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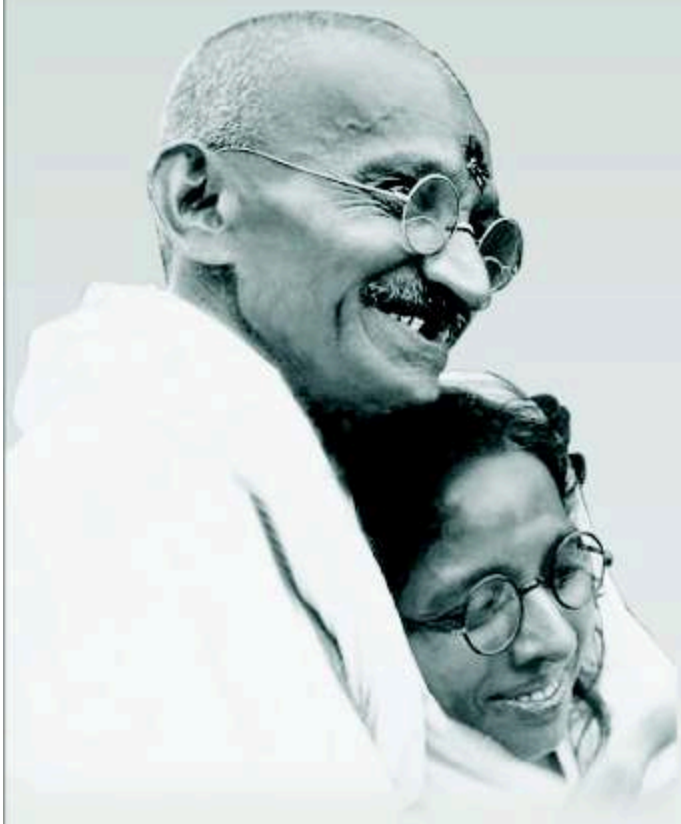

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Control of *Propionibacterium acnes* using Essential oil

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Abstract : In the present investigation the antimicrobial effect of 19 plant essential oils have been studied on *Propionibacterium acnes*, the bacterium causes human skin disease, the Acne vulgaris. Out of 19 essential oil, 5 oils showed inhibitory effect against this bacterium. These plant oils used are from *Thymus vulgaris* (thyme), *Eucalyptus globulus* (eucalyptus), *Syzygium aromaticum* (clove bud), *Cinnamomum verum* (cinnamon) and *Melaleuca alternifolia* (tea tree), they have shown effective inhibition zone in mm as 29.66±0.5, 25.00±0.0, 55.33±0.5, 18.00±0.0, 13.66±0.5 respectively. The anti acne cream was prepared containing 2% of each of the effective essential oil, stearic acid (24.2%), cetyl alcohol(1.20%), triethanolamine (1.32%), propylene glycol(0.95%), isopropyl myristate (1.20%), glycerine (2.00%), borax (1.90%). Anti acne cream formulated showed the anti activity against *P. acnes*.

Key words : *Propionibacterium acnes*, Acne vulgaris, Minimum inhibitory concentration, Anti acne cream.

Introduction

Acne vulgaris is common human skin disease, affecting particularly adolescents but also affect adults as well. Although overall health is not impaired it is not a trivial disease; it produces cutaneous and emotional scars. The symptoms of acne also include whiteheads, blackheads and pimples causing disfiguration ensuing psychological, social and economic problems (Ravichandran *et al.*, 2004).Webster (2002) reviewed the literature on acne vulgaris and reported that it is caused due to follicular inhabitant of *Propionibacterium acnes*, which acts as a harmless commensal incapable of tissue invasion or serious infection. He further suggested that *Propionibacterium acnes* activates complements and became inflammatory when comes in contact with immune system. The organisms produce metabolites as subaceous trigycerides, consuming glycerol fraction by discarding fatty acids. Tropical therapy has been recommended for the management of acne vulgaris with specific limitations (Ravichandran *et al.*, 2004; Jerajani *et al.*, 2004). Gislene *et al.*(2000) reported antibacterial activity of some plant extracts and phytochemicls on antibiotic-resistant and antimicrobial treatment of *P. acnes*. Gubelin *et al*. (2006) isolated from inflammatory acne and suggested antimicrobialability against *Propionibacterium acnes*. Cox *et al*. (2000) studied the role of essential oil of *Melleuca alternifolia* (tea tree oil) against *Propionibacterium acnes* as antimicrobial action. The antibacterial activity of clove essential oil against *Propionibacterium acnes* was noticed by Fu *et al*. (2009). Athkomkulchai *et al*. (2008) and Abbasi *et al*. (2010) gave formulations of anti acne creams from plant oils and reported their efficacy avoiding use of systemic antibiotics.

