The NEHU Journal

Vol. XVIII, No.2, July - December 2020

NEHU

ISSN. 0972 - 8406

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Editor: **Prof. S.R. Joshi**
Department of Biotechnology & Bioinformatics
NEHU, Shillong
Email : editornehujournal@gmail.com

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Editorial

Dear Researchers and Readers

We have been experiencing a "New Normal" in the midst of the unprecedented misery brought about by covid-19 pandemic. As we learn to live in the new normal, there has also been a sense of complacency that has crept in amongst the people with the covid fatigue visible all around. However, till the vaccine is developed and immunity is assured, we cannot be relaxed and complacent about the dreadful virus that has taken innumerable lives across the globe apart from causing economy stress. As we learn more about the virus and expect the vaccine to be available sooner than later, we have learnt more or less to live with the coronavirus disease. In this stressful time when our educational institutions are literally locked for research activities as a measure of safety and precaution, laboratory bench work has not happened since the announcement of the lockdown. In spite of the prevailing situation, I am grateful to researchers who have submitted their manuscript to our journal for consideration of publication. I take this opportunity to express my gratitude to the reviewers for their valuable suggestions and my editorial team for being there whenever I needed their help and assistance.

This volume comes with the collection of articles of multidisciplinary nature with a mix of papers drawn from Life Sciences, Human & Environmental Sciences and Social Sciences.

The present volume begins with an article on "**Induced polyploidization in *Brassica fruticulosa* - a wild relative of Brassicas as potential source for mustard aphid**" by Arun Kumar and Prashant Yadav who illustrate through their research how polyploidy is an important source for acquiring new genetic recombination and creating genetic uniqueness in plants achieved through genetic manipulation associated with the obtainment of some increased enviable traits of the plants providing them greater adaptability to various biotic and abiotic stresses as compared to its diploids counterparts.

Pradnya Kakodkar in the article entitled "**Oral health promotion for the indigenous population in India**" discusses the oral health problems existing

among the indigenous population of India and highlights the different oral health promotion methods to address the disease burden. The study is presented through the adoption of seven different integration methods that have been discussed.

The article on "**Climate change adaptation in mountain community of Mustang district, Nepal**" by Prakash Upadhyay describes how the ethnic Loba people of Mustang are experiencing climate vulnerability in terms of adaptation in agropastoral livelihood. He elaborates on how they have adjusted their agropastoral living with what nature has provided and are coping to the changing climate with customary adaptive measures which includes traditional system of landholding, collective labour and irrigation management, seasonal migration, spiritual connectivity and out-migration for the collective survival of the community amidst the threats of becoming a climate refugee.

Sunita Gehlot and Praveen Gehlot in their article "**Socio-ethnomycological validation of Maru Khumbhi (*Phellorinia herculeana*) occurring in Indian Thar Desert**" brings forth the information on Indian Thar desert being a treasure of trove of maru khumbhi scientifically known as ***Phellorinia herculeana*** and the local rural peoples having the knowledge to utilize this mushroom as food and medicine. They emphasize on the point that the socio-ethnomycological knowledge pertaining to the mushroom is scarcely documented.

In their article "**Phytotoxic assessment of some monoterpenes and their formulation with leaf extract of *Chenopodium ambrosioides***", Saroj Kumari Fagodia, Daizy Rani Batish and Harminder Pal Singh present the findings of the investigation on the phytotoxicity of eight monoterpenes belonging to two major groups, i.e. oxygenated monoterpenes (linalool, citronellol, citronellal, 1, 8-cineole) and monoterpene hydrocarbons (limonene, β-pinene, p-cymene, α-terpinene) and their formulation with *Chenopodium ambrosioides* leaf extract against *Cassia occidentalis* and concluded that some monoterpenes have great scope for the development of new weed control strategies and their further formulation improves efficacy of active compounds, reduced dose usage and save costs of application.

Lily Shylla, S.K. Barik and S.R. Joshi in their research work " **Impact assessment of heavy metal contamination on water quality of underground and open-cast**

coal mines" evaluate how mining of coal as one of the precious natural resources results in hazardous aftermath owing to the built up accumulation of heavy metals that are non-biodegradable and damage the environment. The study evaluates the impact of metal contamination on water from underground and open–cast mines by pollution indices approach in two different seasons.

In the article " **Death rituals and afterlife: The Ao-Naga boatman of the dead in Temsula Ao's poems and the Greek mythological character of Charon**" by Harpreet Kaur Vohra examines the Naga Ao belief of the boatman of the dead and ideas of the afterlife through the poems of Naga writer, Temsula Ao and compares it with the Greek boatman of the dead, Charon by analyzing how these beliefs intersect, and are these intersections larger commentaries on other death rituals and beliefs.

Melinda Nongbet Sohlang and Suktilang Majaw in the article "**Voltage dependent anion channel-mediated apoptosis: Its role in the pathogenesis of diabetic complications**" elucidate how excessive or insufficient apoptosis can lead to various diseases and highlight the case of diabetes enhanced cellular apoptosis associated with micro- and macro-vascular complications citing evidences which suggest the involvement of voltage-dependent anion channel (VDAC) towards increased apoptosis observed in diabetes.

I take this opportunity to thank the contributors for their submissions and the reviewers for their promptness in providing valuable comments. Suggestions and cooperation of the editorial members have always been a source of guidance and strength. I appeal to scholars to submit/continue submitting manuscript(s) for publication in future issues of The NEHU Journal.

Stay Safe
Prof. S.R. Joshi
Editor

ISSN. 0972 - 8406
The NEHU Journal Vol. XVIII, No.2, July - December 2020, pp. 1-11

Induced polyploidization in *Brassica fruticulosa* - a wild relative of Brassicas as potential source for mustard aphid tolerance

Arun Kumar , Prashant Yadav*

Directorate of Rapeseed-Mustard Research, Bharatpur -321 303, Rajasthan
*Corresponding author e-mail: aruncyto@gmail.com

Abstract

Polyploidy is an important source for acquiring new genetic recombination and creating genetic uniqueness in plants. Ploidy manipulation is generally associated with the obtainment of some increased enviable traits of the plants as well as also provides them greater adaptability to various biotic and abiotic stresses as compared to its diploids counterparts. In the present study, successful induction of autotetraploidy has been achieved through seedling treatment of colchicine in B. fruticulosa Cyr. subsp. fruticulosa (2n = 16 FF), a wild relative of cultivated brassicas. The diploid seedlings of B. fruticulosa were treated with different concentrations of aqueous colchicine using the cotton-swab method for 8 -12 hours for 2-3 days. The highest percentage of success was recorded in when the seedlings were treated with 0.2% cochicine for eight hours within two days. The synthesized plant showed remarkable enhancement in several morphological and floral characters making more robust. Induced tetraploid was cytologically distinguished from diploid by the occurrence of 32 chromosomes at diakinesis/metaphase-I with different combinations of univalent, bivalents and multivalent in the form of trivalents and quadrivalents. The anaphase I and II disjunction of bivalents/chromosomes was leading more or less regularly and equally to the formation of seeds from the synthesized plants. Significant enhancement in morphological traits as revealed in colchicine-induced plant and normal meiotic behaviour leading to a good seed set may ultimately result in providing the plant breeder with more variability, especially in mustard aphid crop improvement programmes of brassicas

Key words: Colchicine, Cytology, Meiotic behaviour, Quardivalents.

Introduction

Polyploidy is recognized as a prominent feature in the evolution of higher plants and adaptation, and polyploids have often been selected during the evolution of crop plants (Leitch and Bennet, 2008). From a plant breeder point of view, manipulation in ploidy is significant for genetic improvement in crop plants and often generates variants that may possess useful characteristics and by doubling the gene products it also provides a wider germplasm base for crop improvement studies. Indian mustard [*Brassica juncea*, (L.) Czern and Coss.] is one of most important oil seed crops of India and with yield

potential of 15–30 q/ha and 38–42% oil content. This crop fulfils approximately 27% of vegetable oil requirements of our country. India is considered to be one of the secondary centres of diversity for *B. juncea* where it is well adapted to different variable agroclimatic conditions of northern India (Sharma *et al.,* 2002).

However, this crop often encounters severe biotic and abiotic stresses emerging as a result of unprecedented change in environmental conditions at local, regional and global levels (Chopra *et al*., 1996). Amongst the biotic stresses, mustard aphid, *Lipaphis erysimi* (L.) Kaltenbach is a perpetual annual threat leading to productivity losses up to 70%, depending upon the severity of outbreak (Bandopadhyay *et al*., 2013). Normally the farmers depend on chemical pesticide/ insecticides primarily based on synthetic chemical insecticides for the control of pests and diseases. The use of chemical pesticides has caused a lot of environmental hazards apart from resistance developed by the pests to the chemical. These chemicals, besides aggravating environmental pollution, can also be toxic to friendly insects. Therefore, a resistant cultivar is a more sustainable and environment-friendly option. The development of an insect-resistant cultivar requires a heritable and transferable resistance (Stoner and Shelton, 1988). However, so far cultivated *Brassica* germplasm/cultivars has failed to provide source of tolerance/resistance against mustard aphid.

Fortunately, *Brassica* coenospecies has been bestowed with nearly 100 species and genera of wild and weedy relatives, serving as rich reservoir of genes conferring many agriculturally important traits. These species can be effectively utilized to introduce economically important traits to cultivated species as well as development of potential wide hybrids (Prakash, 2001; Kumar *et al*., 2013, 2015). Notably, *B. fruticulosa*, a wild relative of cultivated brassicas, which is endemic to the Mediterranean coast and can be a potential genetic source for crop improvement because it possesses resistance to cabbage aphid (*Brevicoryne brassicae*) (Cole, 1994a; Ellis and Farrell, 1995; Ellis *et al*. 2000) and a higher concentration of lectins was suggested to be the underlying mechanism of resistance in this species (Cole, 1994b). The insecticidal properties of this species are a promising method of biological control. Through introgressive hybridization, this trait may transferred to other important vegetable cultivars of the genus *Brassica*. The insufficient within-species variability can be addressed by the effective utilization of untapped genetic diversity in wild and weedy relatives of brassicas for breeding and crop improvement (Prakash, 2001).

Attempt to improve crop brassicas, through conventional breeding/intraspecific hybridization programme cannot be expected to genetic variants, since these approaches mobilize only a limited extent of variation. The alternative strategies which have great potential in brassicas to expand gene resources are nonconventional interspecific/ intergeneric hybridization, alteration in ploidy level, structural changes in chromosomes and artificial synthesis of new as well as naturally occurring species. Therefore, the present investigations were conducted with primary objective of to obtain polyploid mutant

genotypes by the implementation of colchicine in *B. fruticulosa* which were verified on basis of significant differences in various cyto-morphological traits of autotetraploid over its diploid counterparts. Thereby, resulting genome doubling or ploidy manipulation could be correlated with the enhancement of various economically important traits and utilization of synthesized autotetraploids in *Brassica* crop improvement programme for future exploitation.

Material and methods

The seeds samples were directly sown in earthen pots filled with peat, perlite and sand (1:1:1, v/v/v). Cotton swab method was employed for colchicinization. Sterilized cotton swabs immersed either in 0. 5, 0.1 or 0.2 % aqueous colchicine were placed on emerging apical tip between two cotyledonary leaves. To avoid an increase in the concentration, colchicine was added drop by drop at regular intervals on the cotton swabs with the help of sterilized syringe. Such treatment was done for 2-3 days for 3-4 hours per day.

The data on various morphological attributes of both diploid and colchicine-induced plants were recorded by physical measurements with centimetre scale or micrometre as the case may be. Length and breadth of stomata has been recorded for this young leaves were peeled off from its dorsal surface, stained with safranin for few minutes and washed thoroughly and mounted in glycerine, observed under microscope. Length and breadth of stomata was measured using ocular micrometer and their mean values were calculated.

For meiotic studies flower buds of an appropriate size were collected from selected mature plant and fixed on the spot in freshly prepared carnoy's fluid (ethanol : chloroform : acetic acid - 6 : 3 : 1), for a minimum of 24 hours at room temperature and subsequently stored in 70% alcohol at 10^0C. Anthers were squashed in 1% acetocarmine solution. On average 25 Pollen Mother Cells (PMC) were analysed at diplotene/diakinesis/metaphase I to estimate the range of chromosome associations and recombinational frequencies by chiasma analysis. At anaphase I/II on average15-20 cells were analysed to study the distributional pattern of chromosomes.

Results

Efficiency of colchicine treatment:

B. fruticulosa seedlings at cotyledonary leaves stage were treated with aqueous colchicine (0.5%, 0.1% and 0.2% v/v) for 2-3 days for 3-4 hours per day. The highest induction percentage (7.14%) of putative polyploidy were recovered with only in 0.2% aqueous colchicine (Counted as the number of colchicine – induced tetraploids recovered from total number of seedlings treated with aqueous colchicine) (Table 1).

Table 1. Efficiency of colchicine treatment in seedlings of *B. fruticulosa*

Colchicine Concentration (%)	Duration of treatment in hour (hr)	No. of days of treatment	No. of seedling treated	No. of seedling survived	No. of colchicine – induced tetraploids	Percentage of tetraploids
0.5	09	3	30	27	-	-
	12	3	30	25	-	-
0.1	09	3	30	24	-	-
	12	3	30	18	-	-
0.2	09	2	30	14	1	7.14
	12	2	30	19	-	-

Morphological observations:

Colchicine-induced tetraploid plant was robost from the initial stages of development and it was maintained till maturity. Plant height, Primary branches, Secondary branches per plant, length of petiole, length and width of leaflet, corolla length and width and siliqua length showed considerable increase in comparison with the corresponding diploid. The number of seeds per siliqua decreased in the synthesized colchicine-induced tetraploid plants. Among the various morphological characters' colchicine-induced tetraploid plant, showed considerable increase in all the morphological attributes accept seeds per siliqua which were less than diploid plant *B. fruticulosa* (Fig 1, Table 2).

Table. 2. Comparative morphological characters and pollen fertility of diploid and colchicine – induced autotetraploid of *B. fruticulosa*

Characters	Diploid 2n=16	Autotetraploid 2n=4x=32
Plant height (cm)	57.3	61.7
Primary branches per plant	5	8
Secondary branches per plant	7	14
Main raceme length (cm)	29.8	31
Length of petiole (cm)	2.0	2.4
Length of odd leaflet (cm)	7.3	11.5
Width of odd leaflet (cm)	6.8	9.4
Stomatal length (µm)	28.9	53.8
Stomatal Breadth (µm)	17.7	28.4
Corolla length (cm)	1.0	1.5
Corolla width (cm)	0.5	0.7
Siliqua length (cm)	3.2	3.5
Seeds per Siliqua	15	13
Pollen diameter (µm)	26.0	51.7
Pollen fertility (%)	97.6	65.5

Stomatal and Palynological observation:

Average length of stomata in autotetrapoloid plant was 53.8 µm, although it was 28.9 µm in diploids. Similar differences were observed in breadth as well which was 28.4 µm autotetrapoloid and 17.7 diploid µm (Figure 1, Table 2). Pollen diameter in autotetraploid and diploid plants was 26.0 and 51.7 µm, respectively. That clearly reveals the difference between pollen sizes in treated as well as non-treated plants. Thus, the majority of pollen grains in autotetraploids were bigger compared to diploids. A significant reduction in the pollen fertility of autotetraploids has been observed (Fig 1, Table 2).

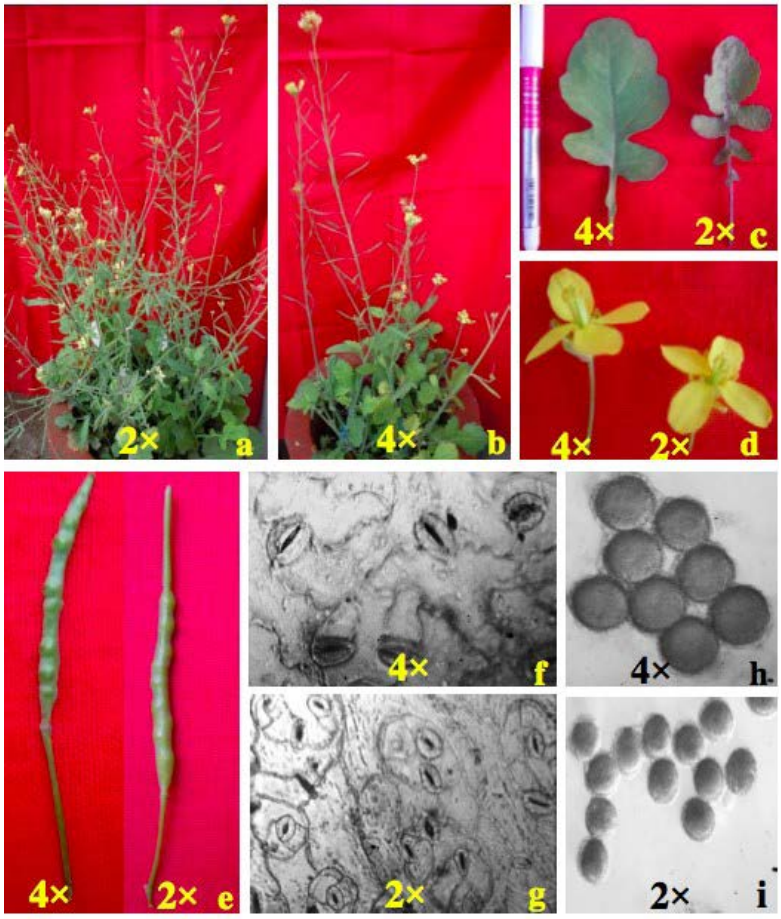


Figure 1: Comparison of morphological attributes in diploid and colchicine – induced tetraploids of *B. fruticulosa.* (a) Diploid plant; (b) Autotetraploid plant; (c) Leaf; (d) Flower; (e) Siliquae (Pods); (f)Stomata of diploid; (g) Stomata of colchcine induced autotetraploid; (h) Pollen grains of colchcine induced autotetraploid; (i) Pollen grains of diploid.

The above morphological observations clearly suggest that the induced autotetraploid plant showed robust, more compact bearing increased vegetative features and reproductive parts, and superior over its diploid counterparts.

Cytology of diploid and colchicine-induced tetraploid:

In diploid *B. fruticulosa* 25 Pollen Mother Cells (PMCs) analyzed at diakinesis/ metaphase I showed normal bivalent formation. The average number of chromosome associations was 8.50II + 0.60I. The equal distribution of chromosomes (8:8) was observed in all the 20 cells analysed at anaphase I (Figure 2 ; Table 3-4).

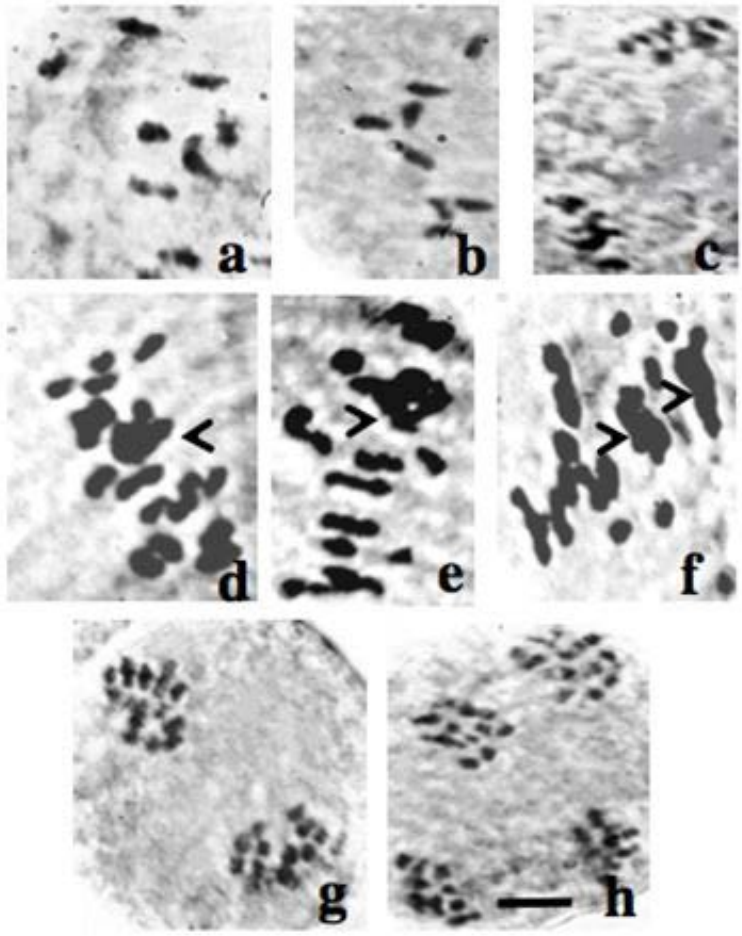


Figure 2: Documentation of cytological examinations in diploid and colchicine – induced tetraploids of *B. Fruticulosa*, Figure (a-c) *B. Fruticulosa* diploid (a) Diakinesis 8II; (b) Metaphase I 8II; (c) Anaphase I 8:8; (d-h) *B. Fruticulosa* induced autotetraploids (d) Diakinesis 1IV+9II+10I; (e) Metaphase I 2IV+10II+4I; (f) Metaphase I 2IV + 11II + 2I; (g) Anaphase I 16:16 (equal distribution); (h) Anaphase II (equal distribution); (Quadrivalents marked by arrow heads); Bar: 10 µm.

Table 3. Average number and range of chromosome associations at diakinesis/metaphase I in diploid and colchicine – induced tetraploids of *B. fruticulosa*

Ploidy level	No. of cells analyzed	Quadrivalents		Bivalents		Univalents	
		Mean	Range	Mean	Range	Mean	Range
Diploid (2x=16)	25	-	-	8.20 ± 0.59	6-8	0.70 ± 1.37	0-2
Tetraploid (4x=32)	25	2.40 ± 0.20	1-2	8.23 ± 0.25	7-10	3.30 ± 0.61	0-10

In autotetraploid plant in total 25 PMCs were analyzed. The average chromosome association determined for this plant was 2.40IV + 8.23II + 3.30I per cell, their range being 1-2, 7-10, 0-10, respectively. The maximum number of cell showed the occurrence of either one or two quadrivalents, besides other chromosome associations. 20 cells analyzed at anaphase I, the distribution of chromosomes (16:16) at anaphase I was found in 17 cells and three cells had unequal distribution of lagging two univalent (Figure 2, Table 3-4).

Table 4. Anaphase I distribution in diploid and colchicine – induced tetraploids of *B. fruticulosa*.

Ploidy level	No. of cells analysed	Chromosome distribution	No. of cells	Percentage
Diploid (2x=16)	20	8:8	20	100.00
Tetraploid (4x=32)	20	16:16 15:17 14:18	17 2 1	85.00 10.00 5.00

Discussion

Induction of polyploidy using colchicine and their cyto-morphological and genetic characterization has been a subject of immense interest among geneticists and plant breeders for a long time (Otto and Whitton, 2000, Reiseberg, 2001, Ramsey and Schemske, 2002, Mable, 2003) Polyploid plants often possess many superior agronomic traits of economic over the diploid plants. For example, ploypoids may have larger leaves and flowers, thicker stems and roots, darker green leaves, an increased width to length ratio of the leaves, increased cell size, leading to larger reproductive and vegetative organs a more compact growth habit and a higher tolerance to environmental stress. On the basis of above mentioned facts, herein present study the utility of various morphological and cytological analyses in distinguishing autotetraploids from diploids has been tested.

In the present study, some morphological features of the colchicine-induced autotetraploids *viz*. plant height, primary and secondary branches, length of petiole, length and width of leaflet, corolla length and width and siliqua length etc. showed an increase in dimension while few characters viz. seed per siliqua followed a reverse trend compare to the diploid plants (Kumar and Dwivedi, 2014).

In plants stomatal analysis and pollen diameter provide an efficient mean for characterization of polyploidy as these characters generally increased in polyploids. Kumar and Dwivedi (2014) produced tetraploid in *Brassica campestris* using colchicine treatment and primarily verified the status of tetraploid via stomatal analysis and found that stomatal analyses could be the most efficient criteria for ploidy confirmation. Similarly increase in the size of pollen diameter has been documented in present case Such observations have been previously demonstrated in a large number of plant species (Srivastava and Raina, 1983; Mishra, 1986; Bewal *et al.,* 2009).

In autotetraploidy, each chromosome theoretically represents itself four times, due to which mostly the quadrivalent associations would be expected. In the present study, meiotic analysis of induced colchiploid of B. fruticulosa showed low quadrivalent frequency and high bivalent average per cell. Several reasons have been put forward to explain the low/high quadrivalent frequency in tetraploids. Sybenga (1975) suggested that the frequency of bivalents and quadrivalents in tetraploids is dependent on the points of pairing initiation. When there is a single point of pairing initiation, bivalents are formed; when there are two points of initiation, both quadrivalents and bivalents are formed in equal frequencies; when the initiation points are more than two, quadrivalents are formed in high frequency as compared to bivalents. The average chromosome association determined for this plant was 2.40IV + 8.23II + 3.30I per cell, their range being 1-2, 7-10, 0-10, respectively. The maximum number of cell showed the occurrence of either one or two quadrivalents, besides other chromosome associations

In the induced tetraploid plant had some cells at anaphase I with unequal distribution and/or lagging bivalents or univalent. Tetraploids always show irregularities and these irregularities are considered to be due to the formation of multivalent associations. It was the common feature in colchitetraploids. The cytological causes, resulting in unviable gametes, include non-disjunction of multivalents, lagging chromosomes, univalents, non-viability and other abnormalities (Verma *et al*., 2017).

Induced Polyploidization of *Brassica fruticulosa* plant obtained in the present investigations are ideal for further, utilization in the hybridization programme with *B. juncea* and other related species for introgression of traits of economic importance, especially for mustard aphid in brassica crop.

Acknowledgments

We sincerely acknowledge Director, Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur - 321 303 (Rajasthan) India, for providing financial support and the facilities to carry out this research work.

References

Bandopadhyay, L., Basu, D. and Sikdhar, S. R. 2013. Identification of genes involved in wild crucifer *Rorippa indica* resistance response on mustard aphid *Lipaphis erysimi* challenge. *PLOS ONE,* 8: e73632.

Bewal, S., Purohit, J., Kumar, A. and Rao, S. R. 2009. Cytogenetical investigations in colchicines – induced tetraploids of *Cyamopsis Tetragonoloba* L. *Czech Journal of Genetics and Plant Breeding,* 45: 143-154.

Chopra, V. L., Kirti, P. B. and Prakash, S. 1996. Accessing and exploiting genes of breeding value of distant relatives of crop Brassicas. *Genetica,* 97: 305–312.

Cole, R. A. 1994a. Locating a resistance mechanism to the cabbage aphid in two wild Brassicas. *Entomologia Experimentalis et Applicata,* 71: 23-31.

Cole, R. A. 1994b. Isolation of a chitin binding lectin, with insecticidal activity in chemically defined synthetic diets, from two wild brassica species with resistance to cabbage aphid *Brevicoryne brassicae*. *Entomologia Experimentalis et Applicata,* 72: 181-187.

Ellis, P. R. and Farrell, J. A. 1995. Resistance to cabbage aphid (*Brevicoryne brassicae*) in six Brassica accessions in New Zealand. *New Zealand Journal of Crop and Horticultural Science,* 23: 25-29.

Ellis, P. R., Kift, N. B., Pink, D.A.C., Jukes, P. L., Lynn, J. and Tatchell, G. M. 2000. Variation in resistance to the cabbage aphid (*Brevicoryne brassicae*) between and within wild and cultivated *Brassica* species. *Genetic Resources and Crop Evolution*, 47: 395–401.

Kumar, A., Singh, B. K., Meena, H. S., Singh, V. V. Singh, Y. P. and Singh, D. 2015. Cytomorphological and molecular characterization of F_1 hybrids between *B. tournefortii* and *B. rapa*. *Cytologia*, *80*: 317-326.

Kumar, A., Singh, B. K., Singh, V. V. and Chauhan, J. S. 2013. Cellular and molecular evidences of synthesis of interspecific hybrids between *Brassica rapa* and *B. fruticulosa* through sexual hybridization. *Australian Journal of Crop Science,* 7: 849-854.

Kumar, G. and Dwivedi K. 2014. Induced Polyploidization in *Brassica campestris* L. (Brassicaceae). *Cytology and Genetics*, 48: 103–110.

Leitch, L. J. and Bennett, M. D. 1997. Polyploidy in angiosperms. *Trends in Plant Science,* 12: 470-476.

Mable, B. K. 2003. Breaking down taxonomic barriers in polyplody research. *Trends in Plant Science*, 8: 582-590.

