg Gopal, Jhade Deenanath, Pandey Harish. *Ficus racemosa* Linn: Its Potentials Food Security and Rural Medicinal Management (Review Article). J. Pharm. Sci. & Res. 2016, Vol. 8(5), 317-322.
- Sharma, Shveta & Sharma, Jagmohan & Kaur, Gurpreet. International journal of drug formulation and research Therapeutic uses of *Elettaria cardomum*. 2018. 10.13140/RG.2.2.12210.91848.
- Singh Shailja and Jaiswal Shalini. Therapeutic Properties of *Ficus regligiosa*. International Journal of Engineering Research and General Science, 2014, Volume 2, Issue 5.
- Thilagavath T. and Kathiravan G. Phytochemical Analysis and Antimicrobial Activity of Ethonolic Leaf Extract of *Ficus racemosa* Linn. Research J. Pharm. and Tech.; 2017: 10(2): 537-540. doi: 10.5958/0974-360X.2017.00107.X
- Tiwari, Dr & Talreja, Shreya. A Pharmacological And Medicinal Study Of Areca Palm And Nuts: An Overview. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2020. 11. 100-108. 10.33887/rjpbcs/2020.11.5.12.
- Tripathi, Ruchita & Kumar, Abhishek & Kumar, Sanjeev & Prakash, Satya & Singh, Anil. *Ficus benghalensis* Linn.: A Tribal Medicine with Vast Commercial Potential. Indian Journal of Agriculture and Allied Sciences. 2015. 1(3).
- Paschim Medinipur, West Bengal-Wikipedia (received on 16.08.08.21)

https://www.mapsofindia.com/maps/westbengal/districts/medinipur.html((received on 16.08.08.21)