The present study is an attempt to use essential oils as antiacne formulations are very good therapeutic use for treating acne.

Materials and Methods

Collection and maintenance of culture -

Pure culture of *Propionibacterium acnes* MTCC 1951 was obtained from Institute of Microbial Technology, Chandigarh, India. The culture was maintained in Peptone yeast extract glucose broth. (Moore and Cato, 1963)

Plant essential oil -

Plant essential oils were obtained from commercial outlet of Dr. Urjita Jain Herbal ltd, Mumbai.

Determination of effect of plant essential oil on growth of *P. acnes.*

Kirby Bauer disc diffusion method was used to determine the effect of plant essential oil on the growth of *P.acnes* (Casida,1993). The broth culture of *P. acnes* was seeded in peptone yeast extract glucose agar containing 1% tween 80. Plates were allowed to solidify. Sterile discs were dipped in plant essential oil and placed onto the agar plates. Erythromycin was used as a control. The plates were incubated at 37°C under anaerobic conditions. The zone of inhibition was observed after 48 hours.

Determination of Minimum inhibitory concentration of effective essential oil –

Broth dilution method was used to determine the Minimum inhibitory concentration. The effective plant essential oil were selected and dilutions were prepared

in the range of 0.1% - 1% using PYG broth containing tween 80. 0.1 ml of the culture was inoculated in each tube. Sterile mineral oil was added in each tube to create anaerobic conditions. The tubes were incubated at 37°C under anaerobic conditions. The results were recorded after 48 hours (Zu *et al.*, 2010).

Formulation of anti acne cream -

Stearic acid was melted and then required quantity of paraffin oil was added to it at a temperature of 70-80°C preventing it from boiling. After mixing, cetyl alcohol was added and the contents were homogenized. With continuous stirring triethanolamine, propylene glycol, isopropyl myristate, glycerine was added in this mixture. Whole material was kept warm during the entire procedure.

In another vessel, essential oil and borax were mixed in distilled water and this suspension was heated at 80-90°C. This suspension was added into the above oily phase mixture and the contents were thoroughly homogenized. Anti acne creams containing 2% of each of the effective essential oil were prepared (Abbasi *et al.*, 2010; Athikomkulchai *et al.*, 2008). The composition of the antiacne cream is given in Table 1.

Results and discussion:

Among the 19 different plant essential oils tested, *Thymus vulgaris* (thyme), *Eucalyptus globulus* (Eucalyptus), *Melaleuca alternifolia* (Tea tree), *Cinnamomum verum* (Cinnamon), and *Syzygium aromaticum* (clove bud) are effective these results have been compiled in Table 2.

MIC value for Thyme was 0.5%, Cinnamon was 0.08%, Eucalyptus was 0.6%, Tea tree was 0.8%. MIC of Clove bud oil could not be determined correctly as the dilutions made were turbid making it difficult to observe the bacterial growth and to determine the MIC value. Similar observation is reported by Carson and Riley (1994) while determining MIC of Tea tree oil. These plant oils are *Thymus vulgaris* (thyme), *Eucalyptus globulus* (eucalyptus), *Syzygium aromaticum* (clove bud), *Cinnamomum verum* (cinnamon) and *Melaleuca alternifolia (*tea tree) have shown effective inhibition zone as 29.66±0.5mm, 25.00±0.0mm, 55.33±0.5mm, 18.00±0.0mm, 13.66±0.5mm respectively. The anti acne cream with each essential oil (2.00%) containing stearic acid (24.2%), cetyl alcohol (1.20%), triethanolamine (1.32%), propylene glycol (0.95%), isopropyl myristate (1.20%), Glycerin (2.00%), Borax (1.90%) was suggested. Anti acne cream formulated showed the anti *P. acnes* activity. In terms of MIC cinnamon oil was found to be most effective.