Mishra, U. 1986. Cytogenetic studies in some members of *Papillionaceae*. Ph. D. Thesis Patna University, Patana.

Prakash, S. 2001. Utilization of wild germplasm of *Brassica* allies in developing cytoplasmic male sterility- fertility restoration systems in Indian mustard *Brassica juncea*, In: H. Liu and T.D. Fu (ed.). *Proceedings of International Symposium on Rapeseed Science,* Science Press, New York.

Ramsey, J. and Schemske, D. W. 2002. Neopolyploidy in flowering plants. Annual *Review of Ecology, Evolution, and Systematics*, 33: 589-639.

Reiseberg, L. H. 2001. Polyploid evolution: keeping the peace at genomic reunions. *Current Biology*, 11: R925-R928.

Sharma, G., Kumar, V. D., Haque, A., Bhat, S.R., Shyam Prakash and Chopra, V.L. 2002. *Brassica* coenospecies: a rich reservoir for genetic resistance to leaf spot caused by *Alternaria brassicae*. *Euphytica*, 125: 411–417.

Srivastava, P. K. and Raina, S. N. 1983. Cytogenetics of *Trphrosia.* VII- Colchicine induced polyploidy in eleven species. *La Cellule*, 74: 79-114.

Stoner, K. A. and Shelton., A. M. 1988. Influence of variety on abundance and within-plant distribution of onion thrips (*Thysanoptera: Thripidae*) on cabbage. *Journal of Economic Entomology*, 81:1190-1195.

Sybenga, J. 1975. *Meiotic Configurations*. Springer-Verlag, Berlin, Heidelberg, New York.

Verma, R.C., Dass, P., Shaikh, N. and Khah. M.A. 2017. Cytogenetic investigations in colchicine induced tetraploid of *Cosmos sulphureus* (Asteraceae). *Chromosome Botany*, 12: 41-45.

ISSN. 0972 - 8406
The NEHU Journal Vol. XVIII, No.2, July - December 2020, pp. 13-20

Oral health promotion for the indigenous population in India

Pradnya Kakodkar
Deputy Director Research, Dr. D. Y. Patil Vidyapeeth,
Sant Tukaram Nagar, Pune, Maharashtra- 411 018, India;
Email: pradnya.kakodkar@gmail.com

Abstract

This article details the oral health problems among the indigenous population of India and the different oral health promotion methods to address the disease burden. Seven different integration methods have been discussed, viz: Community outreach and Engagement model, School based model, Workforce model, Allied health professional model, Mobile Dental Service model, Integrated network and Oral health promotion through the use of traditional methods. Different methods individually or in combination can be implemented for the upliftment of the oral health of the indigenous people.

Keywords: Indigenous population, oral health, folk claim, oral disease, India.

Introduction

Indigenous population are communities that live within, or are attached to, geographically distinct traditional habitats or ancestral territories, and who identify themselves as being part of a distinct cultural group, descended from groups present in the area before modern states were created and current borders defined (WHO). They are culturally distinct societies and communities and there are approximately 476 million Indigenous people worldwide, in over 90 countries (World Bank, 2019). In India alone there are 700 tribal groups with a population of 104 million people, which is only second to the largest African tribes (Census Info, 2011)

The tribal population primarily inhabits rural and remote areas and is among the most vulnerable and marginalized section of the society (Narain, 2019) .While there is an increasing trend in the prevalence of chronic diseases such as diabetes, hypertension and cancer, associated with the use of tobacco, lack of physical activity and consumption of unhealthy diet among the Indian population, a similar increase is also being experienced by the tribal populations in India (Narain, 2019).

Oral Health among indigenous population

Oral health is the mirror of general health. Oral health problems either pose as symptoms or cause exacerbation of general health diseases. Dental decay, periodontal diseases, oral cancer, malocclusion and edentulousness are the important dental problems. Among them, periodontal disease has an association with certain systemic conditions, like atherosclerotic vascular disease, pulmonary disease, diabetes, pregnancy-related complications, osteoporosis, and kidney disease (Kane, 2017). Table 1 details the oral health status among the different tribes in India. It is evident from the table that there is high disease burden. But literature reports scanty data assessing the oral cavity of the indigenous population in India, in comparison to the greater number of tribes in India.

Barriers to Health care

Health care in general is not available to the majority of the tribal population due to the following reasons: Lack of accessibility to health facilities, non-availability of health staff in the health centres, non-availability of essential drugs and equipment's, lack of proper building facilities, difficult terrain and constraints of distance and time , lack of transport and communication facilities and traditional practices and superstitions [Local beliefs, customs, and practices have obstructed health care delivery to the tribals] (Bala & Thiruselvakumar, 2009). Particularly in oral health there is a gap of 80-20 (80% disease is prevalent and only 20% care facility is available). There is low priority for oral health in relation to general health diseases. Lack of professional and political advocacy for oral health and for redistributing resources and poor living conditions. Further, there is dominance of the restorative approach and no knowledge of prevention and importance of oral health.

Owing to the disease burden and the barriers encountered towards oral health care among the indigenous population, following oral health promotion methods have been enlisted.

Oral Health promotion through integration

Integrated care is defined as bringing together inputs, delivery, management and organisation of services related to diagnosis, treatment, care, rehabilitation and health promotion. Integration is possible at different levels:

1.Community outreach and Engagement model. Drs Abhay and Rani Bang engaged the people of the community at Gadchiroli and initiated Shodhgram (a village hospital). The main aim was to reduce Neonatal mortality rate and Infant mortality rate. The interested females from the village were trained as Arogydoot who provided homebased care. There was 70% reduction in the mortality rate. This further laid way for initiation of ASHA workers. The other activities undertaken were alcohol and tobacco control (Kakodkar, 2017).

2.School based model. It is possible to engage the children under one roof at the school. Also, any intervention would be easily accepted if given at the school, which is there area and the children are comfortable in that setting. The teachers can be engaged

for reinforcement and since they are the role models, it will be easy to mould the children. Preventive programs of oral health can be conducted at the school like: Oral health education, pit and fissure program, tobacco preventive and cessation program, toothbrushing program and oral screening program.

3.Workforce model. In this model, on one side, integration involves physicians and nurses screening—and treating—patients with dental needs and on the other side , it empowers dentists and other dental professionals to screen patients for non-dental medical problems such as throat cancer . This way, with whatever workforce available maximum health care is provided.

4.Allied health professional model. Allied health professionals like ASHA workers and the anganwadi workers are involved with the delivery of health or related services pertaining to the identification, evaluation and prevention of diseases and disorders. They carry out duties like aiding in breast feeding and complementary feeding, instituting immunization and supplementary nutrients, identifying childhood disability and childhood blindness. These allied workers can be trained and empowered to become oral health guides and also render oral health care preventive and educative services. Indigenous health workers are in a unique position to deliver culturally competent oral healthcare because they have a contextual understanding of the needs of the community. Literature has shown that empowering the anganwadi worker to become oral health guide can be beneficial (Kakodkar *et al.,* 2015).

5.Mobile Dental Service model Mobile and portable dental units are an effective and efficient way to take the sophisticated dental services to the doorsteps of the rural masses, school premises and urban slums through optimal utilization of dental institutions, dentists and dental auxiliaries (Ganavaidya, 2014). As per the Dental Council of India Guidelines, every dental college should adopt a village and possess one mobile dental van. There are 313 dental colleges in India (DCI) and hence with the mobile dentistry they will be able to provide services to the needy in the nearby remote areas. In a country like India where there is mal distribution of dentists, the mobile dental clinics can be used effectively as an alternative system of delivery of oral health care (Jayprakash *et al.,* 2002)

6.Integrated network: Two examples are cited here, in which service is provided by integrated network of the systemic and oral health.

a. **Immunization with Oral Examination:** Integrating the Oral polio drops activity with oral examination can be easily done by the allied health professional through a very simple Lift Your Lip program. After giving the drops the worker can check the oral cavity of the child for any oral disease or abnormality.

b. **The Fit for School (FIT)** programme integrates school health and Water, Sanitation and Hygiene (WASH) interventions. The handwashing and the toothbrushing group activity can be picked up from this program for implementation in the schools of the remote rural areas. This will involve a group of children who will wash their hands

and brush their teeth together. Doing this activity daily will inculcate a good practice among them. In areas, where there is shortage of water or no proper water system infrastructure the tippy tap system can be instilled. The tippy tap activity has helped the children in a remote area in India (Shukla, 2018). The tippy tap can be generated very easily. Materials used: 5-L unused oil can, roadside three wood sticks, and unused rope of variable length. The children would step on the stick lying on ground, which pulled the can downward and, in this way, the water from the can be used for handwashing. A soap is tied parallel to the can. The tippy tap and handwashing intervention was tested at an anganwadi in India for a period of two months and it was concluded that the children developed handwashing habit before every meal (Shukla, 2018). The group toothbrushing habit can also be effectively implemented in the school. Construction of the common water facility for group activity can be easily done (Figure 1).

7. Oral health promotion through the use of traditional methods

Use of traditional methods like cleaning the mouth with chewing sticks (mango, neem/ miswak, babul, guava, etc), tongue cleaning (coconut leaf, togue cleaner made of steel) and mouth washes (herbal products or Oil pulling) have shown good results in the oral cavity (Baloor, 2014) and are referred to as complementary or alternative medicine. Traditional medicine shows effects in reduction of decay, oral malodor, bleeding gums, dryness of throat, cracked lips and is effective for strengthening teeth, gums and the jaw.

Figure 1: Group toothbrushing program in school

Conclusion

Based on the integrated methods quoted in this article, different methods or combination can be implemented for the upliftment of the oral health of the indigenous people. Probably they disease and health condition which are peculiar to the tribes and also their unique medicinal methods. These people are less explored and are not open into the normal system. Efforts have to be taken to explore their way of living, their health profile and the folk claims.

Future Recommendation:

a. Strengthen the allopathy system of medicine in tribal areas with the extension of the three-tier system of village health workers, auxiliary nurse mid-wife and primary health centres.
b. Validate identified tribal remedies (folk claims) used in different tribal areas.
c. Encourage, document and patent tribal traditional medicines.
d. Promote cultivation of medicinal plants used by them.
e. Promote the formation of a strong force of tribal village health guides through regular training-cum-orientation courses.
f. Strengthen research into diseases affecting tribal and initiate action programs.

Table 1 Details of studies in the literature regarding the oral health problems among the indigenous people in India.

Author (Year)	Aim of the study (sample size)	Results	Conclusion
Kumar *et al*. (2009)	Assessed the oral health status of the Bhil tribal population of Southern Rajasthan (n=1590)	Mean DMFT and DMFS scores were 5.34 ±6.48 and 18.94±35.87. Shallow pockets were prevalent in 40% and deep pockets among 11.6%.	High prevalence of periodontal disease and poor oral hygiene.
Kumar *et al.* (2016)	Assessed the oral health status and treatment needs of Santhals residing in Dhanbad, Jharkhand. (n=921)	DMFT scores among 35–44 and 65–74 years old were 5.21 ± 2.34 and 7.42 ± 4.29 respectively. Used twigs to routinely clean their teeth.	Poor oral hygiene and periodontal status was seen among the tribes.
Valsan *et al.* (2016)	Assessed oral health status and treatment needs of Paniya tribe in Kerala(n=420)	76.9% had periodontal disease. Tooth brushing was reported by 55.5% . Paan chewing, with tobacco or without tobacco, habit was reported by 89.3%. Mean sextant of 2.30-2.55 had calculus. Caries prevalence was 40%. DMFT in the 35-44 years age group was 1.52±1.95 and in 65-74 age group it was 18.47 ± 13.10.	Oral disease burden is very high in Paniya tribes
Vijaykumar *et al.* (2017)	Assessed the oral health status and treatment needs among Sugali tribes.(n=820)	80% had tobacco habits. 49% had dental fluorosis. 10% had shallow pockets and 13% had deep pockets. Mean DMFT for males was 6.03 ± 2.35 and for females was 5.78 ± 2.55.	The tribes were characterized by a lack of awareness about oral health, deep-rooted dental beliefs, high prevalence of dental fluorosis, periodontal disease, dental caries and lack of previous dental care, high treatment needs, and limited access to oral health services.
Shrivastav *et al.* (2018)	Assessed the oral hygiene and periodontal status in the primitive tribe group of Bharias in Patalkot, Madhya Pradesh. (n=462).	OHIS= 2.56 ± 1.36. 35.4 % had periodontal pocket > 6mm and 27.9% people had attachment loss of 6-8mm	Higher prevalence of periodontal diseases and poor oral hygiene status in Bharia people can be attributed mainly to their difficult terrain, isolation, very low literacy level, socioeconomic status, and cultural practices.
Asif *et al.* (2019)	Assessed the oral hygiene practice, oral hygiene and periodontal status of two tribes residing in Bhadrachalam, Telangana (n=1000)	Koya group: OHI-S = 2.56 ± 0.82, 32.6% used toothbrush, 37.4% used twig and 17.6% finger. Mean sextant of 4.11 had calculus and 0.51 shallow pockets. Lambada group: OHIS=2.51 ± 0.93 , 46.4% used toothbrush, 30% used twig and 9.4% finger. Mean sextant of 4.37 had calculus and 0.58 had shallow pockets.	Oral hygiene practice was poor and periodontal status was compromised.

References

Asif, S.M., Naheeda, S., Assiri, K.I., Almubarak, H.M., Kaleem, S.M., Zakirulla, M. *et al.* 2019. Oral hygiene practice and periodontal status among two tribal population of Telangana state, India- an epidemiological study. *BMC Oral Health.* https//doi.org/10.1186/s12903-018-0705-1.

Bala, S.M. and Thiruselvakumar, D. 2009. Overcoming problems in the practice of public health among tribals of India. *Indian Journal of Community Medicine,* 34(4): 283-7.

Boloor, V.A., Hosadurga, R., Rao, A., Jenifer, H. and Pratap, S. 2014. Unconventional dentistry in India - an insight into the traditional methods. *Journal of Traditional and Complementary Medicine,* 4(3): 153-8.

Census Info. 2011. Office of Registrar General & Census Commissioner India. Ministry of Home Affairs, Government of India. https://www.censusindia.gov.in/2011census/HLO/HH14.html, accessed on 02/07/2020.

Dental Council of India (DCI). https://dciindia.gov.in, accessed on 07/07/2020.

Fit for School. https://www.fitforschool.international/fit-approach, accessed on 07/07/2020.

Ganavadiya, R., Chandrashekar, B., Goel, P., Hongal, S. and Jain, M. 2014. Mobile and portable dental services catering to the basic oral health needs of the underserved population in developing countries: a proposed model. *Annals of Medical Health Science and Research*, 4(3): 293-304.

Jayprakash, H., Veeresha, K.L. and Hiremath, S.S. 2002. Mobile dental clinics- A review. *Journal of Indian Association of Public Health Dentistry,* 2: 18-24.

Kakodkar, P., Matsyapal, C.K., Ratnani, N. and Agrawal, R. 2015. Anganwadi workers as oral health guides: An interventional study. *Journal of Scientific Research and Development*, 2: 337.

Kakodkar, P. 2017. Editorial: A tribute to Dr. Abhay Bang. *Journal of Dental Research and Review,* 4: 57.

Kane, S.F. 2017. The effects of oral health on systemic health. *General Dentistry,* 65(6): 30-34.

Kumar, T.S., Dagli, R.J., Mathur, A., Jain, M., Balasubramanyam, G., Prabhu, D. and Kulkarni, S. 2009. Oral health status and practices of dentate Bhil adult tribes of southern Rajasthan, India. *International Dental Journal,* 59(3): 133-140.

Kumar, G., Tripathi, R.M., Dileep, C.L., Trehan, M., Malhotra, S. and Singh, P. 2016. Assessment of oral health status and treatment needs of Santhal tribes of Dhanbad District, Jharkhand. *Journal of International Society of Preventive and Community Dentistry,* 6: 338-43

Narain, J.P. 2019. Health of tribal populations in India: How long can we afford to

neglect?. *Indian Journal of Medical Research,* 49(3): 313-316.

Shrivastav, A., Maurya, R., Shukla, C., Sahu, T., Chauhan, N., Azad, A. and Dubey, A. 2018. Oral hygiene and periodontal status in the primitive hidden tribe of Patalkot, a tribal area in Central India. *Journal of Indian Society of Periodontology,* 22(1): 55-59.

Shukla, M. 2018. Implementing innovative and sustainable methods to tackle grassroot level problems at anganwadi centers in Virpapura Village, Karnataka (India). *Journal of Dental Research and Review,* 5: 139-44.

Valsan, I., Joseph, J., Janakiram, C. and Mohamed, S. 2016. Oral Health Status and Treatment Needs of Paniya Tribes in Kerala. *Journal of Clinical and Diagnostic Research.* doi: 10.7860/JCDR/2016/21535.8631.

Vijayakumar, N., Rohini, C., Reddy, C., Sunkari, M., Kumar, S. and Malar, C.I. 2017. Assessment of Oral Health Status and Treatment Needs among Sugali Tribes in Telangana Region: A Cross-Sectional Study. *International Journal of Oral Health Medical Research,* 3(6): 21-26.

WHO.Indigenous population. https://www.who.int/topics/health_services_indigenous/en/#:~:text=Indigenous%20populations%20are%20communities%20that,were%20created%20and%20current%20borders , accessed on 15/06/2020

World Bank. Indigenous people. https://www.worldbank.org/en/topic/indigenouspeoples, accessed on 15/06/2020.

ISSN. 0972 - 8406
The NEHU Journal Vol. XVIII, No.2, July - December 2020, pp. 22-27

Socio-ethnomycological validation of Maru Khumbhi (*Phellorinia herculeana*) occurring in Indian Thar Desert

Sunita Gehlot[1], Praveen Gehlot[2]*

[1]Department of Education, Lucky Institute of Professional Studies, Jodhpur, Rajasthan-342 003
[2]Department of Botany, Jai Narain Vyas University, Jodhpur, Rajasthan- 342 001
*Corresponding author: drpg73@rediffmail.com

Abstract

Indian Thar desert is treasure of trove of maru khumbhi scientifically known as Phellorinia herculeana. Local rural peoples have the knowledge to utilize this mushroom as food and medicine. However, socio-ethnomycological knowledge pertaining to the mushroom is scarcely documented. Therefore, a survey was conducted for socio-ethenomycological validation of neutraceutical and pharmaceutical value of this wild edible mushroom. A survey was conducted and response of people of different age groups were documented from Barmer, Jaisalmer and Jodhpur districts of Western Rajasthan with the help of a questionnaire containing 22 questions related to various aspects of the mushroom. Survey report revealed that rural folk hunted this mushroom for nutritional as well medicinal purposes during rainy seasons and the excess quantity of the mushroom is sold in the market to earn extra income. The rural people are not aware of sustainable use and conservation of the mushroom. The present study is of significance towards acquired ethnic information and scientific validation of wild mushroom P. herculeana.

Keywords: *Phellorinia herculeana*, Ethnomycology, Sociobiology, Indian Thar desert

Introduction

Desert wild edible mushroom *Phellorinia herculeana* commonly known as 'maru khumbhi' grows on the sandy soil or on sand dunes during the rainy season (Gehlot and Singh, 2015a). It is hunted by local rural populace for the food and medicinal purpose (Gehlot, 2016). *P. herculeana* (maru khumbhi) is only edible gastroid fungus growing in the semi-arid and arid area of Rajasthan (Gehlot and Singh, 2015a). The fruiting body of this mushroom appears individually or in groups during the rainy season. It is usually grown on sandy and sandy loam soil of the sand dunes (Gehlot and Singh, 2015b; Solanki *et al.,* 2016). It is morphologically characterized by white to cream color sporophore with a size of 8 to 12 cm in length. The sporophore consists of a long, thick stipe and round to obclavate pileus. Pileus composed of an outer multilayer sterile wall (peridium) and an inner fertile portion called gleba fully loaded with basidiospores (Gehlot, *et al.,* 2016).

Rural folks of Indian Thar Desert, Rajasthan have different opinions regarding

the application of desert mushroom with special reference to use as food, nutritive value, medicinal purpose, myths and/or belief. Therefore, socio-ethnomycological study was undertaken to sum up indigenous information for scientific documentation/validation.

Materials and Methods

A survey was conducted at gastroid mushroom *P. herculeana* occurring site in Indian Thar desert, Rajasthan. A questionnaire containing 22 questions related to vernacular names, season of occurrence, habitat of growing, method of collection, nutritional value, edibility, culinary method, medicinal properties, how to use the mushroom as medicine, marketing of mushroom, local vendor, price of mushroom, who is consumer, preservation method and other information that rural folks want to share information related to it were prepared for the study. The question paper was given to local people for their responses. Ten people (5 men and 5 women) of different age groups were selected from Barmer, Jaisalmer and Jodhpur districts of Western Rajasthan. A total of 100 question papers were employed for the survey. The data were analyzed through the evaluation and interpretation of answers. Personal interaction and interview with some local persons were also composed. Photography and video were also taken as verification of ethnic acquaintance. Results were tabulated, statistically analyzed and conclusions were drawn as per the method suggested by workers (Kumari *et al.,* 2012; Mridu and Atri, 2015).

Results (Sociobiology)

The rural people of districts of Indian Thar Desert *viz*., Barmer, Jaisalmer and Jodhpur were asked to respond to the following questions related to wild mushroom *P. herculeana*:

Q 1. What is the local name of mushroom given in this photograph?
Ans. 100% people replied it as “maru khumbhi”.

Q 2. What is the scientific name?
Ans. 100% of rural people said they don’t know about it.

Q 3. Is it edible or poisonous?
Ans. 86% of people replied, it is edible, 6% of people replied poisonous while 8% of people replied as don’t know.

Q 4. Where is it growing?
Ans. 95% of people said it is grown on sand dunes and sandy soil during monsoon (rainy) season.

Q 5. When do you collect it?
Ans. 75% of people said it is collected during the rainy season.

Q 6. Who collect wild mushroom?
Ans. Any family member (men, women and children) collect the mushroom from agricultural sites and sand dunes during farming and animal grazing. It is a traditional

practice.

Q 7. Have you applied any specific method for the collection of mushroom?

Ans. People replied that they don't know any specific method for collection but they hunt it by direct pulling out from soil.

Q 8. **Who is selling this mushroom in the market?**

Ans. Mostly the mushroom is collected for domestic purposes. If it is in excess quantity then the grazers sell it to local vendors at the rate of Rs. 100-200/- per kilogram for household income. Local vendors sell the mushrooms to the district vegetable market at about Rs. 400-500/- per kilogram.

Q 9. Who is the consumer of wild mushrooms?

Ans. Generally urban people purchase the mushroom to prepare shahi vegetable and special dishes during rainy days. Standard hotels are also good buyers of this mushroom and serve the mushroom as a special vegetable with high prices. This is considered as royal cuisine. Some foreigners also demand this mushroom as regional specialty.

Q 10. Do you eat wild mushrooms?

Ans. 65% of people said they eat this wild mushroom.

Q 11. Which kind of dishes cook (culinary) in the house?

Ans. 80% of people said that it is used to cook vegetables (Sabji), 10% of people said they eat it directly after removal of the upper layer and soil particles.

Q 12. How does it taste?

Ans. It is very delicious and tastes like cheese. Some people said it is just like a boiled egg.

Q 13. How many days can it be used after harvesting?

Ans. It is traditional practice that it can be used for 2-3 days after harvesting. The mature mushroom having coloured powdery mass is not consumed to avoid any toxicity.

Q 14. How can it preserve for off-season use?

Ans. Some ethnic persons cut mushrooms in small pieces and dried them on the roof of the house in sunlight. These dried pieces are used as a dry vegetable and dry powder is applied on the wounds for healing.

Q 15. How do you prepare a recipe?

Ans. Young mushroom is washed with luke warm water for removing soil particles and contamination of other dust adhered on the surface of pileus and sporophore. It is cut into small pieces followed by shallow frying with usual ingredients including, onion, garlic and red tomato. Red chili powder, coriander powder, turmeric powder and salt are added as spices according to the taste. Some water is also added for proper cooking of mushroom pieces and gravy. Coriander leaves are added for flavor and garnish of the dish.

Q 16. Do you know any medicinal application of this mushroom?

Ans. People suggested several medicinal applications of the mushroom for example, 90% of people said it is used as healthy food, 45% of people said they eat it for energy, 35% of people said it is used for the strengthening of bones. 18% of people said it is used for healing bone crack. 40% of people said it is applied externally on wounds as healing agents and antimicrobial agents. 8% of people said it is used for the treatment of skin disease.

Q 17. How do you use it for medicinal purposes?

Ans. Both fresh and dried mushroom is used as medicine. Fresh mushrooms are directly given to a person suffering from small fractures in the bone. Dried powder of spores is directly applied as ointment on the wound. Traditional herbal doctors recommended it to diabetic and heart disease patients. Some (2-3) people said that it is also given to camel suffering from leg bone fracture.

Q. 18. Do you have any knowledge about using this mushroom in heart disease and diabetes

Ans. Yes, we are using this mushroom to cure heart and diabetes disease.

Q 19. Are you aware of any myths or beliefs regarding mushroom?

Ans. Villagers believed that the growing of mushroom is connected with the lightning flash of the sky during the rainy season. Some people said that the mushroom seeds (Spores or inoculums) came with rains and grow on sand dunes. Some people said that spores are germinated by the effect of electrical discharge during the lightning.

Q 20. Are any myths that Maru khumbhi grows only at the site of snakes resided?

Ans. Maximum people replied that there is not any myth that Maru khumbhi grows only at the site of snakes resided.

Q. 21. How do you know about this knowledge?

Ans. They replied that they got the information from their parents and grandparents.

Q. 22. Is any literature available regarding information about the application of this mushroom?

Ans. No, we have no idea about any literature having the information related to this mushroom.

Discussion

As was outcome during the present socio-ethenomycological survey, *P. herculeana* is known to have various applications. Normally it is cooked as a vegetable due to nutritional value and delicacy. Doshi and Bohra (2000) analyzed the sporophore of *P. herculeana* contains 23.30% crude protein, 9.39% carbohydrates and 1.77% lipid and other nutritive value. The survey report revealed that it is given to people for extra nutrient supplement especially calcium for early recovery of bone crack. Jandaik (1976) studied nutritive elements values of *Phellorinia* and found that it is a rich source of calcium. Singh (1994) also reported that the local rural people used *P. herculeana* for

bone cracks due to its high contents of calcium. The survey report also validates that the dry powder of mature mushroom is used as medicine to heal the external wounds. Panwar and Purohit (2002) studied that *P. herculeana* exhibited very strong antimicrobial activity against human pathogenic bacteria *Pseudomonas aeruginosa* and *Proteus mirabili*s.

Survey revealed that maximum people believed that this mushroom has the potential to cure heart and diabetes disease. This belief is due to fact that mushroom contains potassium that is directly linked with heartbeat and pulse rate while mushroom contains less amount of sugar and a high amount of protein which is beneficial for patients suffering from diabetes. Doshi and Bohra (2000) analyzed the nutritional status of the fruiting body of *P. herculeana* and confirmed the presence of the high amount of different essential amino acids (Lysine, Leucine, Tyrosine, Tryptophan, Phenylalanine and Histidine) with minerals (0.42%, Ca, 0.09% Mg, 0.65% P, and 98% K). During the investigation, it was noticed that this mushroom may be a good source of earning money for poor villagers. It can be collected from the large barren sand dunes and can be sold in the market. Local vendors also sell it in the streets of the city. Gehlot (2016) also stated that women and children of this region are major collectors of mushroom and sell it in the market to increase their income. Gehlot and Singh (2015a) reported that *P. herculeana* is collected from the arid region of Rajasthan and is sold in the other state of India. Due to high price and magnificent demand in market and availability only in monsoon season, some young people took startup to collect natural growing mushroom with the help of daily workers and transfer to the city vegetable market. It is also alarming that collection of mushroom is done in an unscientific manner that may be dangerous to their natural growth and may result in to extinction. Therefore, the conclusion remark that the collection of *Phellorinia* for business purposes should be banned and a conservation policy must be drawn for the protection of *Phellorinia* mushroom. Awareness campaigning is also required for safe and long-lasting use of this mushroom. The state government, forest department and gram panchayats should have to take action immediately through awareness programs to educate rural people toward conservation of mushroom germplasm. The present study may be useful in documenting the indigenous socio-ethnomycological knowledge of Indian Thar Desert.

Acknowledgement

Corresponding author acknowledge Head, Department of Botany, JNV University for laboratory facilities and co-author thankful to Lucky Institute of Professional Studies, Jodhpur for survey and analysis support.