Anti acne cream also showed the activity against *P. acnes*. The study depicted that the anti acne formulations are very good therapeutic compositions for acne. Our observations confirm the findings of Athkomkulchai *et al*. (2008) and Abbasi *et al*. (2010). Anti acne cream containing Eucalyptus oil formulated by Athkomkulchai *et al*. (2008) showed the zone of inhibition of 8.0±0.0 mm whereas in the present study zone size of 14.0±0.0 mm was observed.

Essential oil inhibits respiration and increase permeability of bacterial cytoplasm and yeast plasma membranes. They also cause potassium ion leakage. The ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the most likely source of its lethal action at the minimum inhibitory levels (Cox *et. al.*, 2000).

Clove essential oil exhibited significant activity

Table 1: The composition of anti acne cream

Ingredient	% Weight
Stearic acid	24.20
Cetyl alcohol	1.20
Triethanolamine	1.32
Propylene glycol	0.95
Isopropyl myristate	1.20
Glycerin	2.00
Borax	1.90
Essential oil	2.00

Table 2: Antimicrobial activity of plant oils against *P. acnes*

Plant essential oil	Average Zone in mm
Eucalyptus globulus	25.00±0.0
Matricaria recutita	-
Cinnamomum verum	55.33±0.5
Syzygium aromaticum	18.00±0.0
Pogostemon cablin	-
Vitus vinifera	-
Cedrus atlantica	-
Cymbopogon flexuosus	-
Melaleuca alternifolia	13.66±0.5
Thymus vulgaris	29.66±0.5
Rosmarinus officinalis	-
Curcuma longa	-
Zingiber officinale	-
Piper nigrum	-
Salvia sclarea	-
Azadirachta indica	-
Simmondsia chinensis	-
Cymbopogon martinii	-
Citrus limon	-

against *P. acnes*, promises potential in vivo activity. The bacteriostatic mechanism involves damage to the cell walls and membranes of bacteria. At longer incubation times, cytoplasm proteins may diffuse from the cytoplasm. Alternatively, essential oil might inhibit protein synthesis (Fu *et al*, 2009). The results of the present study support their theory.

The main component of thyme essential oil is thymol and that of cinnamon is eugenol, which possesses notable anti-bacterial and anti-oxidant effects. These two constituents may also be responsible for the antibacterial and cytotoxic activities of thyme or cinnamon essential oil.

In addition to their use for food and cosmetics, the potential of essential oil for the treatment of acne merits further exploration in the future (Zu *et al*. 2010). Traditional herbal treatment in combination with cosmetic care is a well established basis of acne therapy. Some herbal products may have the potential to replace standard chemical therapy in a mild to moderate cases because of their good efficacy and higher tolerability (Reuter *et al.*, 2010).

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Asian J. Exp. Sci., Vol. 26, No. 2, 2012; 05-10

Genomic Analysis of Bacterium Isolated from Amended Coal Fly Ash by 16S rDNA

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Abstract : Fly ash being the problematic solid waste must be managed in eco-friendly way. For the same the present study was conducted to analyse the FA resistant bacteria by16S rDNA PCR. The identified bacterium was *Kocuria* sp HO-9042 with Gen bank Accession No: DQ531634.2. PCR amplification of the 16S rDNA gene from *Kocuria* sp revealed 8F and 1492R sequences with 550bp and 985bp respectively and a consensus sequence of 1401bp. The distribution of 100 BLAST hits on the 1401 query sequence showed the 10 homologs with about 200 nucleotide similarities with the homologues showing close relatedness on the basis of the sequence similarity in the significant alignments. The present investigation suggests that *Kocuria sp* HO-9042 in coal fly ash amended soil will also help in improving soil fertility.

Key words : Fly ash, 16S rDNA, *Kocuria*, PCR, BLAST.

Introduction

Fly ash (FA), a coal combustion residue of thermal power plants has been regarded as a problematic solid waste all over the world (Pandey and Singh, 2010). It is an amorphous ferro-alumino silicate with a matrix very similar to soil. Addition of FA to soil may improve the physico-chemical properties as well as nutritional quality of the soil and the extent of change depends on soil and FA properties. In view of the high cost of disposal and environmental management, utilization of FA in agricultural sector could be a viable option. Its use in agriculture was initially due to its liming potential and the presence of essential nutrients, which promoted plant growth and also alleviated the nutrient deficiency in soils (Mittra *et al*., 2005).

In soil, microorganisms by virtue of the exo-enzymatic activities are considered as primary decomposers playing key role in mineralization and demineralization process facilitating cycling of minerals in biosphere (Rodriquez *et al*., 2011) resulting in the fertility of the soil. Bacterial population can influence carbon or mineral cycles and have the ability to colonize harsh environments. However, little efforts have been made in studying the microbial ecology of such soils. In such soils or sites affected with fly ash, introduction of beneficial soil microorganisms and their establishment, colonization and survival along with their role in improving soil fertility and interaction with plant roots will reveal more information on developing strategies for faster remediation of such sites. Amplification of 16S rDNA and analysis of amplicon diversity using techniques such as TGGE and DGGE are valuable tools for exploring microbial diversity in the natural environments (Macrae, 2000). Consensus oligonucleotides produce DNA bands by agarose gel electrophoresis following PCR amplification. These band patterns provided unambiguous DNA fingerprints of different eubacterial species and strains. Widespread distribution of these repetitive DNA elements in the genomes of various microorganisms and BLAST enable rapid identification of bacterial species and strains, and be useful for the analysis of prokaryotic genomes. As there is paucity of knowledge about the bacterial identification which are resistive to fly ash, the present work was conducted to identify the resistive bacterium in the fly ash amended soil using 16S rDNA.

Materials and Methods

Fly ash for the laboratory experiment was collected from Patratu Thermal Power Plant, Ranchi and was amended with soil from agro-ecosystem of Ranchi University campus. Table 1 depicts the edaphic profile of soil and fly ash. The amendment was done in the proportion of 5% FA. The bacterial culture was prepared using sample from the FA and soil mixture by dilution plate count method (Waksman, 1922). The isolation of bacteria from soil samples was initiated by taking 1g of soil from each composite and transferring it to sterilized test tube for suspension in 9 mL of sterilized deionized water by shaking for 30 mins. 1 mL inoculant was taken from the aliquots of 1: 10^7 dilutions of the primary suspension (1 g soil in 10 ml distilled water). Each dilution was plated in Petri plates (100 mm dia) containing Czapak Dox Agar media for the bacterial culture. The white circular colonies with entire margin and raised elevation were isolated from the bacterial culture by streak method and pure cultured using solid agar media for slant preparation which was used for the genomic analysis.

Table 1: Edaphic profile of soil and fly ash (mean±SD, n=4)

Parameters	Soil	Fly ash
pH	5.81±0.1	6.85±0.31
O. C (g %)	0.31±0.21	0.12±0.11
O.M (g %)	0.54±1.1	0.21±0.3
Nitrogen (mg N/g soil)	0.078±0.41	0.175±0.22
Phosphorus (mg P/g soil)	0.0279±0.17	0.13±0.23
Potassium (mg K/g soil)	1.48±0.41	1.9±0.14
EC(m.Mhos/cm)	0.23±0.04	0.56±0.21

Genomic analysis

DNA extraction and purification

DNA was isolated from the pure culture of the bacterial colony. Tris – EDTA (10mM Tris-HCl, 1mM EDTA; pH 8) buffer and lysozyme (10 mg/mL) were added in the pelleted cells of the dominant isolate and incubated for 30 min at room temp. SDS and proteinase K(10U/µL) were added and incubated at 55°C for 2h. DNA was extracted with phenol, chloroform and iso-amyl alcohol and was precipitated with ethanol and dissolved in TE buffer (Wawer and Muyzer 1995). Its quality was evaluated on 1.2 Agarose Gel which revealed a single band of high molecular weight DNA.

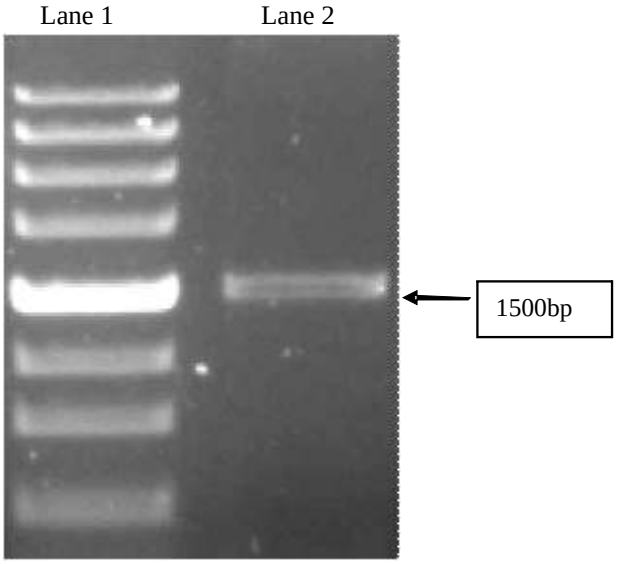
PCR amplification and sequencing 16S rDNA gene