References

Doshi, A. and Bohra, B. 2000. Studies on the gastromycetous fungus *Phellorinia inquinans* (Berk.). *Final project report, ICAR project no. PG/Gr.II/ICAR/14/95-96.PP.81.*

Gehlot, P. 2016. Edible gastromycetes mushrooms of Thar Desert: New economic source for rural upliftment. *Mushroom Research*, 25: 55-56.

Gehlot, P. and Singh, S.K. 2015a. Diversity of Gastromycetes flora in Indian Thar Desert. *Indian Journal of Tropical Biodiversity*, 23: 74-77.

Gehlot, P. and Singh, S.K. 2015b. Soil Characterization of *Podaxis pistillaris* and *Phellorinia inquinans* natural growing sites in Indian Thar desert. *Indian Journal of Tropical Biodiversity*, 23: 82-84.

Gehlot, P., Pathak, R. and Singh, S.K. 2016. *Phellorinia*: A Under exploited Mushroom. *Indian Journal of Tropical Biodiversity,* 24: 1-7.

Jandaik, C.L. 1976. Nutritive value of *Phellorinia inquinans* Berk-an edible Gastromycetes. *Indian Journal Mushroom*, 3: 5-7.

Kumari, B., Atri, N.S. and Upadhyay, R. C. 2012. Culinary status and sociobiology of Termitophilus and Lapiotoid mushrooms of North West India. *World Journal of Agariculturral Science,* 8(4): 415-420.

Mridu and Atri N.S. 2015. *Podaxis pistilaris*-A common wild edible mushroom from Haryana (India) and its Sociobiology. *Kavaka*, 44: 34-37.

Panwar, C. and Purohit, D. K. 2002. Antimicrobial activities of *Podaxis pistilaris* and *Phellorinia inquinans* against *Pseudomonas aeruginosa* and *Proteus mirabilis. Mushroom Research,* 11: 43-44.

Singh, R.D. 1994. Edible mushroom of arid zone of Rajasthan. *Mushroom Research,* 3: 39.

Solanki, D.S., Kumar, S., Sharma, K., Gehlot, P. and Singh, S.K. 2016. Weather prerequisites for fructification of *Phellorinia* mushroom. *Plant Archives,* 16: 986-989.

ISSN. 0972 - 8406
The NEHU Journal Vol. XVIII, No.2, July - December 2020, pp. 29-39

Climate change adaptation in mountain community of Mustang district, Nepal

Prakash Upadhyay

Associate Professor of Anthropology
Tribhuvan University, Prithvi Narayan Campus,
Pokhara, Nepal
Email: prak-socio@hotmail.com

Abstract

The ethnic Loba people of Mustang are experiencing climate vulnerability in terms of adaptation in agropastoral livelihood. They have adjusted their agropastoral living with what nature has provided and are coping to the changing climate with customary adaptive measures which includes traditional system of landholding, collective labour and irrigation management, seasonal migration, spiritual connectivity and out-migration for the collective survival of the community amidst the threats of becoming a climate refugee. Anthropologically climate effects are not just about human capacity to adapt and exercise resilience it is furthermore about their altering sociocultural institutions to readjust and to muddle through with its implications. Such readjustment requires repositioning of intimate human-environment relationships that not only ground and substantiate indigenous worldviews, but also work to maintain and safeguard local landscapes and ethos.

Keywords: Climate refugee, Community-based, Hidden-hunger

Introduction

As a phenomenon of climate change, global warming is the sturdy intensification in the average temperature of earth's atmosphere because of an amplified quantity of heat striking the earth from the sun which is being trapped in atmosphere. A two-degree raise in temperature threatens 25 percent of all plant and animal species on the planet with extinction (Rodenberg, 2009). Cook (2013) argues that while climate change is not solely destructive, the negative impacts of global warming on health and agriculture are greater than the benefits for the majority of the world. According to UNDP Report (2008) climate change cause the most harm to the most vulnerable populations or those who lack the ability to cope with and adapt to climate change because of a lack of access to essential resources. Dankelman (2011) has noted that marginalized inhabitants of remote regions like women, children, the elderly, and the impoverished have less access to and control over resources and therefore are more negatively impacted by climate change. Upadhyay (2015) argues that the variation in climatic parameters are attributed directly or indirectly

to human activities which are increasing the hazard of deforestation, flooding, storms, desertification, soil erosion and the melting glaciers in the Himalayas. Barnett et al. (2005) state that with higher temperatures the runoff peak will shift to winter and early spring, away from summer and autumn when the demand is greatest. Dessler and Parson (2006) squabble that the impact of climate change is not consistent across the globe, and considerable differences can be perceived between different regions, places and communities but the potential risk of vulnerability to climate change is expected to be high in remote mountains.

In an elevation ranging from 1,372 to 8,167 meters and with several high peaks, Mustang District, bordered by Tibetan plateau, is one of the remotest, protected, isolated and sparsely populated districts of Nepal with an area of 3,573 km^2 and a population of 13,452 (CBS, 2012). With a population of 3843 in 1104 households (1.49 persons per $km^{2)}$, the population of Upper Mustang has decreased significantly at the decadal ratio of 1.40; similarly, number of households is decreasing by 1.14 decadal ratios (CBS, 2012). The study area Lomanthang is a rural municipality situated in Upper Mustang inhabited by indigenous Loba community with a total population of 569 – Male 276, Female 293 in total 172 households (CBS, 2012). The household size is 3.30 which are lower than the national average of 4.9 persons (CBS, 2012). The proportion of dependent population is relatively high: 18.6% of the total population is children and 9.7% are aged above 80 years. This population statistic has close relationship with migration rate (19%) from mountain region of Nepal (Suwal, 2014).

Loba people are the followers of Tibetan Buddhism, and are culturally and ethnically of Tibetan origin closely identified with their socio-economic, cultural, linguistic, geographical and historical context with Tibet--a unique example of Tibetan culture (Selter, 2007). The Loba livelihood is based on agropastoral system, seasonal migration, trade and controlled tourism. But the existing rangeland is not sufficient for livestock because majority of the area is barren and cold desert like. Overgrazing is the main factor causing deterioration of rangelands (Schaller and Gu 1994). Declining rainfall has resulted in decline in crop production, crop failure, and more insect attack. But vegetables like string beans, spinach, cauliflower, cabbages which grow at lower elevations with higher temperatures, were growing faster (Bhandari *et al.* 2015). The ever increasing scarcity of drinking and irrigation water, rising temperatures and desertification in mountains has forced a historically and culturally rich subsistence-agriculture and livestock dependent Lobas to migrate to new places for a better life (Shahi, 2013). Low and erratic rainfall pattern, shifting snowfall, decreasing humidity, and increasing air temperature has created water crisis, drying spring and declining soil moisture with a direct negative impact on plant and animals' growth depicting a direct link between the changes in climate parameters and its consequences on agriculture and livelihood (Prasain, 2018). Given this predicament, the key objective of this paper is to assess the climate vulnerability of Loba community of Upper Mustang in terms of their experiences and adaptation in agropastoral livelihood.

The sample of this study includes randomly selected 75 households of Loba farmers with a population of 256 (female 132 and male 124). One household head each from 75 households were selected for the study on the ground that as elderly they are the decision makers in household activities and have good understanding of climate change impacts and adaptation experiences. Survey method was used to collect information on the impact of climate change on crop production and adaptation strategies which supplemented the qualitative ethnographic information collected through observations, interviews and case studies on personal experiences, valuation and responses on climate change impact and adaptation in agriculture and livestock. For primary data collection, fieldwork was carried out from March 11 to April 9, 2019.

Climate Change Impact and Information Sources

According to Department of Hydrology and Meteorology (DHM) Mustang Station, the minimum annual average temperature of Upper Mustang is increasing at a rate of 0.048^0C per year. Summer temperature is increasing at a rate of 0.024^0C per year. In contrast, winter temperature is escalating at a rate of 0.115^0C per year. Autumn average temperature is increasing at a rate of 0.026^0C per year. Spring average temperature is fairly increasing at a rate of 0.032^0C per year. Broadly, the minimum temperature is escalating in all season. The maximum annual average temperature of Upper Mustang is moderately increasing at a rate of 0.0139^0C per year. In contrast winter season's maximum temperature is increasing at an annual rate of 0.139^0C. Autumn seasons' maximum average temperature is increasing at a rate of 0.049^0C per year. Average annual temperature of spring season is fairly escalating at a rate of 0.0673^0C. In general, the maximum temperature is increasing in average in each season.

Escalation of temperature has created diverse problems on ecosystem and biological behaviors of flora and fauna in Lomanthang. Melting glaciers, fluctuations in weather patterns, increasing temperature frequency and intensity of extreme weather events has caused negativity on agriculture and livestock. Accordingly, it has caused more harm to most helpless population or those who lack the ability to cope with and adapt to climate change. Over the last many decades Lobas witnessed environmental changes such as warming, intensified natural disasters and declining precipitation patterns. These have been conflicting with their knowledge and seasonal agricultural schedule.

In addition to natural climate variability, mass deforestation for the construction of new houses and hotels resulted in serious ecological disturbance, accordingly climatic change and its impacts were experienced harshly that forced Lobas attuned to adaptation in agropastoral livelihood system. The situational trauma of climate change influenced the customary ways of living; livelihood efforts were being harder, perceived and experienced in daily life and there were few sources of information on climate change. About 32.5% information on climate change and its impacts were obtained by local people's own observation of variations in climatic phenomenon.

Agropastoral Adaptation and Food Sovereignty

MacDonald (1998) affirms that mountain farming systems are often characterized by diversified practices, a feature which distinguishes them from the standardized and often highly specialized practice of modern agriculture. For the people living in mountains, diversification is a rational strategy for risk reduction (MacDonald 1998, Mishra et al. 2003). Many mountain farming systems are highly flexible in response to environmental as well as socio-economic changes (Bishop 1998, Mishra et al. 2003, Aase et al. 2010). Trans-Himalayan region Lomanthang has limited arable land as well as limited growing season characterized by high levels of risk and vulnerability associated with geographical process causing the lowest per unit of agricultural and livestock production. In a flexible community level unity of agropastoral production system, subsistence agriculture and livestock are the main bases of Loba economy and livelihood which are sturdily associated with factors like accessibility of irrigation water, seed/manure, soil nutrients, human labour and livestock, pasture land, land holding, fuel, food consumption pattern, migration, goods exchange system, services and resources distribution prototype. Recently traditional agropastoral system has incorporated some modern tools/technologies in order to cope with changing climate. Challenges like early ripening of crops, dryness, and scarcity of irrigation water is rampant. In the process of adaptation, they are cultivating several varieties of vegetables and fruits. The altering environment and agriculture system nexus can be comprehended via human ecological analysis that focuses not only on one aspect but on *holism* and all-inclusive way. Kassam (2001) asserts that such correlation can be observed at the relations between humans, and other animals, plants and their habitats.

Nyeleni (2007) state that food sovereignty is the right of people to healthy and culturally appropriate food produced through ecologically sound and sustainable methods, and their right to define their own food and agriculture systems. Agriculture production, consumption, conservation and sustainable use of local varieties embedded in Loba food sovereignty affirms that those who produce, distribute, and consume food should control the mechanisms of food production and distribution encompassing the rights to healthy and culturally appropriate food. For Lobas there is a biological and cultural significance of food. It is a medium for social interaction; production, processing, preparation and consumption are *community-based*. Active participation of male and female, elderly people, children, relatives and neighbors is imperative during cultivation, harvesting, processing and consumption. The agriculture season begins with a ritual called *Sakaluka* in which women plough agriculture field in order to appease the *Snake God* and to get blessings for good weather and harvest. *Sakaluka* is a collective action with social, spiritual and material dimensions to prevent extreme weather events and to express collective solidarity of the community, bringing them together, reaffirming collectivism, promoting solidarity to cope and reconcile with natural phenomena.

Ingrained in local ecology Loba food sovereignty system anticipates providing healthy and culturally appropriate food to community in a harsh climate. In a high mountain ecosystem amidst changing climate, agriculture/livestock system has sustained in relation to local livelihood and food sovereignty. Womenfolk make use of the food primarily to fulfill family and livestock's nutritional needs which constitutes grain, meat, milk and butter containing large proportion of dietary requirement with a conviction that meat generates heat to cope with severe cold. *Tsampa* (barley flour) is used widely used as medicine with buttered tea and *Chhurpi* (dried cheese) for pregnant women, sick, old and children. But in the last few decades, Lobas are gradually losing their food sovereignty. Loba heritage and capacities to produce healthy, high-quality abundant food are being threatened and undermined by neo-liberal market capitalism. Previously, food autonomy had provided them the hope and power to preserve, recover and build on their food producing indigenous knowledge and capacity. But due to alternative income sources from tourism, now, Lobas buy food and drinks imported from Tibet and India. Due to emerging markets, Lobas have started consuming high quality imported rice that has threatened indigenously produced crops and consumption pattern. Previously Loba had the practice of bartering grain with Tibetan salt. But with the decline in grain production and the availability of imported salt in the market, Lobas lost their sovereignty over salt and grain trade in which livestock played a crucial role for carrying loads.

The traditional polyandry system facilitated and organized the family and had supported cultivation and familial barter business. Polyandrous marriage had been a tool for managing the inherited property, livestock and grazing land/forest that had assisted in keeping the paternal property indivisible, maintaining agricultural sustainability and hence keeping vibrant the concept of food sovereignty. But currently, polyandry is on the verge of extinction owing to which the whole sociocultural and economic fabric of the family life is altering. The forces eroding polyandry are modernization, increasing education, weak social bonds, alternative income sources from tourism, and the changing life style.

Aase *et al.* (2010) affirms that Trans-Himalayan farming system constitutes variables such as labour, cultivated fields, domesticated animals, forest and pastures. The Loba people are adapting and integrated in large scale processes such as agriculture and livestock rearing, trade, migration and tourism. Different varieties of crops like buckwheat, naked barley, mustard, potatoes etc have developed social, cultural, commercial, nutritional, and medicinal as well as adaptability to changing ecological system. Few years back there was a high priority to produce cereal crops but now Lobas have become more selective of crops. The value of crops differs according to household demand, changes in consumption pattern and livestock population.

Yak, sheep, goat, horse, mules are the part of pastoral livelihood used for nutritious food items as milk, yogurt, fat, butter, cheese and meat, manure, fuel and for carrying goods. Prior to the development of roadways and modern transportation, Lobas maintained a large number of horses as a means of transportation. But currently goods are

transported by truck and tractors. Change in the pattern of transportation and travel has reduced the quantity of food items for livestock consumption. Previously pea and wheat were the major productions, but nowadays buckwheat and potato are the high valued crops meant for export. Pea and wheat were the popular crop items to feed livestock when the Lobas kept large number of horses, mules, and donkey. But currently pea and wheat consuming animals are diminishing which make a little demand of pea and it is not popular item. Further, the increase in temperature, changes in precipitation patterns, changes in weather events, and reductions in water availability have resulted in reduced agricultural productivity of pea and wheat.

Severely extreme weather events have interrupted food delivery and resulted in price hikes in food prices after extreme events which are expected to be more frequent in future. Brown *et al.* (2015) has stated that productivity depends upon the weather condition and insects attack. Productivity is also sensitive to frost and hailstone. These natural occurrences have challenged Loba peoples' indigenous knowledge on weather that has threatened their food sovereignty due to their marginal geo-climatic situation. Barley is valuable locally due to its high value consumption as *Tsampa* the local staple nutritional food item. Potatoes and Turnip are grown in a significant amount. Rice is not grown locally; imported rice is available in same price as wheat is in Lomanthang. The most overlooked crop in Lomanthang is wheat. Foremost reason is that those people who were buying wheat for daily consumption have shifted to rice which is easily available in market. Adopting the rice as a conspicuous food, young people overlook locally available food which is a course of action towards losing food sovereignty.

The local distribution system of food has changed due to the availability of new food items, changing food habits, changes in economic value of different varieties of grains and the livestock production system. With the arrival of foreign tourists, most food items are served to tourists but the productivity of crops is not high in relation to inputs due to poor soil, inadequate manure and water, low temperature and extreme weather events. More seeds are needed and harvest proportion is also low. The seed has to be very much concentrated while broadcasting, but due to extreme cold winters, seed germination is extremely low. Seeds are also destroyed by birds, rats and insects. Organic pesticide was the first preference of Lobas but climate change generated effects and appearance of new pests on crops and decline in routine crops has forced the farmers to use inorganic pesticides in crops and vegetables. Rise in summer temperature has obligated the Lobas to follow yearly crop rotation to improve soil fertility and to avoid insect. Their responses to climate variability consisted of iterative sequence of improvised strategic adjustments. Based on their expectations of what the climate might be, Loba farmers proceed to carry out agricultural practices based on a well-established crop rotation pattern called *Kar-Nak*. If wheat or naked barley is grown in *Kar* (the previous year), *Nak* crops peas, mustard, or buckwheat is cultivated the next year. Fallow land can be seen all over the place in the lack of irrigation water.

Previously Lobas were not much cautious about grass collection since they had enough winter grazing access for their livestock in Tibet. But, this has halted nowadays due to Chinese government policy to restrict the entry of Loba people's livestock in Tibet. Similarly, owing to decline in food production, food supply to Tibet has stopped. Previously crops and livestock were much valued because they were used for trade in Tibet. But nowadays, the worth of local crops has changed because of the construction of new roads that has made easy access of imported goods. Nevertheless, Lobas have continued practicing agro-pastoralism as their traditional occupational identity, holding land as their ancestral property and gender participation for mobilizing the agropastoral system of subsistence amidst the changing climate. Both women and men are affected by and vulnerable to climate change and global warming, but women often bear more of the burden (UNDP, 2010). Amid patriarchic norms, gender participation in agriculture and livestock rearing is imperative for the Lobas. With heavy work burden, women have higher social status in community, domestic and public spheres.

Beforehand, Lobas lacked the habit of consuming green leafy vegetables. But now in the process of climatic adaptation, varieties of green vegetables are cultivated and consumed. Until few years back asparagus was grown at the altitude of 2200 meters but now it is grown at an altitude of 3750 meters. Now Lobas have started growing courgette, cauliflower, cabbage, carrots, coriander, string bean, bean, turnip as the main vegetable items. Until 2009 courgette could be grown only inside the greenhouse, but nowadays it is grown outside the greenhouse. Cucumber, chili, tomatoes, bitter gourd is grown inside greenhouse. The varieties of crops which were grown during winter are now cultivated during summer season which is due to climate change adaptation. Until few years ago, it took two years to grow big-sized onion but nowadays within a year it grows outside the greenhouse. Previously apples were not grown, but nowadays high quality apples are grown in orchards. Growing reliance on chemical fertilizers has facilitated the growth of apples. Apricot, peach, plum, pear, walnut, grapes are grown, widely consumed locally and sold to tourist resorts during tourist season. Less rainfall resulted in less crop production, crop failure, and more insects attack but had less effect on fruit farming.

The Loba cultural tradition of *Lakti-Chikula* a customary form of labour group rotation is under threat owing to the scarcity of human-resource during the harvest time. Under this customary system of labour exchange, labourers are used in the field to plough, collecting manure, to make canals etc. Seed broadcasting is done by well experienced person of *Lakti-Chikula* group organized in a group and they work in each and every individual household who have participated in the group. This group goes turn by turn to each household based on the arrangement of time. But nowadays, due to the acute shortage of labourers, the Lobas are hiring wage labourers from other districts of Nepal during the harvest season. Since the fast rate of Lobas out-migration amidst the intimidation of becoming a climate refugee, the wage of hired labourer is rising fast which costs more expensive for the farmers to cultivate crops. It is cheaper to buy crops from nearby shop rather than paying wages to farm labourers. This tendency is challenging the agropastoral practices of Lobas.

Water Resource Management

Chaulane (2009) argues that irrigation water management is complex because of its attributes, such as the nature of public goods and the lack of properly defined rights for its use. The collective maintenance of irrigation system in Lomanthang is the continuity of customary norms of adaptation system for water management. But currently due to the scarcity of water, extreme weather events, melting glacier and pollution, the availability of irrigation/drinking water has become problematic. Lobas have a customary system to distribute water at the head-end and tail-end. The Lobas have developed village head system which is to mobilize obligatory labour support from the people who owns land and use water for irrigation and household. Village head take the responsibilities of clans, lineage and kin groups. Labour contribution for irrigation canal maintenance is fixed proportionately on the basis of land holding. Such determination is done on the basis of seed required for cultivable land. If there are households who own less or abundant land they are required to contribute proportionally in the maintenance of irrigation canal. Those houses who even do not own land have to contribute single day labour as they drink water. This indigenously managed irrigation system is the manifestation of local kinship, village headmanship, institution of polyandry, property inheritance system, access to land and agro-pastoral system that give nativity to age old indigenously managed irrigation system ultimately manufactured and protected by wider social structure.

Diminishing polyandry and fragmentation of paternal landholdings has affected the traditional water management practices. Currently as river beds have become deeper through erosion due to frequent Glacial Lake Outburst Flood (GLOF) hence Lobas could not build/repair canals with their technology to bring water up the level to irrigate the field. Irrigation along with agriculture has been affected not only by global warming and extreme weather events, but also by changing sociocultural institutions as polyandry, transformation effects, migration, tourism, Chinese and Nepali governments' policy, road construction and transportation facilities.

Seasonal Migration and Resource Mobilization in a Changing Climate

Seasonal migration during extreme freezing cold season is an apparatus which is due to high altitude, snow fall and severe cold during winter season. Seasonal migration has helped Loba people to mobilize resources other than their cultivated field. Seasonal migration is imperative from religious, economic, and social point of view and has huge impact on agropastoral production and food consumption. Lobas migrate during winter after the end of harvest season. They trade local craft items, herbal medicines in cities of Nepal and India, visit relatives, pay pilgrimage to religious sites, treat sick family members and relatives. Since livestock is a significant link in the agropastoral farming system, during the seasonal migration, livestock is stall-fed (with leaves, grass and crop wastes) by few left-over elderly people at home.

Owing to the absence of family members at home, they save food at home and earn cash by trading goods and services and they buy items such as food, drinks, and clothes. Seasonal migration during winter season is the local strategy to cope with hardship with cold, food and other necessary items. The seasonal migration has implications on traditional joint family structure and polyandry. But the alterations in traditional polyandry marriage and family relationship have caused severe consequences on resources mobilization in agriculture, livestock, and human labour. Owing to the vigor of environmental stressors, Lobas seasonal migration is the needs of livelihood-distressed households. From anthropological perspective, in a warming world amidst altering sociocultural institutions, such migration may alter or fade away completely in the long run.

Conclusion

Even though having contributed the least to greenhouse gas emissions, Lobas are the one most at risk from its consequences due to their close relationship with the fragile ecosystem. Climate change ramifications embedded with altering sociocultural institutions has brought different kinds of risks and prospects, threatened the agropastoral livelihood system, cultural and economic survival, and indigenous knowledge in relation to local habitat and food sovereignty. This connotes that climate change is not something that has come in isolation but amplification of the existing problems of geographical marginalization and the emerging trend of modernization. The adaptation strategies are alternatives such as seasonal migration, value focused agricultural production, seasonal and permanent migration and involvement in tourism.

Climate change is more about human-environment and nurture-nature relations. Managing climate-related risks to agropastoral livelihood requires new information, skills and technologies such as seasonal forecasts, risk analysis and water saving agricultural practices. With supports from related organizations, adaptation can be utilized as good opportunities to avert *climate refugee* predicament and the threat of *hidden-hunger* which is lowering Lobas essential nutrient contents. There is a need of impact identification and to understand changes that have occurred due to changing climate, adapt to them, and make the most of the new-fangled opportunities, while addressing upstream-downstream issues. From anthropological perspective, the effects of climate change are not just about local populations' capacity to adapt and exercise their resilience in the face of exceptional change, it is also about their changing sociocultural institutions to readjust to change and to muddle through with its implications. Such readjustment, necessitate a repositioning of intimate human- environment relationships that not only ground and substantiate indigenous worldviews, but also work to maintain and preserve local landscapes and culture. Adaptation is *management smart* dexterity hence climate adaptation necessitates adaptive progression to effectively manage biodiversity under climate change by expanding adaptive capacity as close to its theoretical, fundamental and practical limits as possible. There is the need for policy interventions at the community level. Community knowledge and participation are keys to make certain the success of such measures.

References

Aase, T. H., Chaudhary, R. P., and Vetaas, O. R. 2010. Farming flexibility and food security under climatic uncertainty: Manang, Nepal Himalaya: Farming flexibility and food security under climatic uncertainty, *Area*, *42* (2), 228–238.http://doi.org/10.1111/j.1475-4762.2009.00911.x accessed on 11/12/2010

Barnett, T. P., *et al*., 2005. Potential impacts of a warming climate on water availability in snow-dominated regions, *Nature*, 438:303 - 309.

Bhandari, T.S., Shrestha, H.L. and Dhital, K.R. 2015. Soil Study in Mustang – ICIMOD. https://www.icimod.org/?q=20204 accessed on 17/11/2015

Bishop, N.H., 1998. *Himalayan Herders*. Orlando: Harcourt Brace & Company : 367.

Brown, M.E., Antle, J.M. and Backlund, P. 2015. Climate Change, Global Food Security, and the U.S. Food System. http://www.usda.gov/oce/climate_change/FoodSecurity2015Assessment/Full. http//DOI: 10.7930/jo86 accessed on 15/12/2015

CBS. (2012). National Population Census 2011. Household and Population by Sex, Ward Level: Mustang. Kathmandu, Nepal: Central Bureau of Statistics, Government of Nepal.

Chaulane, P. 2009. Water, Wealth and Power: A Case Study from Upper Mustang of Nepal, *Occasional Papers in Sociology and Anthropology*, Department of Sociology and Anthropology, Tribhuvan University, 11: 104-125.

Cook, J. 2012. Skeptical Science, It's not bad. http// Skepticalscience.com global-warming-positives-negatives accessed on 22/4/ 2014

Dankelman, I. 2002. Climate change: learning from gender analysis and women's experiences of organizing for sustainable development, In: Masika, Rachel (ed.) *Gender, Development and Climate Change*, London: Oxfam: 21-29

Dessler, A.E., and Parson, E.A. 2006. *The Science and Politics of Global Climate Change: A Guide to the Debate*. Cambridge, Cambridge University Press: 17.

Kassam, K.A.S. 2001. *Human Passing on the Knowledge : Mapping ecology in Wainwright*, Calgary : University of Calgary: Canada: 36.

MacDonald, K.I. 1998. 'Rationality, Representation, and the Risk Mediating Characteristics of a Karakoram Mountain Farming System', *Human Ecology*, 26 (2):287 - 321.

Mishra, C., Prins, H.H.T. and Van Wieren, S.E. 2003. Diversity, Risk Mediation, and Change in a Trans-Himalayan Agro-pastoral System, *Human Ecology*, 31 (4):595 - 609.

Nyeleni Organization 2007. *Nyeleni declaration*. http://nyeleni.org/IMG/pdf/DeclNyeleni-en.pdf accessed on 6/12/2007

Prasain, S. 2018. Climate change adaptation measure on agricultural communities of Dhye in Upper Mustang, Nepal, *Climate Change*, 148(1-2):279–291.

Rodenberg, B. 2009. Climate Change Adaptation from a Gender Perspective: A Cross-cutting Analysis of Development-policy Instruments. German Development Institute: 7.

Schaller, G.B. and Gu, B. 1994. Comparative ecology of ungulates in the Aru Basin of Northwest Tibet, National *Geographic Research and Exploration,* 10: 266-293.

Selter, E. 2007. Upper Mustang: Cultural Heritage of Lo Tso Dhun. UNESCO Kathmandu. https://books.google.com.np/books?id=t2skOgAACAAJ accessed on 19/10/2007

Shahi, P. 2013. Climate change hits Upper Mustang, entire village moving to better area. Kathmandu, Sep 28, The Kathmandu Post Daily: 8

Suwal, B.R. 2014. *Internal migration in Nepal.* Population Monographs of Nepal. National Planning Commission Secretariat, Central Bureau of Statistics, Kathmandu (1):241-283.

United Nations Development Programme (UNDP). 2008. *Fighting climate change: human solidarity in a divided world*. UN Plaza, New York: 28-31.

United Nations Development Programme (UNDP). 2010. The Contribution of UNDP-GEF Adaptation Initiatives Towards MDG3. UNDP-GEF, New York: 3-6.

Upadhyay, P. 2015. Climate Change as Ecological Colonialism: Dilemma of Innocent Victims, *Himalayan Journal of Sociology and Anthropology*, Tribhuvan University, Prithvi Narayan Campus, Pokhara, Nepal, 6:187-202.