Fragments of 16S rDNA gene were amplified by PCR from the above isolated DNA. A single discrete PCR amplicon band of 1500bp was observed when resolved on Agarose Gel. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried with 8F and 1492R primers using BDTv 3.1 Cycle Sequencing Kit on ABI 3730^{x}l Genetic Analyzer. Consensus sequence of 1401bp rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the nrdatabase of NCBI genbank database (Pruitt *et al*., 2005). Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. The nucleotide database was searched with the sequences obtained with NCBI BLAST tool (http:// www.ncbi.nlm.nih.gov/BLAST) (Altuschul e*t al*., 1997)

Results

The bacterial culture of the soil fly ash mixture expressed morphologically different colonies. The circular colonies from the culture on genomic analysis

Figure 1: Agarose gel image showing the 1500bp of 16S rDNA amplicon band



Lane 1 - DNA marker
Lane 2 - 16 S rDNA amplicon band

by 16S rDNA polymerase chain reaction was found to be *Kocuria sp.* HO-9042 (Gen Bank Accession Number: DQ531634.2) on the basis of nucleotide homology. Fragment of 16S rDNA gene was amplified by PCR from the isolated bacterial DNA, revealed a single discrete PCR amplicon band of 1500 bp when resolved on agarose gel (Fig. 1). The forward and reverse primers used for the bacterial DNA sequencing were 8F and 1492R primers revealing two different regions of the 16S rDNA of the bacterium *Kocuria sp.* HO-9042 with 550bp and 985 bp respectively (Fig: 2 & 3). The forward and reverse sequence data revealed a consensus sequence of 1401bp (Fig. 4) referring to the most common nucleotide or amino acid at a particular position after multiple sequences are aligned. The consensus sequence showed which residues were most abundant in the alignment at each position.

Further, the BLAST reports the sequence similarity by 100 blast hits to identify the homologs to the query sequence and infer the unknown bacterium (Fig. 5). 16S rDNA gene of the bacterium was used as the input sequence to determine the bacterial homologs using 1401bp of the query sequence and assessing the similarity. 10 homologous sequences were inferred from the BLAST with similarity of about 200 amino acid or nucleotide sequences with slight variations. The nucleotide database were searched with the sequences obtained using NCBI BLAST tool and showed 99% similarity with 16S rDNA gene of the sampled bacterium in the database sequences. Based on these characteristics and sequence analysis, the isolate was identified as *Kocuria sp* HO-9042.

The significant alignment table (Table 2) revealed the homologous bacteria to the identified bacterium in accordance to the Gen Bank Database. The table depicted the variation in the maximum score of the 10 homologous taxa which were equivalent to the total score with *Kocuria sp* HO-9042 showing 2588 score. The minimum expected value of zero showed the maximum similarity among the homologues revealing them to be the homogenous strains of *Kocuria*. 99% similarity was observed for *Kocuria rosea* strain CT22, Bacterium K2-25 and *Kocuria* sp. CNJ770 PL04 followed by 98% sequence similarity for *Kocuria* sp. RM1, *Actinobacterium* C18 gene, *Kocuria* sp. ljh-7, *Actinobacterium* C20, *Kocuria aegyptia* strain YIM 70003 and *Kocuria* sp. E7 to the identified bacterium *Kocuria* sp. HO-9042.

Figure 2: Forward primer (8F) for *Kocuria sp* HO-9042 of 550bp

TGCAAGTCGAACGATGATCTCCCGCTTGCGGGGGTGATTAGTGGCGAACGGGTGAGTAATACGTGAGTAAC
CTGCCCCTGACTCTGGGATAAGCCTGGGAAACCGGGTCTAATACTGGATACGACTCCTCATCGCATGGTGGG
GGGTGGAAAGGGTTTGACTGGTTTTGGATGGGCTCACGGCCTATCAGCTTGTTGGTGGGGTAATGGCTCACC
AAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCC
TACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGAT
GACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCCACAAGTGACGGTACCTGCAGAAGAAGC
GCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAA
AGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGAAAGCCCGGGGCTCAACCCC

Figure 3: Reverse primer (1492F) for *Kocuria sp* HO-9042 of 985bp

CCTTCGACGGCTCCCTCCCACAAGGGGTTAGGCCACCGGCTTCGGGTGTTACCAACTTTCGTGACTTGACGG
GCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGCTGATCTGCGATTACTAGCGACTCCGACTT
CATGAGGTCGAGTTGCAGACCTCAATCCGAACTGAGACCGGCTTTTTGGGATTAGCTCCACCTCACAGTATC
GCAACCCTTTGTACCGGCCATTGTAGCATGCGTGAAGCCCAAGACATAAGGGGCATGATGATTTGACGTCAT
CCCCACCTTCCTCCGAGTTGACCCCGGCAGTCTCCTATGAGTCCCCACCATCACGTGCTGGCAACATAGAAC
GAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCT
GTCCACCGACCCCGAAGGGAAACCCCATCTCTGGGGTAGTCCGGTGAATGTCAAGCCTTGGTAAGGTTCTT
CGCGTTGCATCGAATTAATCCGCATGCTCCGCCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCCT
TGCGGCCGTACTCCCCAGGCGGGGCACTTAATGCGTTAGCTACGGCGCGGAGAACGTGGAATGTCCCCCAC
ACCTAGTGCCCAACGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCATGCTTTCGCTCCT
CAGCGTCAGTAACAGCCCAGAGACCTGCCTTCGCCATCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTA
CACCAGGAATTCCAGTCTCCCCTACTGCACTCTAGTCTGCCCGTACCCACTGCAGACCCGGGGTTGAGCCCC
GGGCTTTCACAGCAGACGCGACAAACCGCCTACGAGCTCTTTACGCCCAATAATTCCGGACAACGCTTGCG
CCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGCGCTTCTTCT

Figure 4: Consensus Sequence of 16s rDNA gene of *Kocuria sp* HO-9042 (1401 bp)

TGCAAGTCGAACGATGATCTCCCGCTTGCGGGGGTGATTAGTGGCGAACGGGTGAGTAATACGTGAGTAAC
CTGCCCCTGACTCTGGGATAAGCCTGGGAAACCGGGTCTAATACTGGATACGACTCCTCATCGCATGGTGGG
GGGTGGAAAGGGTTTGACTGGTTTTGGATGGGCTCACGGCCTATCAGCTTGTTGGTGGGGTAATGGCTCACC
AAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCC
TACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGAT
GACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCCACAAGTGACGGTACCTGCAGAAGAAGC
GCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAA
AGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGAAAGCCCGGGGCTCAACCCCGGGTCTGCAGTGGGTACG
GGCAGACTAGAGTGCAGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGA
ACACCGATGGCGAAGGCAGGTCTCTGGGCTGTTACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAG
GATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGGACATTCCACGTTCTCCGCG
CCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACAT
TCACCGGACTACCCCAGAGATGGGGTTTCCCTTCGGGGTCGGTGGACAGGTGGTGCATGGTTGTCGTCAGC
TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGTGATGG
TGGGGACTCATAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCT
TATGTCTTGGGCTTCACGCATGCTACAATGGCCGGTACAAAGGGTTGCGATACTGTGAGGTGGAGCTAATCC
CAAAAAGCCGGTCTCAGTTCGGATTGAGGTCTGCAACTCGACCTCATGAAGTCGGAGTCGCTAGTAATCGC
AGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCACGAAAGTTGGT
AACACCCGAAGCCGGTGGCCTAACCCCTTGTGGGAGGGAGCCGTCGAAGG