ISSN. 0972 - 8406
The NEHU Journal Vol. XVIII, No.2, July - December 2020, pp. 41-56

Phytotoxic assessment of some monoterpenes and their formulation with leaf extract of *Chenopodium ambrosioides*

Saroj Kumari Fagodia[1], Daizy Rani Batish[2], Harminder Pal Singh[3]*

[1]Department of Botany, Shri Baldev Ram Mirdha Government College, Nagaur, Rajasthan, India.

[2]Department of Botany, Panjab University, Chandigarh, India.

[3]Department of Environment Studies, Panjab University, Chandigarh, India.

*Corresponding author: fagodia1989@gmail.com

Abstract

The present study investigated the phytotoxicity of eight monoterpenes belonging to two major groups, i.e. oxygenated monoterpenes (linalool, citronellol, citronellal, 1, 8-cineole) and monoterpene hydrocarbons (limonene, β-pinene, p-cymene, α-terpinene) and their formulation with Chenopodium ambrosioides leaf extract against Cassia occidentalis. In a laboratory bioassay, monoterpenes (0.5, 1.0, 2.5 mM) and their combination with leaf extract (0.75%) of Chenopodium ambrosioides were tested on germination and seedling length of the weed. The inhibition was greatest with oxygenated monoterpenes, whereas leaf extract formulation improved phytotoxicity of hydrocarbon monoterpenes as they alone are not much effective. Post-emergence application of monoterpenes (1% and 2.5%, v/v) and their formulation with 2% leaf extract was evaluated on 4-week-old plant by two physiological parameters - photosynthetic efficiency and membrane integrity. Among all the monoterpenes, citronellol was found to be the most effective both in laboratory and green house bioassay, followed by citronellal. Post-emergent phytotoxicity of citronellal and linalool increased several fold by leaf extract formulation. These results were also confirmed by visible injury ranging from chlorosis to necrosis to complete wilting of plants. The study concludes that some monoterpenes have great scope for the development of new weed control strategies and their further formulation improves efficacy of active compounds, reduced dose usage and save costs of application.

Keywords: Membrane integrity, Photosynthetic efficiency, Phytotoxicity, Post-emergent, Visible injur

Introduction

In agriculture system the potential use of naturally derived products as new, effective, reduced-risk alternatives for the weed management is a contemporary issue because intensive use of synthetic herbicides resulted in soil and groundwater contamination,

harm human health and increase herbicidal resistance in weed species (Dayan & Duke, 2014; Bhat *et al.*, 2019). With the rising demand of eco-friendly pest control options, public and private sector organizations have re-focused their search by looking back to natural sources for new biologically active compounds. So, worldwide efforts are being made to identify the new eco-friendly chemicals as a source of natural herbicides. In this direction, essential oils and their major bioactive compounds, *i.e.* monoterpenes, hold a promising potential. The essential oils/monoterpenes exhibit phytotoxic activity against several weeds (Vaughn & Spencer, 1993; Batish *et al.*, 2008; Mutlu *et al*., 2011; Dayan & Duke, 2014; Isman, 2016; Fagodia *et al*., 2017; Pouresmaeil *et al.*, 2020). These volatiles are suitable to prevent environmental poisoning because they possess little or no residual effect and are generally regarded as safe (GRAS) chemicals (Isman, 2000; Tworkoski, 2002; Dayan *et al*., 2009). Various researchers have suggested that single compound may not be as effective as a combination of different allelochemicals which might act additively or synergistically towards the growth inhibition in plants (Vokou *et al*., 2003; Jamil *et al*., 2009; Vasilakoglou *et al*., 2013; Chotsaeng *et al*., 2017). These allelochemicals may prove beneficial in the weed management as they improve efficacy of active compound, reduce dose usage, save costs of application, allows the control of a diverse weed flora, delay development of herbicide resistant weeds and are safe unlike the chemicals being used in agriculture (Duke & Dayan, 2015).

Therefore, an attempt was made to improve the efficacy of eight most abundant monoterpenes present in the essential oils, including oxygenated monoterpenes and monoterpene hydrocarbons, through their formulations with water extract of an allelopathic plant, *Chenopodium ambrosioides* L. The choice of *C. ambrosioides* was based on its known phytotoxicity (Jimenez-osornio *et al*., 1996; Hegazy & Farrag, 2007) and its rich photochemistry (Singh *et al.*, 2008). A great scope exists for the utilization of these compounds in combination with each other. As these formulations are free of toxic or contaminated products, therefore, they are suitable for use in agricultural and food industry. These liquid formulations are more dose and cost-effective bioherbicides due to their fast burn-down action even at lesser volume than their typical required quantity. To the best of our knowledge, there are no previous studies in the open literature on such possible integration of monoterpenes and aqueous leaf extract formulation. Thus, the objectives of this study were:

- To assess the phytotoxic potential of eight monoterpenes (linalool, citronellol, citronellal, 1,8-cineole, limonene, β-pinene, p-cymene and α-terpinene).
- To examine the impact of allelopathic leaf extract (*C. ambrosioides*) on phytotoxic efficacies of monoterpenes both under laboratory and green house conditions against widely growing urban weed - *Cassia occidentalis* L.
- To investigate the mechanisms by which monoterpenes and their formulation act on the weed.

Materials and methods

Chemicals and biological material

Technical grade linalool, citronellol, citronellal, 1,8-cineole, limonene, *β*-pinene, *p*-cymene and *α*-terpinene were purchased from Sigma Co., St. Louis, USA; Lancaster, UK, and Acros Organics, UK. Seeds of *Cassia occidentalis* L. and fresh leaves of *Chenopodium ambrosioides* L. were collected locally from plants growing wild in the campus of Panjab University, Chandigarh, India. Before use, *C. occidentalis* seeds were scarified with sulphuric acid and imbibed overnight in water.

Preparation of C. ambrosioides leaf extract

C. ambrosioides aqueous leaf extract were prepared by soaking required amounts of air dried leaf powder, 0.75g (0.75% for pre-emergent assay) and 2g (2% for post emergent assay) per 100 ml of distilled water at room temperature for 12 h. Thereafter, extracts were filtered through Whatman no. 1 filter paper and used.

Laboratory bioassay

Phytotoxicity of all the eight monoterpenes and their formulation with leaf extract of *C. ambrosioides* was studied on the germination and early growth of *C. occidentalis* under laboratory conditions. Monoterpene solutions (0.5, 1.0 and 2.5 mM) were prepared using Tween-20 (final concentration <0.01%). Distilled water with the same amount of Tween-20 served as a parallel control. Monoterpenes formulations were prepared by making their emulsions (0.5, 1.0 and 2.5 mM) in 0.75% leaf extract of *C. ambrosioides* with the help of Tween-20. 0.75% *C. ambrosioides* leaf extract along with same amount of Tween-20 served as a positive control. Pre-imbibed *C. occidentalis* seeds (15) were placed in Petri dishes (15 cm in diameter) lined with a thin layer of cotton wad and Whatman no.1 filter paper. Each Petri dish was moistened with 10 ml of respective treatment solution. The Petri dishes were then sealed with cello-tape® to avoid loss of the monoterpenes due to volatilization. For each treatment concentration, including controls, five independent Petri dishes were maintained as replicates. All the Petri dishes were kept in a growth chamber set at 25±2 °C and 16/8 h light/dark photoperiod of 240 µ mol photons m^{-2} s^{-1} photon flux density provided with fluorescent tubes and lamps. After 1 week, germination percent and length of the emerged seedlings was measured.

Greenhouse bioassay

To determine the post-emergent activity of monoterpenes and their formulation, plants of *C. occidentalis* were raised from seeds in 15 cm diameter polypropylene pots in a greenhouse. For this, 1500 g of garden soil mixed with sand in a ratio of 3:1 (*w/w*) was filled in each pot and ten seeds of *C. occidentalis* were sown per pot. One week after emergence, these were thinned to 5 plants per pot. Four week old *C. occidentalis* plants were spray treated with 1 and 2.5% solution of all the eight monoterpenes (or distilled

water in case of control) and their formulations: 1% monoterpene formulation (1% Monoterpenes + 2% *C. ambrosioides* leaf extract) and 2.5% monoterpene formulation (2.5% Monoterpenes + 2% *C. ambrosioides* leaf extract). Spray treatment with 2% *C. ambrosioides* leaf extract served as a positive control. Plants were spray-treated with respective solutions at a volume of 140 ml/m^2. For each treatment, five replications were maintained. After 3-days of treatment, the photosynthetic efficiency and membrane integrity were measured from *C. occidentalis* leaves. Further, the test plants were observed for visual injury levels on the basis of chlorotic and necrotic areas developed after the treatment. The injury levels of the plant were rated on a scale of 0 (with no injury) to 4 (with complete mortality no recovery, expressed as ++++).

Estimation of Photosynthetic efficiency (Chlorophyll Fluorescence)

The maximum potential quantum efficiency of PSII of treated as well as control *C. occidentalis* leaves were measured using the OS-30p Chlorophyll Fluorometer (Opti Sciences, USA). For this, a leaf was attached on the leaf holder of the plant efficiency analyser equipment and subjected to dark conditions for about 10 min. Thereafter, its photosynthetic efficiency was calculated from the ratio of F_v/F_m, where F_v is variable chlorophyll fluorescence and F_m is maximum chlorophyll fluorescence in an illuminated leaf. This was repeated five times for each treatment.

Determination of Membrane integrity (REL)

Membrane integrity in terms of relative electrolyte leakage from *C. occidentalis* leaves was studied as per the method of Singh *et al.* (2007). For this, leaves (100 mg) were incubated in 10 ml of distilled water at 25°C for 1 hour in the test tubes and initial conductivity (E_1) of the bathing medium was measured. Thereafter, the test tubes containing leaf tissues were boiled for 15 min to release all the ions. These were then cooled to 25°C and the conductivity (E_2) was measured again. The relative electrolyte leakage was calculated using following formula and expressed in percentage.

$$\% \text{ REL} = (E_1/E_2) \times 100$$

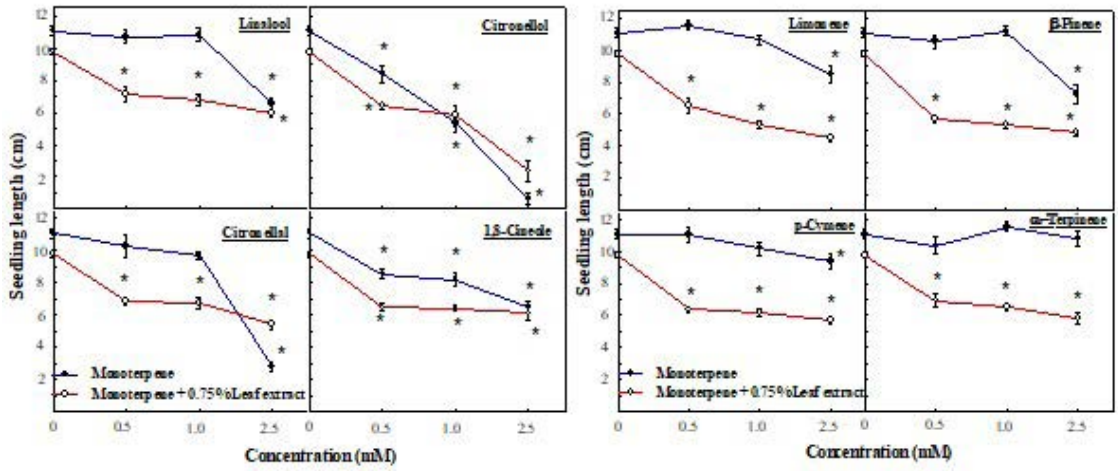
Statistical analysis

All the experiments were conducted in a completely randomised design and were repeated twice. Values are presented as the mean ± SE (standard error) of the repeated experiments. Data was subjected to one-way ANOVA followed by comparison of mean values using post hoc Tukey's test at $P \leq 0.05$ significance level using software programme SPSS (version 16). Graphical representations were made on software programme Sigma plot (Version 8.0).

Results and discussion

Growth studies under laboratory conditions

In response to 0.5 mM and 1.0 mM of monoterpene and its formulation, there was no effect on seed germination of the test weed (data not presented), whereas at 2.5 mM, monoterpenes and their formulation showed a significant effect (Table 1). Monoterpenes when applied alone, germination was inhibited in the order of potency: citronellol > citronellal > 1, 8-cineole > limonene > *β*-pinene > linalool > *p*-cymene > *α*-terpinene. Among the prepared formulations, a positive and significant synergy was observed for *p*-cymene and *α*-terpene. Other monoterpene formulations showed either antagonistic or insignificant effect on the percent germination. Regarding the growth of emerged seedlings, application of monoterpenes showed following order of inhibition: citronellol > citronellal > 1, 8-cineole > linalool > β-pinene > limonene > p-cymene > α-terpinene (Figure 1a and 1b). At 2.5 mM, among all the tested monoterpenes, citronellol was found to be the most effective as it caused 95% reduction in the seedling length over the control, followed by citronellal with 74% inhibition and 1,8-cineole and linalool with 40% inhibition (Figure 1a). On the other hand, the hydrocarbon monoterpenes, α-terpinene, p-cymene, limonene and β-pinene, inhibited the seedling length by 3-34% (Figure 1b). On the whole, oxygenated monoterpenes exhibited high inhibitory potential in comparison to the hydrocarbon monoterpenes. Our observation is corroborated by studies of Vaughn & Spencer (1993), Kordali *et al.* (2007), De Martino *et al.* (2010) and de Oliveira *et al.*, (2018), who also reported the oxygenated monoterpenes are more phytotoxic than hydrocarbon monoterpenes. At ≥ 0.5 mM all monoterpenes formulations caused a significant reduction in the seedling length of *C. occidentalis*. Further, two different types of phenomenon, *i.e.* synergy/antagonism, was observed between monoterpenes and their formulation with *C. ambrosioides* leaf extract on the seedling length of *C. occidentalis*. Monoterpene hydrocarbons showed synergy with *C. ambrosioides* leaf extract as reflected by the improved efficacy. Limonene showed the highest synergy and caused 54% reduction in the seedling length at 2.5 mM concentration. Formulation of *β*-pinene, *p*-cymene and *α*-terpinene also showed improvement in their activity. In case of oxygenated monoterpenes, citronellal and citronellol showed antagonistic effects when formulated with *C. ambrosioides* leaf extract, whereas the activity of linalool and 1,8-cineole remains unaffected by formulation (Figure 1a).



(a) (b)

Figure 1. Effect of monoterpenes and their formulation with 0.75% leaf extract on seedling length of *C. occidentalis* measured 7 days after the treatment. Vertical bars along each data point represent the standard error of the mean. * represents significant difference from their respective controls (Distilled water and 0.75% leaf extract). (a) Oxygenated monoterpenes (b) Hydrocarbon monoterpenes

Table 1. Effect of different monoterpenes and their formulation (0.75% *C. ambrosioides* leaf extract) on percent germination of *C. occidentalis* measured after 7 days. Data presented as mean (%) ± SE. Different alphabets represent significant difference from their respective controls at $P \leq 0.05$. Values within parenthesis indicate percent decrease over + control (only leaf extract).

Treatment (2.5 mM)	Monoterpene	Monoterpene + Leaf extract
Control	100.0 ± 5.4 a	77.0 ± 2.9 a (100 %)
Linalool	76.5 ± 4.3 b	62.4 ± 1.8 b (81.0 %)
Citronellol	5.8 ± 3.1 b	21.2 ± 5.8 b (27.5 %)
Citronellal	25.2 ± 2.7 b	48.9 ± 2.6 b (63.5 %)
1,8-Cineole	58.6 ± 2.8 b	55.0 ± 3.7 b (71.4 %)
Limonene	59.4 ± 3.0 b	62.4 ± 1.8 b (81.0 %)
β-Pinene	65.3 ± 4.9 b	55.0 ± 3.0 b (71.4 %)
p-Cymene	84.9 ± 4.4 a	54.3 ± 2.9 b (70.5 %)
α-Terpinene	97.4 ± 4.6 a	52.4 ± 3.0 b (68.5%)

The synergism between monoterpenes has been evaluated by some workers to prove their better efficacy as potential bioherbicides over individual monoterpenes (Vokou *et al.*, 2003; He *et al.*, 2009; Vasilakoglou *et al*., 2013). Jamil *et al.* (2009) studied the herbicidal potential of sorghum water extracts in combination with the leaf extracts of some allelopathic plants and concluded a better potential of these formulations. Besides, some synthetic herbicides have also been tried in combination with the aqueous extracts of allelopathic plants which have resulted in the reduction of doses of the applied herbicide for weed control (Cheema *et al.,* 2003; Ihsan *et al.,* 2015; Alsaadawi *et al.*, 2020). However, monoterpenes offer a better eco-friendly replacement option over synthetic

herbicides, especially in combination with the aqueous extracts of allelopathic plants. The allelopathic effect of *C. ambrosioides* leaf extract has been attributed to the presence of ascaridole (Jimenez-osornio *et al.*, 1996; Hegazy & Farrag, 2007). However, we did not estimate the nature of these phytotoxins in the present study. Nevertheless, the findings of the present study suggested a positive synergy between monoterpene hydrocarbons and ascaridole as phytotoxicity of the monoterpene hydrocarbons was improved by their formulations with the leaf extract. The mechanism by which these compounds inhibit seed germination and growth remains unclear. However, loss/disruption of mitotic activity might be responsible for the reduction/inhibition of germination and seedling growth of tested plant (Romagni *et al.,* 2000). Koitabashi *et al.* (1997) demonstrated that the essential oils caused accumulation of lipid globules in the cytoplasm and reduced the size of cell organelles possibly due to the inhibition of DNA synthesis or membrane disruption resulting in anatomical and physiological changes.

Effect of Monoterpenes and their formulation on C. occidentalis under greenhouse conditions

In addition to the laboratory bioassay, green house bioassay was also performed to assess the post-emergent activity of monoterpenes and their formulation. Visible injury and physiological parameters, *viz.* photosynthetic efficiency and membrane integrity, was taken into consideration 3 days after treatment. Weed injury in the greenhouse was evaluated on the basis of visual estimates. Injury ratings included four categories: 0 indicated no injury, + minor injury, ++ moderate injury, +++ severe injury but recovery possible, and ++++ severe injury with no possibility of recovery. Injury symptoms increased with increasing concentrations of the volatiles (Table 2). The mature plants of the test weed were severely damaged upon spray of citronellol alone while other monoterpenes resulted in a minor or no injury. After formulation, citronellol exhibited antagonistic relation with the leaf extract, whereas citronellal and linalool act synergistically with the leaf extract. Formulated citronellal became most phytotoxic as it severely damaged the plant to a level where recovery was not possible (Figure 2). Slightly improved phytotoxicity was also shown by 1% limonene formulation. Chlorosis, necrosis, wilting and senescence was observed due to advanced intoxication, which reflects biochemical, physiological and structural changes, appropriately explained by the impairment in photosynthetic activity and increased ion leakage.

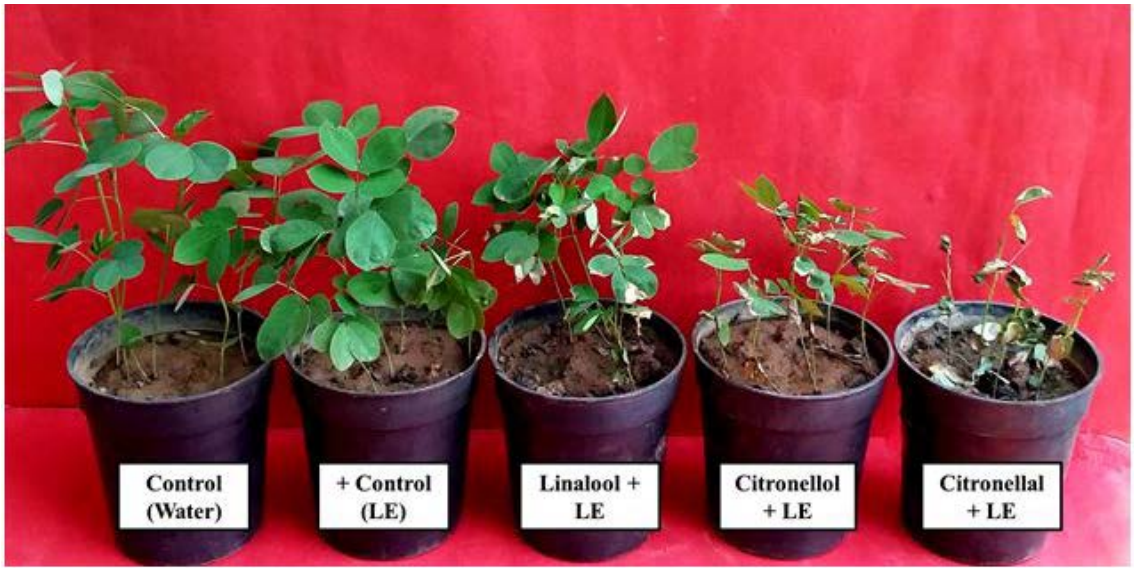


Figure 2. Photograph showing the effect of monoterpenes formulation (2.5% monoterpene + 2% LE) on 4 week old *C. occidentalis* plant 3-days after spray treatment. LE represents C. *ambrosiodies* leaf extract.

Chlorophyll fluorescence or photosynthetic efficiency is a good biomarker to identify mode of action of these phytotoxins. Photosynthetic efficiency in terms of F_v/F_m ratio gives clear evidence about the effect of treatments on chlorophyll content. In the present study, among all the tested monoterpenes only citronellol showed significant decrease in F_v/F_m ratio by 57% at a concentration of 1% and by 84% at 2.5% spray treatment with respect to control. However, in response to other monoterpenes, no significant change was observed (Figure 3A). In the monoterpenes formulation, both synergistic and antagonistic interaction was evidenced. Citronellal and linalool formulation revealed greater inhibition of photosynthetic efficiency as compared to the individual monoterpenes and this suggests a synergistic interaction of citronellal and linalool with 2% *C. ambrosioides* leaf extract.

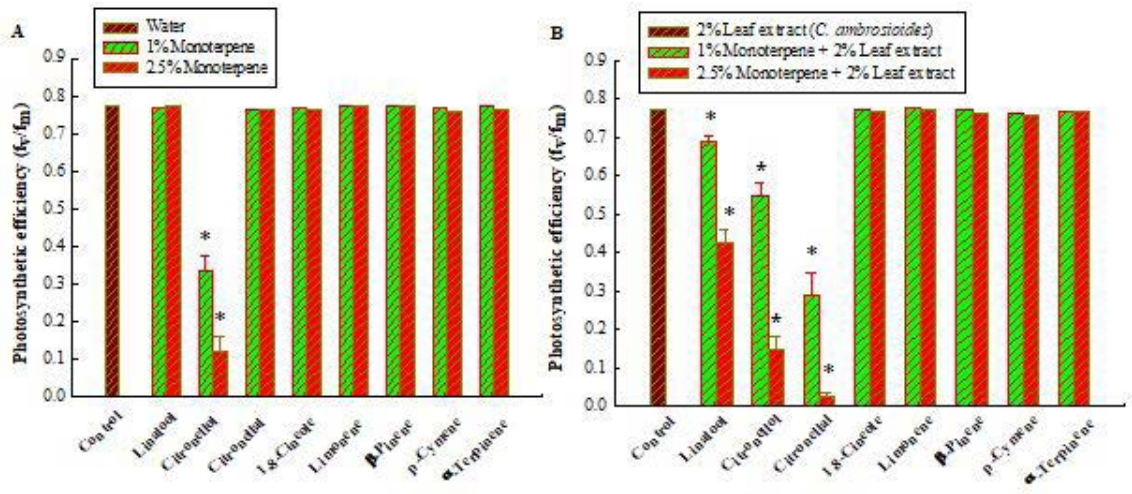


Figure 3. Effect of monoterpenes and their formulation on photosynthetic efficiency (represented as F_v/F_m) of *C. occidentalis* leaves recorded 3 days after spray treatment. Vertical bars represent the standard error of the mean. * represents significant difference from their respective controls at $p \leq 0.05$.

By the spray treatment of citronellal formulation, F_v/F_m ratio was significantly declined by 62% and 97%, respectively, at 1% and 2.5% compared to that of positive control. For linalool formulation, reduction was observed up to 45% at 2.5% spray treatment. On the contrary, 1% citronellol showed antagonism with *C. ambrosioides* leaf extract, reducing its effectivity by 28% in comparison to citronellol alone. However, 2.5% citronellol acted in an independent manner as its activity remained unchanged before and after formulation, and both the treatments showed significant reduction with respect to positive control. However, rest of the monoterpenes formulation were non-effective and caused no significant changes in test plant's photosynthetic efficiency (Figure 3B). Previously, studies have demonstrated post emergent herbicidal potential of essential oils/ monoterpenes (Tworkoski, 2002; Kaur *et al*., 2010; Gouda *et al*., 2016). Chlorophyll fluorescence is often measured to determine the effect of various compounds on the light reaction of photosynthesis and the decrease in F_v/F_m ratio in response to monoterpenes has been reported earlier by some investigators (Dayan *et al*., 2000; Ibrahim *et al*., 2004; Grana *et al*., 2013). Singh *et al*. (2002) reported that monoterpenes reduce/inhibit chlorophyll content in the *C. occidentalis* plant. Loss of chlorophyll might affect photosynthetic machinery as it interferes with chloroplast functioning, membrane stability and stomatal behaviour (Kabanova & Chaika, 2001; Rai *et al*., 2003; Batish *et al*., 2007). Thus, from this study it is clear that among all tested monoterpenes and their formulations only three spray treatments, *i.e.* citronellol, citronellal formulation and linalool formulation, are able

to interfere with photosynthetic machinery of the test plant. The reason for improved activity of citronellal and linalool after formulation is possibly due to presence of double bond in their structure, which offers a potential site for the attachment of functional groups resulting in chemical modifications that could improve the physiochemical and biological properties of the molecule.

Table 2: Symptoms of visible injury (rated on 0-4 scale) on test weed 3-days after treatement with different concentrations (%) of monoterpenes and their formulation with 2% *C. abrosioidies* leaf. Hear 0 stand for no injury, + minor injury, ++ moderate injury, +++ severe injury but recovery possible, ++++ severe injury with no recovery.

Treatment	Monoterpene		Monoterpene + Leaf extract	
	1 %	2.5 %	1 %	2.5 %
Linalool	+	+	++	++
Citronellol	+++	+++	++	+++
Citronellal	+	++	++	++++
1,8-Cineole	+	+	+	+
Limonene	0	+	+	+
β-Pinene	0	+	0	+
p-Cymene	0	+	0	+
α-Terpinene	0	+	0	+
Control	0		0	

Post-emergent application of monoterpenes and their formulations caused damage in the cuticle and cell membrane which lead to the wilting/desiccation of aerial parts. So, in addition to photosynthetic efficiency, membrane permeability was also taken into account to measure the stress induced inside the test plant. All oxygenated monoterpenes at 1% and 2.5% caused a significant ion leakage, except for 1,8-cineole (significant leakage only at 2.5%) (Table 3). All monoterpene hydrocarbons exhibited non-significant effect at 1% and 2.5% concentration except *p*-cymene and *α*-terpene (significant at 2.5%). However, all monoterpenes formulations exhibited greater membrane damage than their respective individual monoterpenes, except citronellol. At 1% monoterpenes formulation, synergy was observed in citronellal, linalool *β*-pinene and *p*-cymene. Likewise, at 2.5% concentration formulation of all monoterpenes except citronellol greater ion leakage was

observed, with the most effective and improved synergy being in citronellal followed by linalool (Table 3). Changes in membrane permeability affects all other physiological and biochemical processes linked to membrane functioning. Thus, to study mode of action of herbicide, monitoring membrane integrity is a good physiological parameter (Dayan & Watson, 2011). The observations revealed in present study are in agreement with earlier reports that essential oils and monoterpenes induce damage and cause cell death in plants (Tworkoski, 2002; Mutlu *et al*., 2011; Kaur *et al*., 2012). Allelopathic compounds are known to depolarize and disrupt cell membranes thereby enhancing their permeability, inducing lipid peroxidation and finally leading to cell death due to production of reactive oxygen species (Singh *et al*., 2006; Mutlu *et al*., 2011). Although the complete action mechanism of these compounds is still not clear.

Table 3. Effect of monoterpenes and their formulation with 2% leaf extract on the relative electrolyte leakage (% REL) in *C. occidentalis* leaves recorded 3 days after treatment. Data presented as mean (%) ± SE. Different alphabets represent significant difference from their respective controls at $P \leq 0.05$

Treatments	Monoterpene		Monoterpene + Leaf extract	
	1%	2.5%	1% + L	2.5% + L
Linalool	8.17 ± 0.08 b	11.94 ± 0.55 c	21.93 ± 0.72 b	32.50 ± 0.94 c
Citronellol	41.54 ± 1.54 b	50.58 ± 0.58 c	26.50 ± 0.50 b	49.29 ± 0.51 c
Citronellal	12.89 ± 0.34 b	18.34 ± 0.21 c	26.50 ± 1.52 b	69.21 ± 1.12 c
1,8-Cineole	3.50 ± 0.75 a	6.45 ± 0.22 b	3.82 ± 0.38 a	8.77 ± 0.22 b
Limonene	2.27 ± 0.07 a	2.95 ± 0.44 a	5.99 ± 0.39 ab	7.54 ± 0.97 b
β-Pinene	2.34 ± 0.21 a	2.44 ± 0.05 a	6.20 ± 0.27 b	10.43 ± 0.24 c
p-Cymene	3.26 ± 0.07 ab	3.82 ± 0.14 b	7.48 ± 0.21 b	9.07 ± 0.40 b
α-Terpinene	3.09 ± 0.67 a	5.52 ± 0.23 b	3.30 ± 0.18 a	10.59 ± 0.22 b
Control	2.65 ± 0.15 a		3.00 ± 0.40 a	

Conclusion

The present study concludes that some monoterpenes and their formulation possesses phytotoxicity, affects germination and seedling growth, causes ion leakage and reduce the photosynthetic activity in test weed. These compounds have great potential to be used as leading chemicals for synthesis of new herbicides for sustainable weed management programmes. According to our knowledge, this is the first report regarding the monoterpenes formulation with allelopathic leaf extract. These liquid formulations are going to be more dose and cost effective bioherbicides. Furthermore, it is meaningful to continue studies with different combinations of essential oils/monoterpenes and allelopathic plant extracts in search of novel bioherbicides.

References

Alsaadawi, I.S., Khaliq, A. and Farooq, M. 2020. Integration of allelopathy and less herbicides effect on weed management in field crops and soil biota: A Review. *Plant Archives*, 20: 225-237.

Batish, D.R., Singh, H.P., Kohli, R.K. and Kaur, S. 2008. Eucalyptus essential oil as natural pesticide. *Forest Ecology Management*, 256: 2166-2174.

Batish, D.R, Singh, H.P., Setia, N., Kohli, R.K. and Yadav, S.S. 2007. Alternative control of little seed canary grass using eucalypt oil. *Agronomy for Sustainable Development,* 27: 171-177.

Bhat, R., Khajuria, M. and Mansotra, D.K. 2019. A systematic review on global environmental risks associated with pesticide application in agriculture. In: Contaminants in Agriculture and Environment: Health Risks and Remediation. *Agro Environ Media-Agriculture and Environmental Science Academy*, Haridwar, India: 96-110.

Cheema, Z.A., Farid, M.S. and Khaliq, A. 2003. Efficacy of concentrated sorgaab in combination with low rates of atrazine for weed control in maize. *Journal of Animal and Plant Sciences,* 13: 48-51.

Chotsaeng, N., Laosinwattana, C. and Charoenying, P. 2017. Herbicidal activities of some allelochemicals and their synergistic behaviors toward *Amaranthus tricolor* L. *Molecules,* 22: 1841.

Dayan, F.E., Cantrell, C.L. and Duke, S.O. 2009. Natural products in crop protection. *Bioorganic & Medicinal Chemistry*, 17: 4022-4034.

Dayan, F.E. and Duke, S.O. 2014. Natural compounds as next generation herbicides. *Plant Physiology*, 166: 1090-1105.

Dayan, F.E., Romagni, J.G. and Duke, S.O. 2000. Investigating the mode of action of natural phytotoxins. *Journal of Chemical Ecology*, 26: 2079-2093.

Dayan, F.E. and Watson, S.B. 2011. Plant cell membrane as a marker for light dependent

and light-independent herbicide mechanisms of action. *Pesticide Biochemistry and Physiology*, 101: 182-190.

De Martino, L., Mancini, E., De Almeda, L.F.R. and De Feo, V. 2010. The antigerminative activity of twenty seven monoterpenes. *Molecules*, 15: 6630-6637.

de Oliveira, M.S., da Costa, W.A., Bezerra, P.N., da Silva Souza Filho, A.P. and de Carvalho Junior, R.N. 2018. Potentially phytotoxic of chemical compounds present in essential oil for invasive plants control: A Mini review. In: R. Radhakrishnan (ed.). *Biological Approaches for Controlling Weeds*, London, United Kingdom: 49-62.

Duke, S.O. and Dayan, F.E. 2015. Discovery of new herbicide modes of action with natural phytotoxins. *American Chemical Society,* 1204: 79-92.

Fagodia, S.K., Singh, H.P., Batish, D.R. and Kohli R.K. 2017. Phytotoxicity and cytotoxicity of *Citrus aurantiifolia* essential oil and its major constituents: Limonene and citral. *Industrial Crops & Products,* 108: 708-715.

Gouda, N.A.A., Saad, M.M.G. and Abdelgaleil, S. 2016. Pre and Post-emergent Herbicidal Activity of Monoterpenes against Barnyard Grass, *Echinochloa crus-galli*. *Weed Science,* 64: 191-200.

Grana, E., Sotelo, T., Tielas, C.D., Reigosa, M.J. and Moreiras, A.M.S. 2013. The phytotoxic potential of the terpenoid citral on seedlings and adult plants. Weed Science, 61: 469-481.

He, H.B., Wang, H.B., Fang, C.X., Lin, Y.Y., Zeng, C.M., Wu, L.Z., Guo, W.C. and Lin, W.X. 2009. Hebicidal effect of a combination of oxygenic terpenoides on *Echinochloa crus-galli*. *Weed Research,* 49: 183-192.

Hegazy, A.K. and Farrag, H.F. 2007. Allelopathic potential of *Chenopodium ambrosioides* on germination and seedling growth of some cultivated and weed plants. *Global Journal of Biotechnology and Biochemistry,* 2: 1-9.

Ibrahim, M.A., Oksanen, E.J. and Holopainen, J.K. 2004. Effects of limonene on the growth and physiology of cabbage (*Brassica oleracea* L.) and carrot (*Daucus carota* L.) plants**.** *Journal of Science of Food and Agriculture,* 84: 1319-1326.

Ihsan, M.Z., Khaliq, A., Mahmood, A., Naeem, M., El-Nakhlawy, F. and Alghabari, F. 2015. Field evaluation of allelopathic plant extracts alongside herbicides on weed management indices and weed–crop regression analysis in maize. *Weed Biology and Management,* 15: 78-86.

Isman, M.B. 2000. Plant essential oils for pest and disease management. *Crop Protection,* 19: 603-608.

Isman, M.B. 2016. Pesticides based on plant essential oils: Phytochemical and practical considerations. In: V.D. Jeliazkov and C.L. Cantrell (ed.). *Medicinal and Aromatic Crops: Production, Phytochemistry and Utilization,* American Chemical Society:

13-26.

Jamil, M., Cheema, Z.A., Mushtaq, M.N., Farooq, M. and Cheema, M.A. 2009. Alternative control of wild oat and canary grass in wheat fields by allelopathic plant water extracts. *Agronomy for Sustainable Development*, 29: 475-482.

Jimenez-osornio, E.M.V.Z.J., Kumamoto, J. and Wasser, C. 1996. Allelopathic Activity of *Chenopodium ambrosioides* L. *Biochemical Systematics and Ecology*, 24: 195-205.

Kabanova, S.N. and Chaika, M.T. 2001. Correlation analysis of Triticale morphology, chlorophyll content and productivity. *Journal of Agronomy and Crop Science,* 186: 281-285.

Kaur, S., Singh, H.P., Batish, D.R. and Kohli, R.K. 2012. *Artemisia scoparia* essential oil inhibited root growth involves reactive oxygen species (ROS)-mediated disruption of oxidative metabolism: *in vivo* ROS detection and alterations in antioxidant enzymes. *Biochemical Systematics and Ecology*, 44: 390-399.

Kaur, S., Singh, H.P., Mittal, S., Batish, D.R. and Kohli, R.K. 2010. Phytotoxic effects of volatile oil from *Artemisia scoparia* against weeds and its possible use as a bioherbicide. *Industrial Crops & Products,* 32: 54-61.

Koitabashi, R., Suzuki, T., Kawazu, T., Sakai, A., Kuroiwa, H. and Kuroiwa, T. 1997. 1,8-Cineole inhibits root growth and DNA synthesis in the root apical meristem of *Brassica campestris* L. *Journal of Plant Research,* 110: 1-6.

Kordali, S., Cakir, A. and Sutay, S. 2007. Inhibitory effects of monoterpenes on seed germination and seedling growth. *Zeitschrift für Naturforschung*, 62: 207-214.

Mutlu, S., Atici, O., Esim, N. and Mete, E. 2011. Essential oils of catmint (*Nepeta meyeri* Benth.) induce oxidative stress in early seedlings of various weeds species. *Acta Physiologiae Plantarum,* 33: 943-951.

Pouresmaeil, M., Nojadeh, M.S., Movafeghi, A. and Maggi, F. 2020. Exploring the bio-control efficacy of *Artemisia fragrans* essential oil on the perennial weed *Convolvulus arvensis*: Inhibitory effects on the photosynthetic machinery and induction of oxidative stress. *Industrial Crops & Products*, 155: 112785.

Rai, V.K., Gupta, S.C. and Singh, B. 2003. Volatile monoterpenes from *Principea utilis* L, leaves inhibit stomatal opening in *Vicia faba* L. *Biologia Plantarum,* 46: 121-124.

Romagni, J.G, Allen, S.N and Dayan, F.E. 2000. Allelopathic effects of volatile cineoles on two weedy plant species. *Journal of Chemical Ecology,* 26: 303-313.

Singh, H.P., Batish, D.R., Kaur, S., Arora, K. and Kohli, R.K. 2006. Alpha-pinene inhibits growth and induces oxidative stress in roots. *Annals of Botany,* 98: 1261-1269.

Singh, H.P., Batish, D.R., Kohli, R.K. and Arora, K. 2007. Arsenic-induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting

from enhanced lipid peroxidation. *Plant Growth Regulation,* 53: 65-73.

Singh, H.P., Batish, D.R., Kohli, R.K., Mittal, S. and Yadav, S. 2008. Chemical composition of essential oil from leaves of *Chenopodium ambrosioides* from Chandigarh, India. *Chemistry of Natural Compounds,* 44: 378-379.

Singh, H.P., Batish, D.R. and Kohli, R.K. 2002. Allelopathic effect of two volatile monoterpenes against bill goat weed (*Ageratum conyzoides* L.). *Crop Protection*, 21: 347-350.

Tworkoski, T. 2002. Herbicide effects of essential oils. *Weed Science,* 50: 425-431.

Vasilakoglou, I., Dhima, K., Paschalidis, K. and Ritzoulis, C. 2013. Herbicidal potential on *Lolium rigidum* of nineteen major essential oil components and their synergy. *Journal of Essential Oil Research*, 25:1-10.

Vaughn, S.F. and Spencer, G.F. 1993. Volatile Monoterpenes as Potential Parent Structures for New Herbicides. *Weed Science,* 41: 114-119.

Vokou, D., Douvli, P., Blionis, G.J. and Halley, J.M. 2003. Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. *Journal of Chemical Ecology*, 29: 2281-2301.

ISSN. 0972 - 8406
The NEHU Journal Vol. XVIII, No.2, July - December 2020, pp. 58-72

Impact assessment of heavy metal contamination on water quality of underground and open-cast coal mines

Lily Shylla[1], S.K. Barik[2], S.R. Joshi[1]*

[1]Microbiology Laboratory, Department of Biotechnology & Bioinformatics.
North-Eastern Hill University, Shillong, India.
[2]Director, CSIR-National Botanical Research Institute, Lucknow, India
[*]Corresponding author: srjoshi2006@yahoo.co.in

Abstract

Production of coal is of utmost importance for energy generation while being crucial for the economic welfare of the nation. Mining of this precious natural resource however results in a hazardous aftermath owing to the built up accumulation of heavy metals that are non-biodegradable and damage the environment. The current study evaluates the impact of metal contamination on water from underground and open–cast mines in two different seasons by pollution indices approach. pH analysis placed all samples under study in the acidic scale with an average range of 2.4-3.65. Samples from open-cast were noted to have more of total dissolved solids compared to the underground mine samples. The concentration of metals in the samples were seen to be in the order Cu < Pb < Cr < Zn < Fe from low to high. The leaching of heavy metals due to the mining operation has led to the contamination of the water bodies as depicted by HPI and HEI values. Higher metal accumulation is noted in the pre-monsoon compared to the post-monsoon season, with samples from underground mines showing slightly lower metal contamination compared to that of the open-cast mines. A feasible and effective technology needs to be integrated into conventional coal mining methods in order to contain and prevent the detrimental leaching of metals contaminating the environment.

Keywords: Metal contamination, Coal mines, Underground, Open-cast, Water pollution indices

Introduction

Coal is the most abundant and essential fossil fuel in India. With increase in demographic pressure, growing economy and a need for improving quality of life, the demand for energy in India is also rising. Mining not only helps in fulfilling the increasing energy demand of scores of industries, but also provides an important opportunity for the economic development of the country (Chaulya and Chakraborty, 1995). Almost 45% of the total energy consumption in India is met by coal, which makes it indispensable in the

welfare of our economy (Energy Statistics, 2017). In India, coal is mined and extracted via two main methods namely, underground mining and open-cast mining. However, coal extraction in India is mostly done by open-cast mining method and it constitutes 93.26% of the total coal production in the country (Coal Directory of India, 2016-17). The process of extracting coal by open-cast mining has increased over the years, as this method incurs less expenditure with minimum wastage (Ghose and Majee, 2001).

Choice of mining method depends on various factors such as geological conditions (type, depth, size and quality of deposit), technological development and degree of mechanisation. Besides these, other factors need to be taken into consideration, such as production cost along, selling price, environmental and social aspects, all of which are also significant in the entire process of mining. All of these factors determine the differences between resources and reserves of coal (Zehirov, 2017). Open-cast method is usually decided for areas with shallow coal seams, while underground mining method is the approach selected to access deeper coal seams (Mukherjee and Pahari, 2019). Both of the mining methods drastically affect the mined area and its surrounding environment with huge amounts of water being discharged onto the surface during the process of mining, in order to facilitate the mining operation. These liquid mine discharges usually contain high concentrations of TDS, TSS and heavy metals which contaminate the surface and ground water of the area (Dhar, 1993; Tiwary, 2001). Coal extraction is an intricate process which generates hazardous toxicants and heavy metals due to their virtue of being highly solubility in nature. Metals such as Fe, Cu, Mn, Co, Ni, Pb, Zn and other mineral dusts are released in the soil and water bodies creating an acidic environment (Chandra and Jain, 2013). The chief cause of coal mine pollution is leaching of acidic mine tailings or run-offs known as 'Acid Mine Drainage' (AMD) discharged into the surroundings. The sulfide ores present in the mines get readily oxidised in presence of air and water to form sulphate-rich acidic drainage resulting in leaching of metals and other metalloids. This causes increased accumulation of inorganic matter and soil acidity while reducing the organic matter content of the natural environment. The discharged effluents from coal mines pick up metals, combined with other mine wastes and leftovers that pollutes the soil and adjacent water bodies thus causing a massive alteration in the dynamics of associated life forms (Johnson and Hallberg, 2005; Zhou *et al.*, 2007). The increase of heavy metals in the environment is said to have a dynamic effect on all living organisms, threatening the food chain, indigenous flora, fauna and native soil microbiota (Yao *et al.*, 2012). In India, a majority of the coal mining districts have been assessed and declared as critically polluted areas by MoEF (CSE, 2012).

Contamination of water bodies in and around coal mines with heavy metals raises an alarm that calls for the need to assess the water quality standard. This can be evaluated using the indexing approach such as the heavy metal pollution index (HPI) and heavy metal evaluation index (HEI), that takes into consideration the overall quality of water with respect to weighted arithmetic quality mean and heavy metal concentrations compared to its respective acceptable standards (Edet and Offiong, 2002). Metal pollution

indices, HPI can be categorized into three main classes *viz* low (< 300), medium (300-600) and high level (> 600); HEI values are also placed in three categories *viz* low (< 150), medium (150-300) and high (> 300), respectively (Bhuiyan *et al.,* 2010; Mahato *et al.,* 2017). In the current study, water samples from both underground and open-cast coal mines have been considered in order to assess the impact of heavy metal contamination on the overall quality of water.

Materials and methods

Study sites and sample collection

Two types of coal mines, underground and open-cast were targeted for the collection of samples in order to assess the impact of metal contamination on water quality. Underground mines are in Khliehriat, Jaintia hills district of Meghalaya and open-cast mines are located in Ledo, Tinsukia district of Assam, India (Figure 1). Four water samples each, were collected from the two mine sites – MW1, MW2, MW3 & MW4 represent underground mines whereas AW1, AW2, AW3 & AW4 represent open-cast mines. Samples were collected in sterilized sample bottles by immersing the bottles about 10 cm below the water surface and brought to the laboratory for analysis. The samples were collected in two seasons, pre-monsoon and post-monsoon seasons.

Figure 1: Underground and open-cast methods of coal mining in Meghalaya and Assam

Determination of physicochemical parameters

Three main parameters were considered for this analysis – pH, Total dissolved solids (TDS) and Electrical conductivity (EC). The pH of the samples were recorded by means of DIC μ pH meter (GOLD 533, Digital Instrumental Corp) calibrated using standard buffers, washing the pH probe between measurements using sterilized deionized water (Rayment and Higginson, 1992). TDS of samples was measured using pre-calibrated EcoTestr TDS (Eutech instruments). For EC, DiST® 4 EC Tester (HANNA Instruments) was used by standardisation of probe using 0.01M KCl solution and conductance recorded.

Estimation of Heavy metals

Five heavy metals were analyzed in the water samples namely iron (Fe), zinc (Zn), copper (Cu), lead (Pb) and chromium (Cr). The presence of metals in the samples was carried out following the protocol of acid digestion. Accordingly the samples were filtered through Whatman filter paper No.42 and the pH adjusted to pH<2 with HNO_3. The sample (500 ml) was heated allowing evaporation and finally concentrated to a residual volume of 50 ml. The concentrate was filtered and subjected to metal estimation using *ICP-OES* (Thermo Scientific iCAP 7600). The concentration of metals was expressed in parts per million (ppm) using a standard formula (Radulescu *et al.,* 2014).

Determination of water pollution indices

Two main pollution indices were considered in the study namely Heavy metal pollution index (HPI) and Heavy metal evaluation index (HEI). The indices are a measure of total quality affected by the presence of different heavy metals in the water samples.

For determination of HPI, two equations are given:

$$\text{Eq. 1} \quad Qi = \sum_{i=1}^{n} \frac{\{Mi(-)Ii\}}{(Si-Ii)} \text{ x } 100 \quad ; \quad \text{Eq. 2} \qquad \text{HPI} = \frac{\sum_{i=1}^{n} Wi\,Qi}{\sum_{i=1}^{n} Wi}$$

For sub index ***Qi***: *Mi* - monitored value for the heavy metal; *Ii* - ideal desirable value; *Si* - standard value of the i^{th} parameter.

For **HPI**: *Wi* - unit weightage of i^{th} parameter, a value inversely proportional to Si of the metal (Prasad *et al.* 2014).

For determination of HEI, $\text{HEI} = \sum_{i=1}^{n} \frac{Hc}{Hmac}$

H_c - monitored value; H_{mac} - maximum admissible concentration (MAC) of the i^{th} parameter (Edet and Offiong, 2002).

Results and Discussion

Physicochemical profile of the samples

Three main parameters were taken into consideration for physicochemical profiling of the water samples *viz* pH, total dissolved solids and electrical conductivity.

Table 1: Physicochemcial profile of water samples from the mines in two seasons

Underground coal mine samples								
	MW1		**MW2**		**MW3**		**MW4**	
Parameters	PRE	POS	PRE	POS	PRE	POS	PRE	POS
pH	3.63	2.63	3.24	2.41	3.57	2.51	3.65	2.54
TDS	561	246	990	401	710	358	607	315
EC	807	702	1597	978	1568	786	1046	780
Open-cast coal mine samples								
	AW1		**AW2**		**AW3**		**AW4**	
Parameters	PRE	POS	PRE	POS	PRE	POS	PRE	POS
pH	3.21	2.7	3.11	2.54	3.13	2.9	3.16	2.9
TDS	1860	1662	2360	2126	2040	1984	2240	1864
EC	3780	3340	4482	3961	4422	3501	4284	3263

PRE – Pre-Monsoon; POS – Post-monsoon; BDL –below detection level; TDS – Total dissolved solids (mg/L); EC- Electrical conductivity (µS/cm).

For underground mines, the highest pH recorded was 3.65 and an average reading of pH 3.02±0.19. The average pH reading for the open-cast samples was 2.95±0.08 and the highest reading being 3.21. The pH of the samples is noted to be slightly lower and more acidic during the post-monsoon than the pre-monsoon season, similar to that reported by Sahoo *et al.* (2011). The overall pH of water bodies in and around coal mines is acidic and much lower than the prescribed limit of pH 6-8 (WHO, 2011). Low pH due to coal mine overburdens has been reported in several studies (Dowarah *et al.,* 2009; Rai *et al.,* 2011). Increased acidity is attributed to the acid drainage and mine spoils leaching into the water systems in the vicinity of the mines (Cherry *et al.,* 2001). The geology of the rock composition and mineral deposits also add to the acidification of mining effluents (Dutta and Agarwal, 2002). The average total dissolved solids (TDS) of the samples for the pre and post monsoon seasons was found to be 717±96.18 and 330±33.04 mg/L in underground mine samples whereas 2125±110.26 and 1909±98.21 mg/L in the open-cast

samples, respectively. The average electrical conductivity (EC) of the underground mine samples was recorded to be 1254.5±195.64 and 811.5±58.70 µS/cm, whereas that of open-cast samples was 4242±159.48 and 3516.25±156.32 µS/cm, for the pre and post monsoon seasons respectively (Table 1). The results corroborate with the past findings reporting EC of coal mine streams to be approximately 3630 µS/cm (Lyngdoh and Kayang, 2012). High conductance of water bodies surrounding coal mines has been suggested in earlier studies and this could serve as an indicator for mining activities indicating contamination (Soucek *et al.*, 2000). The TDS and EC of all samples is seen to be higher in the open-cast mine as compared to the underground mine samples; also higher values are seen in the pre-monsoon than the post-monsoon season. TDS and EC are positively correlated but are however inversely related to pH. High TDS and EC level with decrease in pH indicates severe water pollution (Islam *et al.,* 2017).

Impact of metal contamination on water quality

The methods employed in coal extraction are in many regards crude and unscientific which tend to have a serious damaging impact on the environment. Coal mine sites and the adjacent areas are most affected with mine spoils containing heavy metals and other contaminants percolating into the soil and water systems. In this current study, water from underground and open-cast mines was assessed for the impact of contamination caused by heavy metals, based on water pollution indices - heavy metal pollution index (HPI) and heavy metal evaluation index (HEI). Four samples each, collected from the two types of mines for pre and post monsoon seasons, were analyzed for the presence of heavy metals.

Table 2: Heavy metal concentration of water samples from the mines in two seasons

Underground coal mine samples								
Samples	**MW1**		**MW2**		**MW3**		**MW4**	
	PRE	POS	PRE	POS	PRE	POS	PRE	POS
Fe	47.34	36.64	93.03	56.18	95.23	56.18	78.55	52.83
Zn	2.740	1.347	3.160	1.870	4.01	2.101	4.101	2.013
Cu	0.002	BDL	0.003	0.001	0.004	0.001	0.004	0.002
Pb	0.062	0.035	0.031	0.012	0.037	0.019	0.044	0.021
Cr	0.055	0.031	0.063	0.036	0.071	0.041	0.107	0.087

Open-cast coal mine samples								
Samples	**AW1**		**AW2**		**AW3**		**AW4**	
	PRE	POS	PRE	POS	PRE	POS	PRE	POS
Fe	132.2	80.02	113.1	68.12	96.70	45.30	145.3	95.45
Zn	2.400	1.007	1.671	0.875	0.922	0.783	3.901	1.745
Cu	0.004	0.002	0.002	0.001	0.002	BDL	0.007	0.003
Pb	0.052	0.030	0.047	0.022	0.025	0.018	0.071	0.047
Cr	0.216	0.162	0.144	0.113	0.112	0.095	0.194	0.143

Heavy metals (mg/L); PRE – Pre-Monsoon; POS – Post-monsoon; BDL –below detection level

The concentration of metals in the samples were found to be in the order Cu < Pb < Cr < Zn < Fe from low to high, with Fe being highest in concentration for all the samples (Table 2). The analysis of water from coal mine area of Damodar River India reported a similar finding where Fe is said to be one of the main heavy metal contaminants found in high concentrations in water bodies of coal mine sites (Mahato *et al.,* 2017). The overall pollution indices calculated for the samples revealed open-cast mine samples to have higher values in terms of HPI (482.62) and HEI (103.98) than that of underground mine samples with HPI (363.69) and HEI (69.17), respectively (Table 3). Metal pollution indices for each individual samples from both mine types, along with mean indices, for the two seasons were evaluated and tabulated. The mean HPI of underground mine samples was noted to be 473.73 and 253.67, and mean HEI of 84.603 and 53.729; whereas open-cast samples showed mean HPI of 598.12 and 367.08 and mean HEI of 130.18 and 77.78, for pre and post monsoon season, respectively (Table 4). The pollution index values exhibited by open-cast samples were noted to exceed that of underground samples by 100 units, indicating a comparatively higher metal concentration and contamination in open-cast mines. The analysis according to seasonal variation showed pre-monsoon to have higher level of water contamination than the post-monsoon season.

Table 3: Mean HPI and HEI calculation of all the samples collected from the mines in two seasons

Underground coal mine samples							
Heavy Metals	***Mi*** Mean value (µg/L)	***Si*** Standard permissi-ble value (µg/L)	***Ii*** Ideal desirable value (µg/L)	***Wi*** Unit weightage	***Qi*** Sub index	***Wi*** x ***Qi***	H_c / H_{mac}
Fe	64497.5	1000	300	0.001	9171.071	9.171071	64.4975
Zn	2667.75	15000	5000	0.00006	23.3225	0.0014	0.17785
Cu	2.125	1500	50	0.0006	3.30172	0.00198	0.001417
Pb	32.625	10	0	0.1	326.25	32.625	3.2625
Cr	61.375	50	0	0.02	122.75	2.455	1.2275
Σ *Wi* = 0.12166; Σ *WiQi* = 44.247. **HPI** = 363.69. **HEI** = 69.17.							
Open-cast coal mine samples							
Heavy Metals	***Mi***	***Si***	***Ii***	***Wi***	***Qi***	***Wi*** x ***Qi***	H_c / H_{mac}
Fe	97023.75	1000	300	0.001	13817.68	13.81768	97.02375
Zn	1663	15000	5000	0.00006	33.37	0.002002	0.110867
Cu	3	1500	50	0.0006	3.241379	0.001945	0.002
Pb	39	10	0	0.1	390	39	3.9
Cr	147.375	50	0	0.02	294.75	5.895	2.9475
Σ *Wi* = 0.12166; Σ *WiQi* = 58.716. **HPI** = 482.62. **HEI** = 103.98.							