Figure 5: 100 BLAST hits of *Kocuria sp* HO-9042

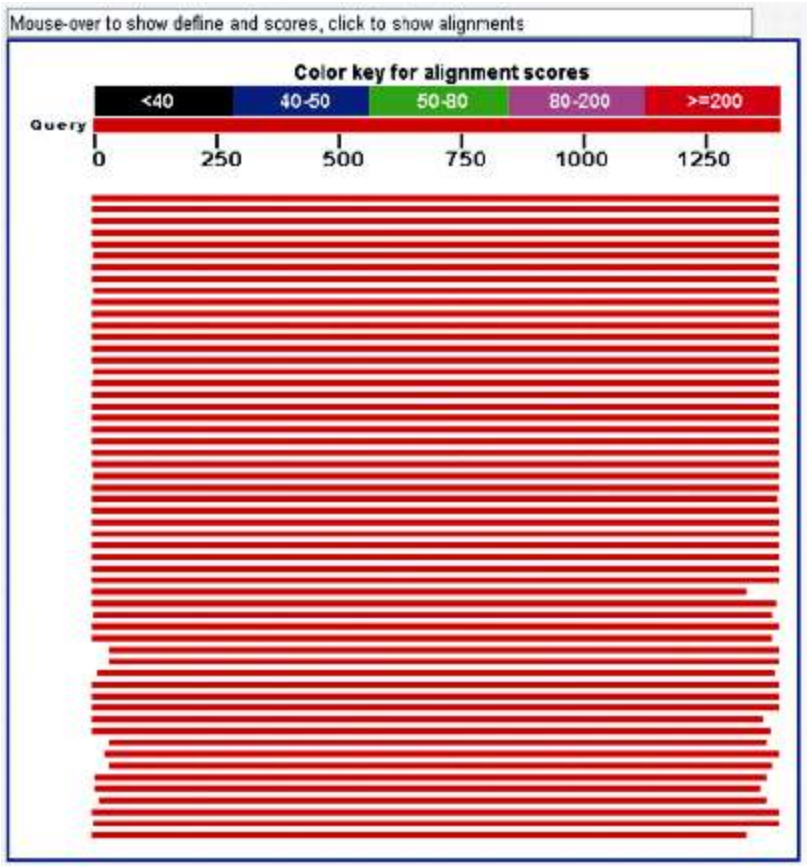


Table 2: Significant alignment table revealing 10 homologs of *Kucoria sp* HO-9042

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
DQ531634.2	*Kocuria* sp. HO-9042	2588	2588	100%	0.0	100%
EU660350.1	*Kocuria rosea* strain CT22	2555	2555	100%	0.0	99%
AY345428.1	Bacterium K2-25	2553	2553	100%	0.0	99%
DQ448711.1	*Kocuria* sp. CNJ770 PL04	2510	2510	100%	0.0	99%
EF675625.1	*Kocuria* sp. RM1	2497	2497	100%	0.0	98%
AB302331.1	*Actinobacterium* C18 gene	2481	2481	99%	0.0	98%
GU217694.1	*Kocuria* sp. ljh-7	2475	2475	100%	0.0	98%
AB330815.1	Actinobacterium C20	2471	2471	99%	0.0	98%
DQ059617.1	*Kocuria aegyptia* strain YIM 70003	2459	2459	99%	0.0	98%
EU372971.1	*Kocuria* sp. E7	2453	2453	100%	0.0	98%

Discussion

Genomic DNA extraction, PCR mediated amplification of the 16S rDNA and sequencing of the PCR products to analyse the sampled bacterium is a highly accepted technique (Kovacs *et al*., 1999). PCR amplification of 16S rDNA using consensus bacterial primers and separation of the resultant PCR amplicons constitute the most popular molecular ecology techniques used to describe soil bacterial ecology (Macrae, 2000). Similar assessment may be done using 16S rRNA genes as in case of *Arthrobacter luteolus* (Emmanuel *et al.,* 2012)

The genus *Kocuria* (Stackebrandt *et al.,* 1995) contains four species, i.e. the type species *Kocuria rosea, Kocuria varians, Kocuria kristinae* and *Kocuria erythromyxa.* All were originally placed in the genus *Micrococcus.* Following 16S rDNA analysis the *Micrococcus,* species were shown to form an individual cluster within the *Arthrobacter-Micrococcus* line of descent (Stackebrandt *et al.*, 1995; Koch *et al.,* 1994); a cluster later described as the family *Micrococcaceae* (Stackebrandt *et al.,* 1997). Members of the genus *Kocuria* were isolated from a wide variety of natural sources including mammalian skin, soil, the rhizosphere, fermented foods, clinical specimens, fresh water and marine sediments. The *Kocuria* strains are circular, non motile gram positive, aerobic, non-encapsulated, non-halophilic, non-endospore forming, with the presence of the fatty acid anteiso $C_{15:0}$ and MK-7 (H_2) and MK-8 (H_2) as the major menaquinones (Zhou *et al.,* 2008).