HPI- Heavy metal pollution index; HEI- Heavy metal evaluation index. Standards BIS IS10500: 2012, WHO (2011)

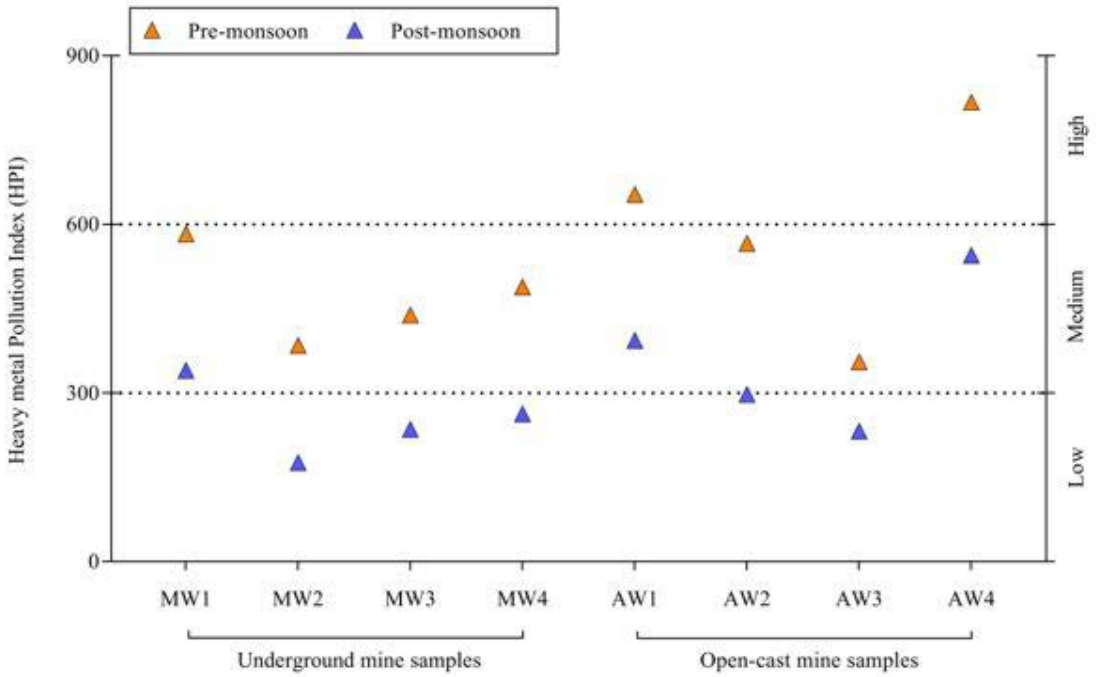


Figure 2: Categorization of water quality for samples from underground and open-cast coal mines based on heavy metal Pollution Index (HPI).

A similar pattern was observed by Singh and Kamal (2017) where they reported lower metal concentration in coal mine water bodies during the post-monsoon and higher in the pre-monsoon season. This could be due to the heavy rains during monsoon seasons causing strong water currents washing away most of the suspended and dissolved matter along with other debris. All water samples of pre-monsoon season were seen to fall in the medium range of pollution, while most samples of post-monsoon being in the low pollution range, according to their respective HPI index. Only two samples belonging to the open-cast mines were placed in the high pollution range (Figure 2). In the case of HEI, all the samples were noted to fall in the low pollution range with not much distinction seen between the two types of mine samples (Figure 3). These findings corroborates with the study by Bhuiyan *et al.* (2010) reporting similar HPI and HEI index values for quality analysis of water samples from coal mines. In a number of developing countries, water pollution indices have been successfully employed for determination of water quality assumed to be burdened by metal contamination (Edet and Offiong, 2002; Prasad and Mondal, 2008).

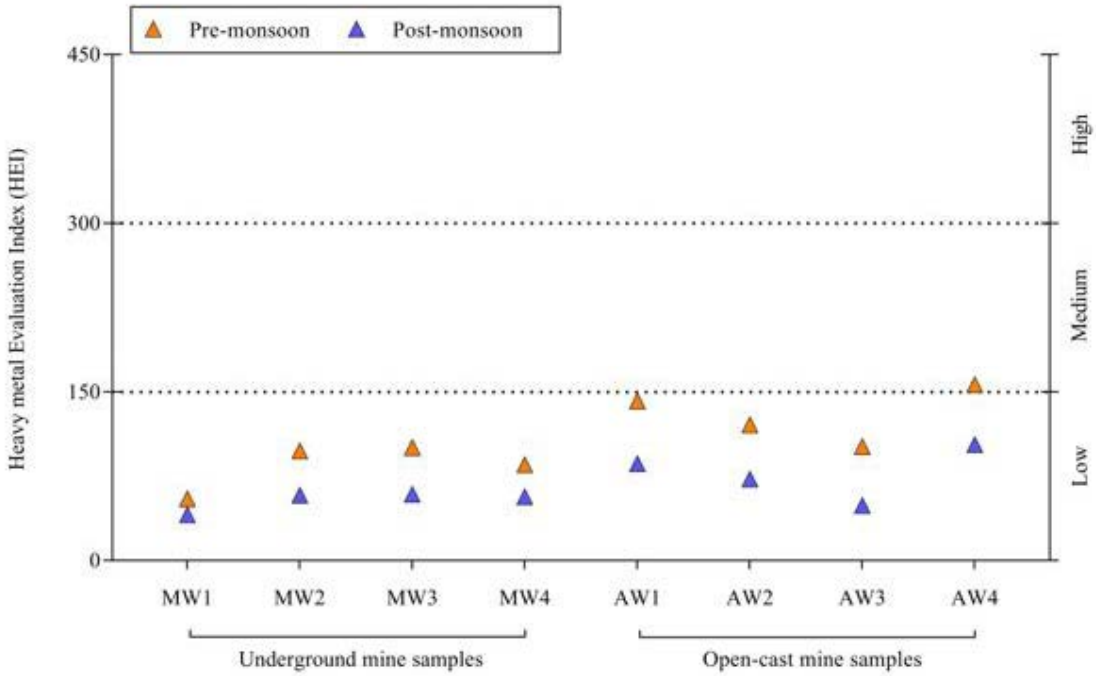


Figure 3: Categorization of water quality for samples from underground and open-cast coal mines based on Heavy metal Evaluation Index (HEI)

Table 4: Water pollution indices HPI and HEI of the water samples

Underground mine samples						
Samples	Pre-Monsoon			Post-Monsoon		
	HPI	Mean Dev	% Deviation	**HPI**	Mean Dev	% Deviation
MW1	582.909	109.1	23.05	340.516	86.84	34.24
MW2	384.383	-89.35	-18.86	176.056	-77.61	-30.60
MW3	438.919	-34.81	-7.35	235.238	-18.43	-7.27
MW4	488.707	14.97	3.16	262.868	9.198	3.63
	Mean 473.73			Mean 253.67		
Samples	Pre-Monsoon			Post-Monsoon		
	HEI	Mean Dev	% Deviation	**HEI**	Mean Dev	% Deviation
MW1	54.825	-29.77	-35.20	40.849	-12.88	-23.97
MW2	97.602	12.99	15.36	58.225	4.496	8.37
MW3	100.62	16.01	18.93	59.040	5.311	9.88
MW4	85.366	0.763	0.90	56.805	3.076	5.73
	Mean 84.603			Mean 53.729		
Open-cast mine samples						
Samples	Pre-Monsoon			Post-Monsoon		
	HPI	Mean Dev	% Deviation	**HPI**	Mean Dev	% Deviation
AW1	653.348	55.17	9.23	393.498	26.41	7.20
AW2	566.154	-32.01	-5.35	297.658	-69.42	-18.91
AW3	355.547	-242.6	-40.56	232.066	-135.0	-36.78
AW4	817.662	219.5	36.70	545.099	178.0	48.50
	Mean 598.12			Mean 367.08		
Samples	Pre-Monsoon			Post-Monsoon		
	HEI	Mean Dev	% Deviation	**HEI**	Mean Dev	% Deviation
AW1	141.882	11.70	8.99	86.328	8.542	10.98
AW2	120.792	-9.388	-7.21	72.639	-5.147	-6.62
AW3	101.502	-28.67	-22.03	49.052	-28.73	-36.94
AW4	156.544	26.36	20.25	103.128	25.34	32.58
	Mean 130.18			Mean 77.78		

HPI (Heavy metal pollution index): Low <300, Medium 300-600, High >600; HEI (Heavy metal evaluation index): Low <150, Medium 150-300, High >300 (Bhuiyan *et al.,* 2010).

Results suggest higher contamination of water bodies caused by open-cast mining of coal rather than underground mining. However, both types of mining operations are seen to cause uncontrolled leaching of these non-biodegradable heavy metals rapidly accumulating in the environment and finding their way into adjacent water and soil systems. Unscientific methods of coal mining are bound to cause detrimental impacts on water quality making it unsafe for domestic use or consumption. These contaminants continue to persist in the environment and are getting incorporated in food chain affecting biotic communities and vital ecological processes (Duarte *et al.,* 2008). This calls for the need of an innovative technology for controlling and restraining the careless leaching of mine effluents that could be integrated along with conventional mining procedures in order to reduce the harmful consequences related to mining of coal.

Conclusion

The complex and unmonitored methods of coal extraction has caused severe damage to the coal mining areas due to leaching of mine effluents into the surrounding environment. Such areas face an acute shortage of clean and safe water for consumption and domestic use. This study was taken up with the objective to assess the impact of heavy metals on the overall quality of water from underground and open-cast coal mines. The analysis was performed on metal-based water pollution indices HPI and HEI. The pollution index values revealed higher contamination in the pre-monsoon than post-monsoon season. Majority of the mine water samples fall in the medium range of pollution, with samples from underground mines showing slightly lower metal contamination in comparison to that of the open-cast mines. The degree of metal discharged from the mines could be influenced by the type of mining method employed for the extraction of coal. This study serves to provide a baseline information of the difference in the water quality impacted by metal leaching from underground and open-cast mines.

Acknowledgements

Authors acknowledge the financial support received from MoEF& CC, Govt of India (MoEFCC- NMHS/LG-2016/005) in the form of the research project. Authors acknowledge the support of DST-FIST[SR/FST/LSI-666/2016(C)] and UGC-SAP[F.4-7/2016/DRS-1(SAP-II)] for financial support to the parent department.

References

Bhuiyan, M.A.H., Islam, M.A., Dampare, S.B., Parvez, L. and Suzuki, S. 2010. Evaluation of hazardous metal pollution in irrigation and drinking water systems in the vicinity of a coal mine area of northwestern Bangladesh. *Journal of Hazardous Materials*, 179: 1065-1077.

BIS (Bureau of Indian Standards). 2012. Drinking water specifications 2nd revision. *Bureau of Indian Standards* (IS 10500: 2012). New Delhi.

Centre for Science and Environment, CSE .2012. Coal mining, pp 1-5. http://www.cseindia.org/userfiles/fsheet2.pdf.

Chandra, A., and Jain, M. 2013. Evaluation of Heavy Metals Contamination due to Overburden Leachate in Groundwater of Coal Mining Area. Journal of Chemical, Biological and Physical Sciences, 3(3):2317-2322.

Chaulya, S.K. and Chakraborty, M.K. 1995. Perspective of new national mineral policy and environmental control for mineral sector. In: *Proceedings of national seminar on status of mineral exploitation in India*, New Delhi, India, pp 114-123.

Cherry, D.S., Currie, R.J., Soucek, D.J., Latimer, H.A. and Trent, G.C. 2001. An integrative assessment of a watershed impacted by abandoned mined land discharges. *Environmental Pollution*, 111(3):377-388.

Coal Directory of India. (FY 1998-99 to 2016-17). Government of India, Ministry Of Coal, Coal Controller's Organisation Kolkata. http://www.coalcontroller.gov.in/pages/display/16-coal-directory.

Dhar, B.B. 1993. Environmental Scenario in Indian Mining Industry', in Chaudhary and Shiv Kumar (eds.), *Environmental Management, Geo Water and Engineering Aspects, Balkema Rotterdam*, pp. 615-619.

Dowarah, J., Deka-Boruah, H.P., Gogoi, J., Pathak, N., Saikia, N. and Handique, A.K. 2009. Eco-restoration of a high sulphur coal mine overburden dumping site in northeast India: A case study. *Journal of Earth System Science*, 118(5):597-608.

Duarte, S., Pascoal, C., Alves, A., Correia, A. and Cassio, F. 2008. Copper and zinc mixtures induce shifts in microbial communities and reduce leaf litter decomposition in streams. *Freshwater Biology*, 53:91-101.

Dutta, R.K. and Agarwal, M. 2002. Effect of tree plantations on the soil characteristics and microbial activity of coal mine spoil land. *Tropical Ecology*, 43:315-324.

Edet, A.E. and Offiong, O.E. 2002. Evaluation of water quality pollution indices for heavy metal contamination monitoring. A study case from Akpabuyo-Odukpani area, Lower Cross River Basin (southeastern Nigeria). *Geo Journal*, 57:295-304.

Energy Statistics. 2017. Annual Report of Central Statistics Office, New Delhi, Ministry

of Statistics and Programme Implementation, Government of India. http://www.mospi.nic.in/sites/default/files/publication_reports/Energy_Statistics_2017r.pdf.pdf

Ghose, M.K. and Majee, S.R. 2001. Air Pollution Due to OC Coal Mining and It's Control in Indian Context. *Journal of Scientific and Industrial Research*, 60:786-797.

Islam, R., Faysal, S.M. *et al*. 2017. Assessment of pH and Total Dissolved Substances (TDS) in the Commercially Available Bottled Drinking Water. *IOSR Journal of Nursing and health Science*, 6(5):35-40.

Johnson, D.B. and Hallberg, K.B. 2005. Acid mine drainage remediation options: A review. *Science of the Total Environment* 338:3-14.

Lyngdoh, I. and Kayang, H. 2012. Impact of coal mine drainage on water quality and microbial ecology of streams in Jaintia hills, Meghalaya. *International Journal of Current Research*, 4(2):002-007.

Mahato, M.K., Singh, G., Singh, P.K., Singh, A.K. and Tiwari, A.K. 2017. Assessment of Mine Water Quality Using Heavy Metal Pollution Index in a Coal Mining Area of Damodar River Basin, India. *Bulletin of Environmental Contamination and Toxicology*, 99(1):54-61.

Mukherjee, S., Pahari, D.P. 2019. Underground and Opencast Coal Mining Methods in India: A Comparative Assessment. *Space and Culture India* 7(1):39-55.

Prasad, B. and Mondal, K.K. 2008. The impact of filling an abandoned open cast mine with fly ash on groundwater quality: a case study. *Mine Water and the Environment*, 27:40-45.

Prasad, B., Kumari, P., Bano, S. and Kumari, S. 2014. Ground water quality evaluation near mining area and development of heavy metal pollution index. *Applied Water Science*, 4:11-17.

Radulescu, C., Dulama, I.D., Stihi, C., Ionita, I., Chilian, A., Necula, C. and Chelarescu, E.D. 2014. Determination of heavy metal levels in water and therapeutic mud by atomic absorption spectrometry. *Romanian Journal of Physics*, 59(9–10):1057-1066.

Rai, A.K., Paul, B. and Singh, G. 2011. A study on physic chemical properties of overburden dump materials from selected coal mining areas of Jharia coalfields, Jharkhand, India. *International Journal of Environmental Science*, 1(6):1350-1360.

Rayment, G.E. and Higginson, F.R. 1992. Australian soil and land survey handbook Soil chemical methods: Australasia. In: *Australian soil and land survey handbook. Port Melbourne*: Inkata Press, pp 1-3

Sahoo, P.K., Tripathy, S., Equeenuddin, S.M. and Panigrahi, M.K. 2011. Geochemical characteristics of coal mine discharge vis-à-vis behavior of rare earth elements at Jaintia Hills coalfield, northeastern India. *Journal of Geochemical Exploration*,

112:235-243.

Singh, G. and Kamal, R.K. 2017. Heavy metal contamination and its indexing approach for groundwater of Goa mining region, India. *Applied Water Science*, 7:1479-1485.

Soucek, D.J., Cherry, D.S., Currie, R.J., Latimer, H.A. and Trent, G.C. 2000. Laboratory and field validation in an integrative assessment of an acid mine drainage impacted watershed. *Environmental Toxicology and Chemistry*, 19(4):1036-1043.

Tiwary, R.K. 2001. Environmental Impact of Coal Mining on Water Regime and Its Management. *Water Air and Soil Pollution*, 132:185-199.

W.H.O (World Health Organization) .2011. Guidelines for drinking water quality. World Health Organization, Geneva.

Yao, Z., Li, J., Xie, H. and Yu, C. 2012. Review on Remediation Technologies of Soil Contaminated by Heavy Metals. *Procedia Environmental Sciences*, 16:722-729.

Zehirov, S., Kaykov, D. and Koprev, I. 2017. A Review of Combining Open-Pit and Underground Mining Methods around the World.Part II, Mining, Technology and Mineral Processing. *Journal of Mining Science*, 60:17-20.

Zhou, J., Dang, Z., Cai, M.F. and Liu, C.Q. 2007. Soil heavy metal pollution around the Dabaoshan mine, Guangdong province, China. *Pedosphere*, 17(5):588-594.

ISSN. 0972 - 8406
The NEHU Journal Vol. XVIII, No.2, July - December 2020, pp. 74-85

Death rituals and afterlife: The Ao-Naga boatman of the dead in Temsula Ao`s poems and the Greek mythological character of Charon

Harpreet Kaur Vohra

Panjab University Regional Centre,
Ludhiana-141001, India
Email: harvohra@rediffmail.com

Abstract

Death rituals and beliefs about the afterlife are uniquely marked by striking similarities on the one hand and also exhibit great variations across cultures, religions and geographies on the other hand. The paper intends to examine the Naga Ao belief of the boatman of the dead and ideas of the afterlife through the poems of Naga writer, Temsula Ao and compare it with the Greek boatman of the dead, Charon. How do these beliefs intersect, and are these intersections larger commentaries on other death rituals and beliefs. What are the reasons that the afterlife holds such sway in myth, ritual and religion? The epistemological and mythical underpinnings of these viewpoints open up myriad interpretations and also offer travelling back in time to exhibit that several ideas about death and the afterlife surpass usual compartmentalization of ethnicity, race, culture and religion owing to their highly enigmatic nature and also to their inability of scientific corroboration and concrete connections with human ontology. The purpose of this comparative analysis is thus to prove that the commonality between the Ao Boatman of the Dead and the Greek boatman Charon does not necessarily indicate any kind of exchange between the cultures but the ever- lingering strong human desire to adequately prepare the dead for the afterlife with the help of a spiritual guide on that onward journey. The dead cannot be left to fend for themselves and hence the boatman is emblematic of death as guided tour which is part of the debt that the living owe to the dead.

Key words: Death rituals, afterlife, boatman of the dead, Naga practices, Greek mythology, Charon

Introduction

Temsula Ao in her poem *Nowhere Boatman* crafts this mythical figure culled from an Ao-Naga belief of a boatman whose primary function is to ferry dead souls across the river between the Land of the Living and the Land of the Dead. This boatman bears several similarities to Charon, the Greek mythological character who ferries souls across the river Styx. This similarity with Greek mythology is quite remarkable, as interactions between the tribe and the Greeks do not seem to have been possible at all. However, an equally opposite fact also holds good that such ideas of soul-ferryman and death-journey

cut across cultures. Another interesting aspect of the boatman is that he does not do his job gratis but for a fee. Hopkinson and Kimberley articulate that the idea of the ferryman of Netherworld is not just prevalent in civilizations like that of Egypt and Greece but also found in other ancient cultures like the Yorubas of south Nigeria, Mesopotamia, Ancient Europe, Rome, and Norse of Denmark (Hopkinson *et al*., 2008). They further mention that the practice of paying the ferryman with coins was disparaged as pagan practice but despite the power of the Catholic Church, this belief of paying the boatman survived into medieval and post-medieval times, though at a reduced rate.

Temsula Ao is celebrated for her poetry on Ao beliefs and practices and her collection of poems *Books of Songs* (2013) has an entire section *Songs from the Other Life* of poems revolving around Ao beliefs and customs which range from mythical imaginings about the first Ao people, death, afterlife, companion spirits and the like. A poem of the same collection, *Soul Bird,* tells of how, after death, the soul takes the form of a hawk and thus sighting one after a person's death was a good omen. These myths bear several similarities with Greek folklore pertaining to mysteries of death and afterlife and such an understanding enhances the etiology of an otherwise inexplicable geography of "this world" and "that world" and "underworld".

GJV Prasad in his introduction to Temsula Ao's poetry collection *Book of Songs* remarks of her that "she is a poet who sings of her life and that of her people. She searches for the past that has disappeared into the mists of time, for it is in the very unrealisability of that history that her people's troubled present arises; the disjunctions and dislocations in their past have given rise to their mistranslated and tense present" (Ao, 2013). *The Nowhere Boatman* is a poem of death (like the eponymous book itself) and brings before the reader the belief of the Ao people in the mystical boatman ferrying souls through the river of the dead and through this journey also negotiates several questions of identity of the Aos. Devastuti Sharma says that while Ao accepts that preserving unspoiled tribal culture is not attainable, she also acknowledges that this results in " cultural chaos" and this is what is perhaps hinted at in *Nowhere Boatman* where she describes the ageless boatman as being stuck on the river between two irreconcilable worlds, referring to the oscillation between perhaps, the conflicts and indecisiveness occurring in the minds of people while choosing between "indigenous identities and their globalized counterparts"(Sharma, 2015).

Poetry based on rituals, whether religious or folk have acted as important indicators of the relationship that communities have with the larger universe they inhabit. It points to beliefs that have established long lasting relationships of the individual to his/her environment and have gone far beyond temporal spaces to inhabit the transcendental plane. While ritual poetry in early Japan, for example, participated in more general complex beliefs and practices involving the magico-religious powers of special works (Ebersole, 1989), Margaret Alexiou's classic 1974 work *The Ritual Lament in Greek Tradition* discusses the ritual lament as essentially functional in nature being only one part of a complex tradition of ritual custom and belief (Alexiou, 2002). Barabara Babcock in

her essay *Too Many, too few: Ritual Modes of Signification* says that Anthropologists like aestheticians, have generally identified art and ritual with "order and unity" defining the latter, for example as an "attempt to inspire logical necessity upon the vagrant affairs of the world" (Babcock, 1978). Neil Price, in his research on Viking-Age mortuary drama says that Viking-Age burials may have involved complex elements of mortuary theatre, ritual narratives literally enacted at the graveside, providing a poetic passage for the individual dead into a world of ancestral stories (Price, 2018).

Death Rituals: Anthropological Explanations

The discipline of Anthropology has been the site where rituals relating to death have been closely imagined. While such rituals differ greatly in manifestation across cultures and religions, anthropological interventions will reveal that the purpose of such rituals, bear a similarity of rationale, thus easily rending the culture/geography/religious divide. Death and death rituals has been the subject of study across cultures and beliefs. In many traditions, the symbolism of tombs and wombs are never far apart. "Death is an ending – we are indeed all going to die – but it is also a moment of transformation, potentiality, and beginning" (Simpson, 2018). The work of ritual officiants and the participants they assemble enable the dead to be resurrected and regenerated in ways that are meaningful to those that are left behind. In this regard, Robert Hertz's intensive work on death rituals and his essay "A Contribution to the Study of the Collective Representation of Death" which was written in 1960 still continues to be one of the most read articles on the subject.

He has also said that in most primitive societies the dead bodies are only stored, so to speak, in the tomb where they are first placed. After a time they are given a new funeral and they receive the final funerary rites which are due to them. This difference in Custom is not,.. a mere accident (Hertz, 1960). Death rituals go back to what people hold sacred and how they wish to 'arrange' for the dead. In June 2005 Pierre Lemonnier and Eric Venbrux organized a conference of the European Society for Oceanists (ESfO) in Marseille on 'Spiritual material: Objects and change in mortuary ritual,' where they returned to the basic tenet of Hertz's theory that ''to make a material object or living being pass from this world to the next, to free or create the soul, it must be destroyed. [...] As the visible object vanishes it is reconstructed in the beyond, transformed to a greater or lesser degree'' (Hertz, 1960). There are cases in which the deceased's intimate possessions are destroyed, but also instances in which objects of the dead are kept as relics or heirloom (Venbruex, 2007).When death rituals are performed, the expectation that the dead would be reborn into a new world and in a new body become paramount concerns. Every life-cycle ritual implies the passage of one group to another: an exclusion, i.e. a death, and a new integration, i.e. a rebirth The rebirth which occurs at death is not only a denial but also a "reassertion of society and a renewal of life and creative power" (Bloch and Parry, 1982). The journey motif also points to this direction-a journey to a new place, among newer souls.

The afterlife is a pivotal concern that follows several death rituals and most rites

of passage are seen as a path to this objective. Douglas Davies, in Death, Ritual and Beliefs says that both Hertz and Hocart placed great importance on mortuary rituals. While Hertz believes they serve some 'biological function of religion', one that saves man from surrender to death and destruction and reinforces the desire for life, Hocart placed great emphasis on ritual that helped people in 'securing life' or in 'promising life' (Davies, 2017). The death-life continuum occupies centre stage and making all necessary arrangements in terms of material possessions to be buried with the dead or elaborate rituals, all serve the purpose of preparing for what lies beyond. It is the duty of the family and the community to help the dead qualify to enjoy a journey of transcendence and hope. Thus, even after death, hope always exists and adequate measures have to be taken to allow the transition from the dead to the living.

The image of the Ao boatman has conspicuous similarities with the Greek mythical character Charon who takes souls across the river Acheron. The Encyclopedia of Death and Dying defines Charon of Greek mythology as a ferryman of the dead. Hermes (the messenger of the gods) brings to him the souls of the deceased, and he ferries them across the river Acheron to Hades (Hell). Only the dead who are suitably buried or burned and who pay the obolus (silver coin) for their passage are accepted on his boat, which is why in ancient Greek burial rites the corpse always had an obolus placed under its tongue. A rather sombre and severe character, Charon does not hesitate to dislodge from his boat, without pity, the souls whose bodies received improper burial or cremation.

Reference to the fear of languishing in No-Man's land between the dead and the living have found echoes in several works of literature like *Antigone* by Sophocles and almost unforgettable among them all is Dante's *The Divine Comedy* and its description of Charon:

> And lo! toward us in a bark
> Comes on an old man, hoary white with eld,
> Crying, "Woe to you, wicked spirits! hope not
> Ever to see the sky again".

Even a legal luminary like Lord Alfred Denning in his book *The Discipline of Law* has used the imagery of the river Styx to talk about "the group of ghosts of dissatisfied testators…wait on the other bank of the Styx to receive the judicial personages," as these lawyers had misconstrued their wills, making their kin suffer (Levinson and Mailloux, 1988).

The Aos place the entrance to the world of the Dead which lies under the earth… and call the line of white rock leading up to it *layasuphu* (Mills, 1973). On their way to the land of the dead, the souls must cross a stream called Lungritsu, the boundary between the living and the dead. If a person reaches this stream, but does not cross it, he/she can return to the land of the living. The boatman of the dead has to be paid some coins (like Charon) to cross the other side of the river, where it is believed, they begin another

existence (Ao, 2013).

Temsula Ao in her poem *Nowhere Boatman*, fictionalizes the journey of the dead souls while she shifts the focus of her attention to the life of the boatman. She invests in him a primeval existence and a sorrowful longing, quite literally transferring the pain of the dead souls onto his own. The poem begins with the boatman saying that his passengers often ask him how long he has been plying this trade on the river between the "land of the living and the land of the dead," a question he finds strange as he represents that which is ageless and he is not sure whether he belongs to the world of the dead or to the world of the living. He knows that he is ageless and timeless and he also knows that he can expect no deliverance from the primeval job that he has been doing. Temsula turns the image of the boatman into a character who seems to want deliverance himself- an inverted conundrum. He sees the valueless meaning of the coins that jingle in his boat:

A soul without a status,
Is how I see myself?
Fated to ply my trade
On this designated route. (38-41)
A little later he again ruminates on his status:
When my own immaterial
Existence knows not
Whether it can claim
Any kinship with the living
Or one day join the dead
Or on a final crossing. (51-56)

The image of the river separating the world of the dead and the world of the living is an idea intrinsic to Greek belief about death and the journey to heaven. Like them, the Ao Nagas believe that there is the world of the dead souls (Asuyim). In between the world of the dead and the world of the living there is a boundary line. The boundary line is a river called Lungritsu or Bitter water, as mentioned earlier (Vatsyayan, 1995). The boatman seems to be languishing on the borders himself:

What advantage
This petty exchange
For the un-remitting service
Of re-locating well-defined souls
In preordained spaces? (46-50)
And some lines later:
Yet I continue to exist.
An ageless, nameless

Indispensable anomaly.

There is much controversy about the etymology of the word Ao, although according to local tradition as well as was observed by J.P. Mills, it is a corruption of the word Aor meaning going across the river Dikhu, a boundary river (Imchen, 1993) although there are many other explanations of the term as well. Crossing rivers however acts as a rite of passage in several traditions and in the Ao belief of life after death, a journey across the river becomes emblematic of physical transposition. The belief of crossing the river was an ancient belief much like the belief in Charon ferrying souls across Acheron.

Naga Practices of the Dead

Beliefs and practices related to death form an important part of the collective consciousness of every people. In tribal communities these beliefs and practices involve distinct and elaborate practices related to disposal of the death and belief in the afterlife. Among the Naga tribes, several practices were held to be common, but a great degree of variation existed among them in their practices. As early as 1854, Francis Jenkins made brief comments on the Angami funeral custom and on the manner of disposal of their enemy skulls. Angami is another major tribe of Nagaland and one of the most widely researched tribes of India. The Ao practice of the dead shares some similarities with that of other tribes of Nagaland. The Aos, Konyaks, Ponhurys, and Wanchos expose their dead on platforms close to the dwelling-houses of the living and often keep the corpse for a long time in the house (Kanungo, 2011). The corpse would be allowed to fall on the platform. In rich households the bodies were left to be smoke-dried on a platform for five and six days for women and men respectively and then on a fixed day, the corpse is taken to the burial ground and put on a machan and left till the machan rotted.

The traditional practices of the Nagas, like that of several tribes of the North Eastern region were affected by the coming of Christianity. Several of the practices that were followed for centuries were declared pagan-like by the missionaries. There were however, some points of convergence with regard to the perceptions of God, Heaven and Hell. The Nagas believed that there was a world to aspire for after the death of a human being and this translated roughly to the perception of Christian paradise. Hence even burial rituals prepared the dead for a life beyond, far from the definitions of human existence. Nagas believed in life after death and that the human spirits go either to the land of the dead or to paradise. The land of the dead was believed to be a dead and colourless underworld where one had to labour and cultivate whereas paradise was a land of complete abundance and bliss. With such a strong concept of paradise as part of Naga beliefs, the later Naga Christians believed that it was through Jesus Christ that they could attain paradise in the life after (Bendangjungshi, 2011).

Various myths related to the departed souls exist among the Aos. One strong belief is that the departed souls pass through ‘Asür rikhüm’, which is known as ‘the gate of departed souls’. It is about one kilometer from Longkhum village. The souls after passing through this gate come to another place called ‘Longri-tzü-lenden’ (Longri-bitter, tzü-

water; lenden-valley). It is believed that the water in this valley is bitter because the departed souls quench their thirst at this place. They drink not only the water but also wash their heads with 'Shitsüng' (a kind of bitter seed which is used like soap). After crossing Longkhum village, they come upon a hill in Wokha where the female departed souls dry their clothes. The Aos called this particular place 'Lazasübo' (a place where maidens dry clothes). Since all the departed souls cross Longkhum village, people believe that a person should visit Longkhum village not only once but twice, so that they can bring back their souls who are believed to be left behind on their first visit.

Several religions of the world comprehend death as a kind of "journey between two worlds that can literally take time and involve challenges and obstacles" (Eller, 2007). Eller also tells us about how the Konyak Nagas declared that the dead needed to carry weapons on their trip to the future home, since each would meet and fight again all of the warriors that they had killed in battle while they were alive. In the afterlife, they believed conditions were similar to life and men were always reunited with their first wife (47). Temsula Ao in another poem *Soul-Bird*, tells of a Ao- Naga belief that when a person dies, the soul takes the form of bird, more specifically a hawk, an insect or even a caterpillar. The sighting of hawks soon after a person's death is considered to be a good omen suggesting "the transition is complete and it is the last appearance of the loved one on earth" (Ao, 201).

She slowly turns heavenwards (said by grandmother)
As her red-rimmed eyes
Settle on the circling silhouette
And then with a sudden
Unseemly whoop
She draws me closer
Whispering in my ear,
"See that keening bird in the sky?
That's your mother's soul
Saying her final good-bye
It is over
Come, let us go home now" (30-41)

There are several similarities between the beliefs related to death and rituals between the Ao Nagas and tribes of Borneo and Fiji as well. On the face of it, it appears to be coincidental but on close analysis it does not seen to be merely fortuitous. There could have been some exchanges between these different people during those times- not just casual but long standing in terms of some amount of acculturation. Similarities in ritual and beliefs of the afterlife can also be attributed to common beliefs of indigenous societies. The other God of the Aos - Tsungrem was Meyutsungba, the God of the "Land

of the Dead". The belief of the people in existence of such a place for the dead was derived from the concept of the soul or tanula as the Aos call it which leaves the mortal body at death and travels to this land for another existence (Subba and Som, 2005).

Just as much as race, religion and ethnicities dictate diversities in beliefs, rituals and collective knowledge, it is also surprising how many similarities could exist in the realm of human experience. The similarity between the boatman of the dead in Greek mythology- Charon and the boatman of the dead in the Ao-Naga tradition is a striking similarity which creates scope for several anthropological interventions. The Ao rituals of the dead were elaborate like the Greeks, thus preparing the soul for inhabiting another world after traversing the land of the living.

The Greek Boatman of the Dead

In early Greek sources, some dead persons are led to the realm of the dead by Hermes in his role as escorter of souls (psychopompos). Hermes enchanted the souls of slain men with his wand and the souls fluttered after him like many birds. The image of Charon as a ferryman of the dead souls into the other world appeared at a later date (Hansen, 2004). In later sources we hear of the ferryman Charon, who for a fee transports the newly dead across the stream that separates the realm of the living from that of the dead. In order that the deceased person has the necessary fare, kinfolk placed a coin in the mouth of the corpse. In the absence of purses and pockets, the mouth was one of the places that Greeks carried coins in daily life, so that it was natural to equip a body with Charon's fair (Hopkinson *et al*.,2008).

Charon as a character finds mention in several works like Shakespeare's *Troilus and Cressida*, Ben Johnson's *Cataline* and more famously ''Dante's The *Divine Comedy*' among others. At Delphi there was a building called Lesche with the painting of Polygnotus portraying Charon. He seems to have derived his inspiration from the poem *Minyad* in which there is a passage about Theseus and Pirithous: "Then the bark of the dead which the old ferryman Charon was wont to guide, they found not at its moorings" (Sullivan, 1950).

Bzinkowski speaks of Charon in such a manner: The image of Dante's Charon is undoubtedly the one that has haunted European imagination and inspired painters such as Gustave Doré (1832-1883), French engraver and illustrator known, among other things, for his illustrations of *Divine Comedy*, or Alexander Litovchenko (1835-1890), a Russian painter, who was awarded a lesser gold medal for the picture of Charon transporting the dead (Bzinkowski, 2009).

The infernal ferryman is a recurrent figure, who takes the traveller to the land of the dead and in creating this journey, also creates a concept of physical location informed by the conviction that the dead must be somewhere and this concept is pervasive. (Fletcher, 2019). Philip Hardie observed that for both Virgil and Spenser, "the mythological underworld is a vivid setting for issues of cosmic, psychological, theological and moral

order and disorder" (Fletcher, 2019). The Boatman of the dead- Charon also appears as a mysterious personage who is man and yet, not alive and who is a soul himself, yet to be delivered. The interesting requirement of money that Charon collects as fare deepens the wordly versus the spiritual debate as Charon has no use for money, but money is an important rite of passage. This goes back to the Greek belief that materialistic objects would be required by the dead when they passed into a new world and all arrangements had to be made for the new life that the dead were to lead. The river and the journey across it also have similarities in many cultures as journeying across seas and rivers, like the quintessential treks up mountains to religious places, were seen as almost mandatory pilgrimage journey and essential for the pilgrim's progress.

Greek Rituals of the Dead

Death, in Greek belief, was never a mere event; it was a transition- one that required elaborate preparation and rituals to be afterworld-ready. These included not merely spiritual preparedness but also attention to physical requirements and protection of the dead body. Emily Vemeule says that the Greek traditions of burial have "the longest surviving and most powerful of all traditions" which saw no visible change from the later Bronze Age to the Hellenistic World and apart from the "intervention of the church into private conduct", it is unchanged even today (*Aspects of Death in Early Greek Art and Poetry*, 1979). The dead were considered sacred entities and their influence existed far beyond the limits of the cemeteries. Herodotus provides a textbook example of how relations between the living and the dead were meant to function. He said that the dead demand proper funerals which "ought to include gifts which they can use in the afterlife". The living for their part can "expect the dead's cooperation so long as they keep the dead happy" (Johnston, 1999). Death rituals are first and foremost rites of passage, and Greek death rituals very often seem "quite foreign, bizarre and "exotic" to people from the north of Europe and the US" (Haland, 2014). Yet they form a part of everyday practice.

The rituals that the Greeks performed for the dead consisted of both elaborate as well as systematic procedures. Prothesis, Ekphora, and Commemorative Feasts were generally the stages of preparing the body, burial and after-burial celebrations. As part of Prothesis, the body was left in a breath of air. Thereafter it was bathed and anointed. To ensure successful passage across the river Styx, a coin was placed in the deceased's mouth to pay for the ferry. Throughout this time, haunting laments could be heard by both family members as well as by professionals hired to lament. In the early hours of the morning following prothesis, the dead was carried by pallbearers or by a horse-drawn carriage along the streets in order to reach the cemetery for the burial. This procession, called ekphora, included musicians, friends, and family, all involved in lament and general expression of grief. The ancient Greeks were greatly encouraged to mourn publically in solidarity with the dead. Once the procession reached the cemetery, the deceased was placed in a larnax, a small, often elaborately carved box. From there, it was either cremated on a funeral pyre or interred, the grave marked by a commemorative stele to ensure that the departed would not soon be forgotten. Following the burial or cremation, a feast hosted was a mark of

gratitude to those who participated in the funeral rites. Owing to the importance given to perpetual remembrance of the dead, there was an emphasis placed on visiting the grave, particularly on the 3rd, 9th, 13th, and 30th days after the burial took place. The tomb was decorated and offerings were made in the form of food and wine (*A History of Greek Funerals*).

There even was an elaborate manner in which the dead body had to be laid on the floor and all rituals were sacrosanct in this matter. As soon as the "moment of dying" was over, the body was prepared for the wake. The mouth and the eyes were closed and the body was washed, anointed and dressed by the women, usually in white. The body was then laid on a bier with the feet towards the door. Sometimes it was strewn with marjoram, celery leaves and other herbs, believed to ward off evil spirits (Alexiou, 2002). The Libations that were to be given to the dead were also elaborate and had to be administered in a certain way. Readers of Greek literature have seen its importance in *Antigone and The Euminides*. The Furies and their unappeasing nature were also closely tied to the libations. The libation, in general terms is a liquid poured out as a consecration. It was done to the accompaniment of simple prayer. Patton says that in chthonian sacrifice it sinks to the ground- if "honey, milk or oil, literally understood as nourishment for the dead, or if water, their bath; it may also head to the underworld in honour of the powers who dwell below" (*Religion of the Gods*, 2009).

The importance accorded to the dead in Greek traditions account for the elaborate rituals and the endearing connections that the dead were to have with the living also engendered this reverence. This dictated several beliefs and practices which have kept the life-death continuum going for millennia.

Conclusion

Thus, the similarities between the Ao boatman and the Greek boatman of the dead bring out significant mythological, theological and temporal aspects about death, the afterlife and ways of equipping a human being for these experiences. Temsula Ao, by depicting the boatman as a languishing figure also makes the character symbolic of certain questions related to identity, the tribe and indigenous concerns. The many efforts that the living make to provide the dead with the wherewithal for the afterlife adequately proves that the living and the dead can never be severed. This unique relationship is perpetuated both through ritualistic practice and commemorative ceremony, thus showing both the enduring as well as enigmatic nature of death and rituals related to it. The vision of the afterlife and journey as leitmotif only assist in the understanding of this complex matrix. Death, by its very definition states a closure, but death rituals and the boatmen of the dead present in themselves, a vision of life after death and that continuum which has intrigued civilizations and cultures.

References

A History of Greek Funerals. https://basicfunerals.ca/cultural-funerals/history-greek-funerals/

Ahmed, R. and Sayed, A.R. 2016. The Celestial Ferryman in Ancient Egyptian Religion: Sailor of the Dead. *Journal of the General Union of Arab Archaeologists*, 1(1):126-165.

Alexiou, M. 2002. *The Ritual Lament in Greek Tradition*. Maryland: Rowman and Littlefield

Allen, M. translator. 1995. *The Divine Comedy*. By Alighieri Dante, New York: Everyman's Library Classics.

Ao, T. 2013. *Book of Songs*. Delhi: Heritage. Babcock, A. B. 1978. Too many, too few: Ritual Modes of Signification. *Semiotica*, 23(3/4): 291-302.

Bendangjungshi. 2011. *Confessing Christ in the Naga context: Towards a Liberating Ecclesiology*. Berlin: Contact Zone.

Bloch, M. and Parry, J., editors. 1982. *Death and the Regeneration of Life*. New York: Cambridge University Press.

Bzinkowski, M. 2009. Charon Psychopompos? Tracing the Continuty of the Idea of a Ferryman of the Dead in Greek Culture. *Classica Cracoviensia*, I3:17-33.

Chandra, N.D.R, and Sentinaro, I. 2010. A Discourse on Ao Naga Folktales. *Journal of Literature, Culture and Media Studies*, II (I): 227- 236.

Davies, Douglas. 2017. *Death, Ritual and Belief: The Rhetoric of Funerary Rites*. London: Bloomsbury.

Ebersole. G.L. 1989. *Ritual Poetry and the Politics of Death in Early Japan*. New Jersey: Princeton University Press.

Eller, J.D. 2007. *Introducing Anthropology of Religion*. New York: Routledge.

Fletcher, J. 2019. *Myths of the Underworld in Contemporary Culture*. Oxford: OUP.

Haland, E.J. 2014. *Rituals of Death and Dying in Modern and Ancient Greece: Writing History from a Female Perspective*. US: Cambridge Scholars.

Hansen, W.F. 2004. *Handbook of Classical Mythology*. Oxford: Oxford University Press.

Hertz, Robert. 1960. *Death and the Right Hand*. Illinois: The Free Press.

Hopkinson, K, *et al*. 2008. For Whom the Coin Tolls: Green Stained Teeth and Jaws in Medieval and Post-Medieval Spanish Burials. *Dental Anthropology*, 21(1) :12-17.

Imchen, P. 1993. *Ancient Naga Religion and Culture*. New Delhi: Har-Anand. Johnston, S.I.1999. *Restless Dead: Encounters between the Living and the Dead in Ancient Greece*. California: University of California.

Kanungo, A. K. 2011. Ornaments of the Dead among the Nagas. *Journal of the Borneo International Beads Conference*. 75-104.

Kastenbaum, R. 2003. Encyclopaedia of Death and Dying. Macmillan E-book. http://www.deathreference.com/Ce-Da/Charon-and-the-River-Styx.html.

Levinson, S., and Mailloux, N. 1988. *Interpreting Law and Literature: A Hermeneutic Reader*. Illinois: North western University Press.

Mills, J.P. 1973. The *Ao Nagas*. Bombay: OUP.

Patton, K.C.. 2009. *Religion of the Gods: Ritual, Paradox and Reflexivity*. USA: OUP.

Price, N. 2018. Passing into Poetry: Viking-Age Mortuary Drama and the Origins of Norse Mythology. *Medieval Archaeology*, 54(1) 123-156. doi: 10.1179/1745817 0X12790370815779.

Sharma, D. 2015. Temsula Ao as an Exemplary Contemporary Tribal Poet of Northeast India: A Reading of Selected Poems. *Drishti*, IV(I):.32-36.

Simpson, B. 2018. "Death". In *The Cambridge Encyclopedia of Anthropology*. F. Stein *et al.,* editors. http://doi.org/10.29164/18death.

Sullivan, F. A. 1950. Charon, the Ferryman of the Dead. *The Classical Journal*, 46(1):11-17.

Subba, T.B. and Som, S. (Ed). 2005. *Verrier Elwin and the Tribal Question in India*. New Delhi: Orient Longman.

Vatsyayan, K. 1995. *Prakrti: The Nature of Matter*. Indira Gandhi National Centre for the Arts.

Vemeule, E. 1979. *Aspects of Death in Early Greek Art and Poetry*. California: University of California.

Venbruex, E. 2007. Robert Hertz's Seminal Essay and Mortuary Rites in the Pacific Ocean. *Journal de la Societe des Oceanistes*, 124(1): 5-10.

ISSN. 0972 - 8406
The NEHU Journal Vol. XVIII, No.2, July - December 2020, pp. 87-100

Voltage dependent anion channel-mediated apoptosis: Its role in the pathogenesis of diabetic complications

*Melinda Nongbet Sohlang, Suktilang Majaw**

Department of Biotechnology and Bioinformatics
North-Eastern Hill University, Shillong-793022, Meghalaya, India

*Corresponding author : smajaw.nehu@gmail.com/smajaw@nehu.ac.in

Abstract

Apoptosis or programme cell death is a normal phenomenon required for maintaining cell homeostasis. Several studies indicate that excessive or insufficient apoptosis can lead to various diseases. In diabetes enhanced cellular apoptosis has been associated with micro and macro-vascular complications. Accumulating evidences suggest the involvement of voltage-dependent anion channel (VDAC) towards increased apoptosis observed in diabetes. VDAC, a multifunctional mitochondrial porin is a protein located on the outer mitochondrial membrane which contributes to apoptosis either through Ca^{2+} overload into mitochondria or by release of apoptotic protein from the mitochondria by opening the mitochondrial permeability transition pore (mPTP) or rupture of the mitochondrial outer membrane. Here, we provide a short review on VDAC-mediated apoptosis and its pathogenic role in the progression of diabetic complications.

Keywords: Apoptosis, diabetes, voltage-dependent anion channel, diabetic complications.

Introduction

Diabetes is a metabolic disorder characterized by hyperglycemic condition caused by deficiency in the secretion of insulin, ineffective insulin action or both (American Diabetes Association, 2009). There are two major forms of diabetes: type 1 (T1D) and type 2 (T2D). T1D results from insufficient insulin production due to destruction of β cells of the pancreas (American Diabetes Association, 2009). T2D results from impaired insulin secretion and insulin resistance and is associated with obesity, lack of exercise, stress, as well as aging. In both types of diabetes, the late diabetic complications in different tissues arise from chronic or intermittent hyperglycemia (Forbes and Cooper, 2013). Hyperglycemia-induced cell death due to apoptosis appears to play a pivotal role in micro-and macro-vascular complications such as angiopathy, retinopathy, neuropathy, atherosclerosis, impaired wound healing and periodontitis (Allen *et al.,* 2005). The present review will discuss the role of cellular death in diabetes with particular emphasis on apoptosis-mediated *via* voltage-dependent anion channel (VDAC) in the pathogenesis of diabetes as a disease.

Apoptosis in diabetes

Apoptosis or programme cell death is a coordinated series of events which is tightly regulated and occurs as a homeostatic mechanism to maintain cell population in tissues (Lee and Pervaiz, 2007). Apoptosis also occurs as a defence mechanism such as in immune reactions or when cells are damaged by disease or noxious agents. However, when apoptosis malfunctions, it results into a variety of pathological states where failure in apoptosis could lead to cancer, autoimmune diseases while excessive apoptosis could lead to cell loss such as in HIV/AIDS, neurodegenerative diseases and diabetes mellitus (Lee and Pervaiz, 2007). There are many mechanisms and pathways that can initiate apoptosis but the two distinct pathways are (i) extrinsic or death receptor pathway and (ii) intrinsic or mitochondria-mediated pathway as shown in Figure 1.

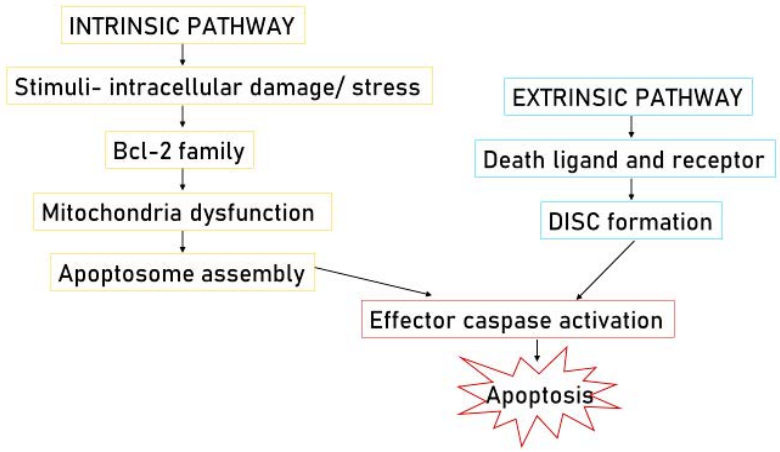


Figure 1. The intrinsic and extrinsic pathway of apoptosis. Apoptosis can occur through either one of the two pathways: extrinsic (death ligand and receptor mediated which involve TNF, Fas as some of the ligands with formation of death-inducing signalling complex, DISC being a critical step in Fas-mediated apoptosis). The latter pathway results from mitochondrial dysfunction which releases cytochrome *c* and subsequent activation of the caspases.

Extrinsic pathway is triggered following signals through death receptors such as tumour necrosis factor (TNF) family death receptors; the intrinsic pathway is activated by cellular stress such as oxidative stress (Zhaoyu and El-Deiry, 2005), Ca^{2+} overload and is regulated primarily by the pro-apoptotic and the anti-apoptotic members of the Bcl-2 family. Both pathways activate a cascade of proteolytic enzymes called caspases that mediate the rapid dismantling of cellular organelles and architecture although caspase-independent pathways have also been reported (Leist and Jaattela, 2001).

Several reports suggest the occurrence of enhanced apoptosis during diabetes i.e., in both T1D and T2D. Among which, apoptosis of β cells has been discussed most widely under

diabetes (Wali *et al.*, 2013). In T1D, β-cells are destroyed by immunological mechanisms, whereas in T2D metabolic abnormalities contribute to β-cell failure and subsequent apoptosis. Therefore, regardless of the diabetes type, highly conserved intracellular pathways of apoptosis are triggered. In T1D and T2D, β -cell death occurs by both the extrinsic and intrinsic pathway (Cnop *et al.,* 2005). Similarly, in other cells/tissues, both the pathways have been found to play a role under diabetes. Reports of apoptosis in other cells/tissues such as renal (Habib, 2013), hepatocytes (Schattenberg and Schuchmann, 2009), cardiomyocyte (Ouyang *et al.*, 2014), retina cells (Barber *et al.*, 2011) have been shown to cause diabetic complications such as diabetic nephropathy, diabetic neuropathy, liver injury, diabetic cardiomyopathy, diabetic retinopathy respectively (Feenstra *et al.*, 2013). The mechanisms that lead to diabetes-induced cell death are complex and are not fully understood but several lines of evidences have implicated that high glucose promotes the upregulation or activation of several molecules involved in different pro-apoptotic pathways and there are no specific pathways for high glucose-induced cell death. Many studies have demonstrated hyperglycemia-induced or glucose-induced intrinsic apoptosis in beta-cells (McKenzie *et al.,* 2010; Wali *et al.*, 2013) endothelial cells (Peng *et al.,* 2013), mesangial cells (Mishra *et al.,* 2005) etc. In intrinsic mitochondria apoptosis pathway cytochrome *c* is released from the intermembrane space of mitochondria through a process called mitochondrial outer membrane permeabilization (MOMP). MOMP is regulated by Bcl-2 protein family. The balance of pro- and anti-apoptotic Bcl-2 proteins in mitochondriał and endoplasmic reticulum membranes regulates MOMP in part by regulating calcium compartmentalization. Bcl-2, Bcl-XL and Mcl-1 prevent MOMP whereas pro-apoptotic members, Bax and Bak activate MOMP (Kroemer *et al.*, 2007). Once released, cytochrome *c* binds Apaf-1 (Apoptotic proteases activating factor), dATP and procaspase-9 creating a complex called apoptosome which activates procaspase-9 to cleave into caspase-9 generating caspase-3 eventually causing apoptosis. MOMP can also be initiated by an abrupt increase in the permeability to ions and small solutes of the inner mitochondrial membrane (IMM) by a process known as mitochondrial permeability transition (MPT). MPT appears to be mediated by a multiprotein complex that is assembled at the juxtaposition sites between the outer mitochondria membrane (OMM) and inner membrane (IMM), the so-called permeability transition pore complex (PTPC) (Kroemer *et al.*, 2007). One of the main components of the PTPC, is the mitochondrial voltage-dependent anion channel (VDAC) (Kroemer *et al.*, 2007) also known as mitochondrial porin which is the most abundant protein in OMM. Several studies have reported that VDAC is associated with T2D (Turko and Murad, 2003; Mostyn *et al.,* 2004) which is an important link between diabetes and mitochondrial function. VDAC has also been found to play a role in coronary endothelial cell dysfunction in T1D mice (Sepassi *et al.,* 2013). Thus, VDAC might contribute to apoptosis related to diabetes and is consequently the main focus of this review.

VDAC and its isoforms

VDAC acts as a channel for the transfer of ions and other small metabolites from outside into the mitochondria and as a selective channel for cations and uncharged molecules. In its open state, it allows the passage of hydrophilic molecules of oxidative phosphorylation substrates (pyruvate, oxaloacetate, malate, succinate, ATP, ADP, inorganic phosphate), urea cycle substrates and exchange of methyl groups (Colombini, 2004; Vander Heiden *et al.,* 2000). However, under special conditions these channels can be closed which reduces the permeability of OMM thereby, blocking metabolite exchange (Colombini, 2004; Holmuhamedov and Lemasters, 2009). VDAC exist as three isoforms VDAC1, 2 and 3 (Caterino *et al.,* 2017) and their amino acid sequences has already been determined. The three proteins have similar structure, conductance (Shoshan-Barmatz *et al.*, 2017), voltage-gating properties with molecular weights of 30-35 kDa and shares approximately 70% identity. All three can be found in most tissues with VDAC1 being the most abundant and VDAC3 being the least common form. All the three isoforms are encoded by distinct genes located on different chromosomes and they share the same exon-intron organization (De Pinto *et al.,* 2010). These VDACs complement each other and have some functional redundancy (Teplova *et al.*, 2011) although, they are significantly different with relation to functionality (Shoshan-Barmatz *et al.,* 2010; De Pinto *et al.,* 2010; De Pinto *et al.,* 2016). Table 1 shows the function of different VDAC isoforms.

Table 1. VDAC isoforms and functions

VDAC isoforms	VDAC function	References
VDAC1	Form oligomeric pores associated with stress and mitochondria DNA (mtDNA) release in diseases	Kim *et al.,* 2019
	Involved in transmission of Ca^{2+} signals to mitochondria during apoptosis	De Stefani *et al.,* 2011; Shoshan-Barmatz *et al.,* 2018
	Important for ROS control	Reina *et al.,* 2010
	Regulator of mitochondrial membrane permeabilization and apoptosis	Tajeddine *et al.,* 2008; Yuan *et al.,* 2008
	Interacts with hexokinase and Bcl-2 to mediate anti-apoptotic activity	Abu-Hamad *et al.,* 2009, Zhang *et al.,* 2019b

VDAC2	Important for ROS control	Reina *et al.,* 2010
	Abundant in the outer dense fiber and might be involved in the regulation of sperm motility	Hinsch *et al.,* 2004
	Porin2 isoform in *Drosophila melanogaster* suggested to be involved in sperm maturation	Guarino *et al.,* 2006
VDAC3	Possible involvement in mtDNA through formation of oligomeric pores	Kim *et al.,* 2019
	Abundant at the outer dense fiber of sperm flagellum and might be involved in the regulation of sperm motility	Hinsch *et al.,* 2004
	Deficient VDAC3 produces healthy infertile mice maybe due to immotile sperm	Sampson *et al.,* 2001
	Functional alteration of complex IV of the heart	Anflous-Pharayra *et al.,* 2011
	Moderates centriole assembly and recruits Msp1 protein to centrosomes	Majumder *et al.,* 2012

Structurally VDACs (primarily VDAC1) are β-barrel-forming transmembrane channels and through biochemical studies, it was shown to composed of one helix and 13 β-sheets with some relatively long loops between β-sheets which was proposed to play a role in protein-protein interaction (Colombini, 2004). However, crystal structure showed a β-barrel is formed by 19 β-sheets with the N-terminal α-helix lying within the pore (Ujwal *et al.,* 2008). Several studies suggest the N-terminal region mobility is involved in channel gating and serve as the interaction site of apoptosis-regulating proteins of the Bcl-2 family (Abu-Hamad *et al.,* 2009; Arbel *et al.*, 2012) and hexokinase (Arzoine, 2008). The N-terminal segment was also proposed to regulate cytochrome *c* release and subsequent apoptosis (Abu-Hamad *et al.,* 2009).

Role of VDAC-mediated apoptosis in diabetic complications

Chronic hyperglycemia and associated risk factors lead to irreversible diabetic complications affecting kidney, retina, peripheral nerves, brain and cardiovascular system. Diabetes mellitus both type 1 and type 2 are associated with an enhanced apoptosis of different cells and tissues which accelerates the occurrence of diabetic complications. Studies have shown that high glucose-mediated apoptotic cell death is relevant to diabetic complications as high glucose causes activation of several proteins involved in the apoptotic cell death (Allen *et al.*, 2005). There are several reports which have explained the possible mechanism by which high glucose increases the rate of apoptosis such as high glucose induced oxidative and nitrosative stress, *via* MAPK, p53 including mitochondria-dependent and-independent mechanisms particularly involving Bax or Bad. In previous

reports, high glucose in mesangial cells was shown to initiate oxidative-stress-induced apoptosis *via* Bax-mediated mitochondrial permeability and cytochrome *c* release (Allen *et al.,* 2005). In a process carried out by insulin-like growth factor-I (IGF-I), high glucose-induced apoptosis was prevented by IGF-I causing phosphorylation of Bad at Ser^{112}(Kang *et al.,* 2003).

Previous studies have demonstrated that high glucose exposure of pancreatic β-cells results into cell dysfunction and cell death. One of the possible factors as described by the study performed by Kim *et al*.(2005), is through decreasing glucokinase (GCK) or hexokinase IV protein expression and its interactions with VDAC which correlates with decrease in Bad phosphorylation. GCK belongs to the hexokinase family that converts glucose to glucose-6-phosphate by transferring a phosphate group from ATP to glucose, the initial step of glucose metabolism (Nordlie *et al.*, 1999). Glucose regulates Bad phosphorylation (Danial *et al.,* 2003) and phosphorylated Bad promotes interactions between hexokinase and VDAC necessary for pumping of ATP from mitochondria (Majewski *et al.,* 2004). Decreased Bad phosphorylation increases the chances of apoptosis. Bad is a member of the Bcl-2 family which induces apoptosis by inhibiting antiapoptotic Bcl-2 family members Bcl-xL, Bcl-2, allowing, Bak and Bax, pro-apoptotic proteins to aggregate and induce release of cytochrome *c* and eventually to apoptosis (Bergmann, 2002). High glucose exposure decreases the association of GCK to mitochondria, thereby increasing the interaction between Bax and mitochondria eventually leading to Bax oligomerisation, cytochrome *c* release followed by β-cell apoptosis (Kim *et al.,* 2005). Zhang *et al.* (2019a) reported that VDAC1 overexpression in T2D causes ATP loss in β- cell which on direct inhibition of VDAC1 restored glucose-stimulated insulin secretion (GSIS) and prevented development of diabetes in db/db mice. Study performed by Ahmed *et al.* (2010), on prolonged exposure of INS1E cell lines to high glucose was accompanied with marked expression of the VDAC1 and a reduction of VDAC2 suggesting that VDAC1 and VDAC2 expression could represent a consequence of early step in the β-cell dysfunction that could be an important target process to prevent altered insulin secretion and β-cell apoptosis. In other studies, hyperglycemia have shown to increase VDAC1 expression in β-cells (Salehi, 2010), in kidney (Gong *et al.,* 2009) including mouse coronary endothelial cells (MCECs) isolated from diabetic mice showed increased VDAC expression. VDAC inhibition restored the increased Ca^{2+}, O^{2-} and mPTP opening activity in diabetic MCECs suggesting normalising VDAC protein may decrease the incidence of cardiac ischemia in diabetes by decreasing endothelial cell apoptosis and increase in capillary density in the hearts (Sasaki *et al.,* 2012). Li *et al.* (2016) reported that IncRNA H19/miR-675 axis regulates cardiomyocyte apoptosis by targeting VDAC1 in diabetic cardiomyopathy. In retina, VDAC is localised to mitochondria predominantly at photoreceptors where results suggest that VDAC is involved in the regulation of cytosolic and mitochondrial Ca^{2+} concentration and mPTP assembly and/or activation. In retinal neurons especially ganglion cells, ischemia, diabetes apoptotic events appear to be promoted by an abnormal concentration of intracellular Ca^{2+}. VDAC being permeable to Ca^{2+} and as a component of mPTP may be involved in inducing mitochondrial swelling.

Therefore, VDAC may play a key role in processes of retinal degeneration that result from ATP depletion and Ca^{2+} overload. In one of the previous studies performed by Jones *et al.,* 2008 showed VDAC as a potential target of O-linked beta-N-acetylglucosamine (O-GlcNAc) modification. O-GlcNAc modification is a type of post-translation modification that modulates cellular function. In this study, they found that the adult mouse heart with elevated O-GlcNAc levels had more O-GlcNAc-modified VDAC and were found to be more resistant to calcium-induced swelling which was critical for survival providing a mechanism of cardioprotection. Increased apoptosis occurred in the tubular cells of db/db mice which on Mito Q administration were inhibited. Mito Q is a mitochondria-targeted antioxidant comprising of coenzyme Q10 and TPP cations. VDAC expression was found to increase in tubular of db/db mice.

However, there are contradictory results showing decreased VDAC on the mitochondrial kidneys of diabetic rats where the up-regulation of VDAC on α-Lipoic acid treatment exerted a protective role against mitochondrial injury (Wang *et al.*, 2013). In another study involving Zucker diabetic fatty obese (ZDFO) rats with early T2D, VDAC protein expression were significantly lower in the cerebral microvessels indicating deterioration of mitochondrial function in the cerebral vasculature during at an early stage of T2D (Merdzo *et al*., 2017). The lower VDAC in the cerebral microvessels of the ZDFO rats might represent a compensatory mechanism to minimize the detrimental consequences of increased oxidative stress in T2D.

Inhibition of VDAC in diabetes treatment

Inhibition of VDAC and VDAC-mediated apoptosis offers a potential strategy for combating diseases like diabetes. There are many known inhibitors of VDAC which affects its voltage gating (Holmuhamedov and Lemasters, 2009; Ben-Hail *et al.*, 2016), however, still few experimental data are available with respect to their use for diabetes treatment. Metformin, an antidiabetic drug has been shown to counteract VDAC1 induction by direct blocking it in db/db mice and restored the impaired ATP generation along with glucose-stimulated insulin secretion in T2D islets (Zhang *et al.* 2019a). In the study by Zhang *et al.* (2019a), two other VDAC inhibitors, VBIT-4 and AKOS (AKOS022075291) were also studied. VBIT-4 and AKOS are novel group of piperarazine- and piperidine-based compounds which directly interact with VDAC1 thereby inhibiting VDAC activities that are associated with metabolite transport, oligomerization as well as activities related to changes in intracellular calcium levels, reactive oxygen species and mitochondrial membrane potential due to mitochondria dysfunction. Recent study by Pittala *et al.* (2020) demonstrated that treatment of T2D model with VDAC1-based peptide, R-Tf-D-LP4 restored the elevated blood glucose levels and caused an increase in number and average size of islets and their insulin content suggesting that this peptide has potential for diabetes treatment. Patent filed by the inventors (Salehi *et al.*, 2018) on 2017-12-21, disclosed the use of substituted piprazine and piperidine derivatives as specific inhibitors of VDAC1 for preventing the progression of and treating prediabetes

and diabetes.

Apart from chemical compounds, natural plant products are another alternative for treating diabetes and diabetic complications without adverse side effects. This alternative has been one of the areas of interest for targeting VDAC in the treatment of diabetes and other diseases. Curcumin, an active compound found in turmeric, has glucose-lowering effect and improve β-cell function in T2D (Wickenberg *et al.,* 2010). This promising antidiabetic property has been postulated to be due to the ability to bind to and inhibit VDAC (De Marchi *et al.,* 2020). Further, methyl jasmonate, a cyclopentanone lipid-belonging to the family of plant stress hormones, can detach hexokinase from VDAC1 from mitochondria causing a dissociated glycolysis, decrease in ATP and release of cytochrome *c* ultimately leading to cell death (Goldin *et al.,* 2007; 2008). Therefore, targeting VDAC particularly in the β-cell, can prove beneficial in restoring normal β-cell functions in diabetes.

Conclusion

Diabetes is associated with enhanced apoptotic cell death which has been linked to a spectrum of diabetic complications. VDAC a key mitochondrial membrane protein involved in apoptosis has been explored for its contribution to the pathogenesis of diabetes. Several studies have demonstrated that inhibition of VDAC prevents progression of diabetes. Thus, targeting VDAC may provide a novel therapeutic strategy for the treatment of diabetes and its complications.

Conflict of interest

The authors claim that there is no conflict of interest.

Acknowledgement

Melinda Nongbet Sohlang acknowledges the financial support received from Department of Biotechnology, Government of India in the form of DBT-JRF fellowship.

References

Abu-Hamad, S., Arbel, N., Calo, D., Arzoine L., Israelson, A., Keinan, N., Ben-Romano, R., Friedman, O. and Shoshan-Barmatz, V. 2009. The VDAC1 N-terminus is Essential Both for Apoptosis and the Protective Effect of Anti-apoptotic Proteins. *Journal of Cell Science,* 122: 1906-1916.

Ahmed, M., Muhammed, S.J., Kessler, B. and Salehi, A. 2010. Mitochondrial Proteome Analysis Reveals Altered Expression of Voltage Dependent Anion Channels in Pancreatic β-cells Exposed to High Glucose. *Islets*, 2(5): 283-292.

Allen, D.A., Yaqoob, M.M. and Harwood, S.M. 2005. Mechanisms of High-glucose-induced Apoptosis and its Relationship to Diabetic Complications. *The Journal of Nutritional Biochemistry,* 16(12): 705-713.

American Diabetes Association. 2009. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, 32(Suppl 1): S62-S67.

Anflous-Pharayra, K., Lee, N., Armstrong, D.L. and Craigen, W.J. 2011. VDAC3 has Differing Mitochondrial Functions in Two Types of Striated Muscles. *Biochimica et Biophysica Acta,* 1807: 150-156.

Arbel, N., Ben-Hail, D. and Shoshan-Barmatz, V. 2012. Mediation of the Antiapoptotic Activity of Bcl-xL Protein upon Interaction with VDAC1 Protein. *Journal of Biological Chemistry,* 287: 23152-23161.

Arzoine, L., Zilberberg, N., Ben-Romano, R. and Shoshan-Barmatz, V. 2008. Voltage-Dependent Anion Channel 1-based Peptides Interact with Hexokinase to Prevent Its Anti-apoptotic Activity. *Journal of Biological Chemistry,* 284: 3946-3955.

Barber, A.J., Gardner, T.W. and Abcouwer, S.F. 2011. The Significance of Vascular and Neural Apoptosis to the Pathology of Diabetic Retinopathy. *Investigative Ophthalmology & Visual Science,* 52(2): 1156-1163.

Ben-Hail, D., Begas-Shvartz, R., Shalev, M., Shteinfer-Kuzmine, A., Gruzman, A., Reina, S., De Pinto, V. and Shoshan-Barmatz, V. 2016. Novel Compounds Targeting the Mitochondrial Protein VDAC1 Inhibit Apoptosis and Protect Against Mitochondria Dysfunction. *Journal of Biological Chemistry,* 291: 24986-25003.

Bergmann, A. 2002. Survival Signaling Goes BAD. *Developmental Cell*, 3(5): 607-608.

Caterino, M., Ruoppolo, M., Mandola, A., Costanzo, M., Orru, S. and Imperlini, E. 2017. Protein–Protein Interaction Networks as a New Perspective to Evaluate Distinct Functional Roles of Voltage-Dependent Anion Channel Isoforms. *Molecular BioSystems,* 13: 2466-2476.

Cnop, M., Welsh, N., Jonas, J.C., Jo¨rns, A., Lenzen, S. and Eizirik, D.L. 2005. Mechanisms of Pancreatic Beta-Cell Death in Type 1 and Type 2 Diabetes: Many Differences, Few Similarities. *Diabetes*, 54: S97-S107.

Colombini, M. 2004. VDAC: The Channel at the Interface Between Mitochondria and the Cytosol. *Molecular & Cellular Biochemistry*, 256 : 107-115.

Danial, N.N., Gramm, C.F., Scorrano, L., Zhang, C.Y., Krauss, S., Ranger, A.M., Datta, S.R., Greenberg, M.E., Licklider, L.J., Lowell, B.B., Gygi, S.P. and Korsmeyer, S.J. 2003. BAD and Glucokinase Reside in a Mitochondrial Complex that Integrates Glycolysis and Apoptosis. *Nature*, 424: 952-956.

De Marchi, U., Fernandez-Martinez, S., de la Fuente, S., Wiederkehr, A. and Santo-Domingo, J. 2020. Mitochondrial Ion Channels in Pancreatic Beta-cells: Novel Pharmacological Targets for the Treatment of Type 2 Diabetes. *Brazilian Journal of Pharmacology*, doi:10.1111/bph.15018 (2020) (Epub ahead of print).

De Pinto, V., Guarino, F., Guarnera, A., Messina, A., Reina, S., Tomasello, F.M., Palermo, V., Mazzoni, C. 2010. Characterization of Human VDAC Isoforms: A Peculiar

Function for VDAC3?. *Biochimica et Biophysica Acta*, 1797(6-7): 1268-1275.

De Pinto, V., Reina, S., Gupta, A., Messina, A. and Mahalakshmi, R. 2016. Role of Cysteines in Mammalian VDAC Isoforms' Function. *Biochimica et Biophysica Acta,* 1857: 1219-1227.

De Stefani, D., Bononi, A., Romagnoli, A., Messina, A., De Pinto, V., Pinton, P. and Rizzuto R. 2012. VDAC1 selectively Transfers Apoptotic $Ca^{(2+)}$ Signals to Mitochondria. *Cell Death Differentiation*, 19(2): 267-273.

Feenstra, D.J., Yego, E.C. and Mohr, S. 2013. Modes of Retinal Death in Diabetic Retinopathy. *Journal of Clinical & Experimental Ophthalmology*, 4(5): 298.

Forbes, J.M. and Cooper, M.E. 2013. Mechanism of Diabetic Complications. *Physiological Reviews,* 93(1): 137-188.

Goldin, N., Arzoine, L., Heyfets, A., Israelson, A., Zaslavsky, Z., Bravman, T., Bronner, V., Notcovich, A., Shoshan-Barmatz, V. and Flescher, E. 2008. Methyl Jasmonate Binds to and Detaches Mitochondria-bound Hexokinase. *Oncogene*, 27(34): 4636-4643.

Goldin, N., Heyfets, A., Reischer, D. and Flescher, E. 2007. Mitochondria-mediated ATP Depletion by Anti-cancer Agents of the Jasmonate Family. *Journal of Bioenergetics & Biomembranes,* 39: 51-57.

Gong, D., Chen, X., Middleditch, M., Huang, L., Vazhoor Amarsingh, G., Reddy, S., Lu, J., Zhang, S., Ruggiero, K., Phillips, A.R. and Cooper, G.J. 2009. Quantitative Proteomic Profiling Identifies New Renal Targets of Copper (II)-selective Chelation in the Reversal of Diabetic Nephropathy in Rats. *Proteomics*, 9: 4309-4320.

Guarino, F., Specchia, V., Zapparoli, G., Messina, A., Aiello, R., Bozzetti, M.P. and De Pinto, V. 2006. Expression and Localization in Spermatozoa of the Mitochondrial Porin Isoform 2 in *Drosophila melanogaster*. *Biochemical & Biophysical Research Communications*, 346: 665-670.

Habib, S.L. 2013. Diabetes and Renal Tubular Cell Apoptosis. *World Journal of Diabetes*, 4(2): 27-30.

Hinsch, K.D., De Pinto, V., Aires, V.A., Schneider, X., Messina, A. and Hinsch, E. 2004. Voltage-dependent Anion-selective Channels VDAC2 and VDAC3 are Abundant Proteins in Bovine Outer Dense Fibers, a Cytoskeletal Component of the Sperm Flagellum. *Journal of Biological Chemistry*, 79: 15281-15288.

Holmuhamedov, E. and Lemasters, J.J. 2009. Ethanol Exposure Decreases Mitochondrial Outer Membrane Permeability in Cultured Rat Hepatocytes. *Archives of Biochemistry & Biophysics*, 481: 226-233.

Jones, S.P., Zachara, N.E., Ngoh, G.A., Hill, B.G., Teshima, Y., Bhatnagar, A., Hart, G.W. and Marban, E. 2008. Cardioprotection by N-acetylglucosamine linkage to Cellular Proteins. *Circulation*, 117: 1172-1182.

Kang, B.P., Urbonas, A., Baddoo, A., Baskin, S., Malhotra, A. and Meggs, L.G. 2003. IGF-1 Inhibits the Mitochondrial Apoptosis Program in Mesangial Cells Exposed to High Glucose. *American Journal of Physiology- Renal Physiology*, 285: F1013-F1024.

Kim, J., Gupta, R., Blanco, L.P., Yang, S., Shteinfer-Kuzmine, A., Wang, K., Zhu, J., Yoon, H.E., Wang, X., Kerkhofs, M., Kang, H., Brown, A.L., Park, S., Xu, X., van Rilland, E.Z., Kim, M.K., Cohen, J.I., Kaplan, M.J., Shoshan-Barmatz, V. and Chung, J.H. 2019. VDAC Oligomers Form Mitochondrial Pores to Release mtDNA Fragments and Promote Lupus-like Disease. *Science*, 366: 1531-1536.

Kim, W.H., Lee, J.W., Suh, Y.H., Hong, S.H., Choi, J.S., Lim, J.H., Song, J.H., Gao, B. and Jung, M.H. 2005. Exposure to chronic high glucose induces β-cell apoptosis through decreased interaction of glucokinase with mitochondria. *Diabetes*, 54(9): 2602-2611.

Kroemer, G., Galluzzi, L. and Brenner, C. 2007. Mitochondrial Membrane Permeabilization in Cell Death. *Physiological Reviews,* 87:99-163.

Lee, S.C. and Pervaiz, S. 2007. Apoptosis in the Pathophysiology of Diabetes Mellitus. *International Journal of Biochemistry & Cell Biology*, 39(3): 497-504.

Leist, M. and Jaattela, M. 2001. Four Deaths and a Funeral: from Caspases to Alternative Mechanism. *Nature Reviews Molecular Cell Biology,* 2: 589-598.

Li, X., Wang, H., Yao, B., Xu, W., Chen, J. and Zhou, X. 2016. lncRNA H19/miR-675 axis Regulates Cardiomyocyte Apoptosis by Targeting VDAC1 in Diabetic Cardiomyopathy. *Scientific Reports*, 6: 36340.

Majewski, N., Nogueira, V., Robey, R.B. and Hay, N. 2004. Akt Inhibited Apoptosis Downstream of BID Cleavage *via* a Glucose-dependent Mechanism Involving Mitochondrial Hexokinases. *Molecular & Cellular Biology*, 24: 730-740.

Majumder, S., Slabodnick, M., Pike, A., Marquardt, J. and Fisk, H.A. 2012. VDAC3 Regulates Centriole Assembly by Targeting Mps1 to Centrosomes. *Cell Cycle*, 11: 3666 -3678.

McKenzie, M.D., Jamieson, E., Jansen, E.S., Scott, C.L., Huang, D.C., Bouillet, P., Allison, J., Kay, T.W., Strasser, A. and Thomas, H.E. 2010. Glucose Induces Pancreatic Islet Cell Apoptosis that Requires the BH3-only Proteins bim and puma and Multi-BH Domain Protein Bax. *Diabetes*, 59: 644-652.

Merdzo, I., Rutkai, I., Sure, V.N., McNulty, C.A., Katakam, P.V. and Busij, D.W. 2017. Imaired Mitochondrial Respiration in Large Cerebral Arteries of Rats with Type 2 Diabetes. *Journal of Vascular Research*, 54(1): 1-12.

Mishra, R., Emancipator, S.N., Kern, T. and Simonson, M.S. 2005. High Glucose Evokes an Intrinsic Proapoptotic Signaling Pathway in Mesangial Cells. Kidney International, 67(1): 82-93.

Mostyn, A., Pearce, S., Stephenson, T. and Symonds, M.E. 2004. Hormonal and Nutritional

Regulation of Adipose Tissue Mitochondrial Development and Function in the Newborn. Experimental and Clinical Endocrinology and Diabetes, 112: 2-9.

Nordlie, R.C., Foster, J.D. and Lange, A.J. 1999. Regulation of Glucose Production by the Liver. *Annual Reviews in Nutrition*, 19: 379-406.

Ouyang, C., You, J. and Xie, Z. 2014. The Interplay Between Autophagy and Apoptosis in the Diabetic Heart. *Journal of Molecular and Cellular Cardiology*, 71:71-80.

Peng, C., Ma, J., Gao, X., Tian, P., Li, W. and Zhang, L. 2013. High Glucose Induced Oxidative Stress and Apoptosis in Cardiac Microvascular Endothelial Cells Are Regulated by FoxO3a. *PLoS ONE*, 8(11): e79739.

Pittala, S., Levy, I., De, S., Kumar Pandey, S., Melnikov, N., Hyman, T., & Shoshan-Barmatz, V. 2020. The VDAC1-based R-Tf-D-LP4 Peptide as a Potential Treatment for Diabetes Mellitus. *Cells*, 9(2): 481.

Reina, S., Palermo, V., Guarnera, A., Guarino, F., Messina, A., Mazzoni, C. and De Pinto, V. 2010. Swapping of the N-terminus of VDAC1 with VDAC3 restores Full Activity of the Channel and Confers Anti-aging Features to the Cell. *FEBS Letters*, 584: 2837-2844.

Salehi, A. 2010. Mitochondrial Proteome Analysis Reveals Altered Expression of Voltage Dependent Anion Channels in Pancreatic Beta-cells Exposed to High Glucose. *Islets*, 2: 283-292.

Salehi, A., Wollheim C.B. and Shoshan-Barmatz, V. 2018. The National Institute for Biotechnology in the Negev Ltd. PCT/IL2017/051379 filed December 21, 2017 and issued June 28, 2018.

Sampson, M.J., Decker, W.K., Beaudet, A.L., Ruitenbeek, W., Armstrong, D., Hicks, M.J. and Craigen, W.J. 2001. Immotile Sperm and Infertility in Mice Lacking Mitochondrial Voltage-dependent Anion Channel Type 3. *Journal of Biological Chemistry*, 276: 39206-39212.

Sasaki, K., Donthamsetty, R., Heldak, M., Cho, Y.E., Scott, B.T. and Makino, A. 2012. VDAC: Old Protein with New Roles in Diabetes. *American Journal of Physiology Cell Physiology,* 303 (10): C1055-C1060.

Schattenberg, J.M. and Schuchmann, M. 2009. Diabetes and Apoptosis. *Liver*, 14: 1459.

Sepassi, L.,Sasaki, K., Heldak, M. and Makino, A. 2013. Role of VDAC in Coronary Endothelial Cell Dysfunction in Type 1 Diabetic Mice. *The FASEB Journal*, 27: 913.2-913.2.

Shoshan-Barmatz, V., De Pinto, V., Zweckstetter, M., Raviv, Z., Keinan, N. and Arbel, N. 2010. VDAC, a Multi-functional Mtochondrial Protein Regulating Cell Life and Death. *Molecular Aspects of Medicine*, 31(3): 227-285.

Shoshan-Barmatz, V., Kmita, H. and Lemasters, J.J. 2017. Editorial: Uncovering

the Function of the Mitochondrial Protein VDAC in Health and Disease: From Structure- Function to Novel Therapeutic Strategies. *Frontiers in Oncology*, 7: 320.

Shoshan-Barmatz, V., Krelin, Y. and Shteinfer-Kuzmine, A. 2018. VDAC1 Functions in Ca2+ Homeostasis and Cell Life and Death in Health and Disease. *Cell Calcium*, 69: 81-100.

Tajeddine, N., Galluzzi, L., Kepp, O., Hangen, E., Morselli, E., Senovilla, L., Araujo, N., Pinna, G., Larochette, N., Zamzami, N., Modjtahedi, N., Harel-Bellan, A. and Kroemer, G. 2008. Hierarchical Involvement of Bak, VDAC1 and Bax in Cisplatin-induced Cell Death. *Oncogene*, 27: 4221-4232

Teplova, V.V., Odinokova, I.V. and Holmuhamedov, E.L. 2011. Isoforms of Voltage-dependent Anion Channel of the Outer Mitochondrial Membrane and Experimental Models to Study Their Physiological Role. *Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology*, 5(2): 111-120.

Turko, I.V. and Murad, F. 2003. Quantitative Protein Profiling in Heart Mitochondria from Diabetic Rats. *Journal of Biological Chemistry*, 278: 35844-35849.

Ujwal, R., Cascio, D., Colletier, J.P., Faham, S., Zhang, J., Toro, L., Ping, P. and Abramson, J. 2008. The Crystal Structure of Mouse VDAC1 at 2.3 A Resolution Reveals Mechanistic Insights into Metabolite Gating. *Proceedings of the National Academy of Sciences USA*, 105: 17742-17747.

Vander Heiden, M.G., Chandel, N.S., Li, X.X., Schumacker, P.T., Colombini, M. and Thompson C.B. 2000. Outer Mitochondrial Membrane Permeability Can Regulate Coupled Respiration and Cell Survival. *Proceedings of the National Academy of Sciences USA*, 97: 4666-4671.

Wali, J.A., Masters, S.L. and Thomas, H.E. 2013. Linking Metabolic Abnormalities to Apoptotic Pathway in beta Cells in Type 2 Diabetes. *Cells*, 2(2): 266-283.

Wang, L., Wu, C. G., Fang, C. Q., Gao, J., Liu, Y. Z., Chen, Y., Chen, Y. N., and Xu, Z. G. 2013. The protective effect of α-Lipoic acid on mitochondria in the kidney of diabetic rats. *International Journal of Clinical and Experimental Medicine*, 6(2): 90-97.

Wickenberg, J., Ingemansson, S.L. and Hlebowicz, J. 2010. Effects of *Curcuma longa* (turmeric) on Postprandial Plasma Glucose and Insulin in Healthy Subjects. *Nutrition Journal*, 9: 43.

Yuan, S., Fu, Y., Wang, X., Shi, H., Huang, Y., Song, X., Li, L., Song, N. and Luo, Y. 2008. Voltage-Dependent Anion Channel 1 is Involved in Endostatin-induced Endothelial Cell Apoptosis. *The FASEB Journal*, 22: 2809-2820.

Zhang, E., Mohammed Al-Amily, I., Mohammed, S., Luan, C., Asplund, O., Ahmed, M., Ye, Y., Ben-Hail, D., Soni, A., Vishnu, N., Bompada, P., De Marinis, Y., Groop, L., Shoshan-Barmatz, V., Renström, E., Wollheim, C. B., and Salehi, A. 2019a.

Preserving Insulin Secretion in Diabetes by Inhibiting VDAC1 Overexpression and Surface Translocation in β Cells. *Cell Metabolism*, 29(1): 64-77.e6.

Zhang, J., Guo, Y., Ge, W., Zhou, X. and Pan, M. 2019b. High Glucose Induces Apoptosis of HUVECs in a Mitochondriadependent Manner by Suppressing Hexokinase 2 Expression. *Experimental and Therapeutic Medicine*, 18: 621-629.

Zhaoyu, J. and El-Deiry, W.S. 2005. Overview of Cell Death Signalling Pathways. *Cancer Biology & Therapy*, 4(2): 147-171.

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Form IV Rule 8

Place of Publication	:	NEHU Permanent Campus Shillong-793022
Periodicity	:	Biannual
Printer's Name	:	Editor, NEHU Journal
Address	:	Permanent Campus Shillong-793022
Publisher's Name	:	Editor, NEHU Journal
Nationality	:	Indian
Address	:	Permanent Campus Shillong-793022
Editor's Name	:	S.R. Joshi
Nationality	:	Indian
Address	:	Department of Biotechnology and Bioinformatics Permanent Campus, NEHU Shillong-793022
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Journal Article

Mayoux, L. 1999. 'Questioning Virtuous Spirals: Microfinance and Women's Empowerment in Africa', *Journal of International Development*, 11(7): 957-984.

McCaffrey, D.F., Ridgeway, G. and Morral, A.R. 2004. 'Propensity Score Estimation with Booted Regression for Evaluating Causal Effects in Observational Studies', *Psychological Methods*, 9(4): 403-425.

Working Papers / Discussion Papers

Krishna, A. 2003. 'Social Capital, Community Driven Development and Empowerment:

A Short Note on Concepts and Operations', Working Paper No. 33077, World Bank: 34.

Government and Institutional Documents

B L Center for Development Research and Action (BLCDRA). 2005. *Micro finance and Empowerment of Scheduled Caste Women: An Impact Study of Self-help Groups in Uttar Pradesh and Uttaranchal.*

Government of India, New Delhi: 56.

Conference and Seminar Papers

Scandizzo, P., Gaiha, R. and Imai, K. 2005. 'Option Values, Switches and Wages - An Analysis of the Employment Guarantee Scheme in India', presented at the Conference on *Social Protection for Chronic Poverty Risk, Needs, and Rights: Protecting What? How?*, organized by IDPM, University of Manchester, 23-24 February 2005.

Online sources

Islam, A. 2009. 'Medium and Long-term Participation in Microfinance: An Evaluation Using a Large Panel Dataset from Bangladesh'. Department of Economics, Monash University,Australia:12.https://editorialexpress.com/cgi-in/conference/download.cgi?db, accessed on 12/12/2012.

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