The sequence similarity values determined for the type strains of members of the genus *Kocuria* ranged between 95.8 and 98.6% (Kovacs *et al*., 1999). The present work result was also in accordance to it showing similar similarity index. The sequence similarity of *K rosea* to other strain was also found to be about 98.1% (Mayilraj *et al.,* 2006). The phenotypic features and complete sequence of 16S rDNA revealed that *Kocuria sp*. HO-9042 strain showed 99% sequence similarity with *Kocuria rosea* strain CT22 as reported by Stackebrandt *et al*. (1995) and 98% sequence similarity with *Kocuria sp.* RM1 and *Kocuria aegyptia* strain 71M 70003 as found by Li *et al*. (2006).

The isolates contained all the signature nucleotides that define the family *Micrococcuceae* to which the genus *Kocuria* belongs phylogenetically (Stackebrandt *et al.,* 1997). A total of 1350 nucleotides present in all strains between positions 41 and 1458 (*E. coli* positions) were used for the analysis were also in configuration to the present study where 1401bp between 8F and 1492R sequences were used.

K. rosea was highly similar to the bacterium *Kocuria sp* HO- 9042 with 99% DNA DNA similarity index. *K. erythromyxa* was not included because, on the

basis of the high 16S rDNA similarity, it can be considered a close relative of *K. rosea* (Kovacs *et al.*, 1999). The type strain *Kocuria rosea* has been reported to cause catheter related bacterium (Altuntas et al., 2004) and the majority of strains are non-pathogenic. Micro-organisms in the soil have the capability to degrade hydrocarbons and act as major agents for remediation of contaminated soil (Widada *et al*., 2002). Presence of *Kocuria sp* HO-9042 in coal fly ash amended soil will also help in the remedial activity as bacterial population have the ability to colonize harsh environments and play their role in improving soil fertility. Therefore its application to the amended FA will be beneficial on the context to the fertility of soil. Being non- pathogenic, the incorporation in the FA amended soil will be harmless. This will help in the utilization of fly ash in agricultural purpose thereby lessening the disposal problems of this emerging solid waste.

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Study of Levels of Heavy Metal in Soil under Amravati Municipal Juridiction, Maharashtra (India)

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Abstract : The study involves the atomic absorption analysis of six selected heavy metals lead, cadmium, zinc, copper and chromium pollution in the soil sample of Amravati municipality, Maharashtra. A total of 160 surface soil samples (0-5 cm.) and 160 sub-surface soil sample (10-15 cm.) were collected from industrial, agricultural, road side and residential area. The result shows that the level of cadmium and copper exceeded the recommended level. Lead and zinc pollution was found only in the industrial area cobalt pollution was found in the industrial and road side area. There was no chromium pollution in the soil samples analyzed.

Key Words : Atomic Absorption Spectroscopy, Heavy metals, pH, Organic content

Introduction

As far as the impact of the environmental pollution is concerned, heavy metals are known to be most harmful like most organic pollutants. The metals are not biodegradable or perishable. When heavy metals are carried into the soil, they will accumulate there with time and enter into the biosphere or the food chain causing harm to human health (Xing and Ching, 2004).

The high density, heavily populated areas with activities, such as sewage and sludge disposal, with high concentration of traffic and automobile combustion are prone to danger of soil contamination with heavy metals especially Mn, Cu, Ni, Fe and Zn (Maharaju, 2010).

The Index of geo-accumulation, enrichment and contamination factor invariable shows that the soil from residential area is moderately contaminated with Cr, Ni and Pb (Cu to some extent). The agricultural soil indicated relatively less contamination indices and it is presumed that plant/crop uptake of these elements along with other macro and micro nutrients during its growth has effectively removed the toxic metals from the soil (Dasaram *et al*., 2011)

The numbers of factor are responsible for the type of pollution *viz.*, geo-climatic conditions, rate of urbanization, improper waste management, other anthropogenic causes etc. almost all these elements that contaminated the system get readily absorbed by plants and then to animal and are relatively toxic at levels slightly above than required for maintaining normal metabolic activities of the body (Chakraborthy *et al*., 2004). This work aimed to increase the awareness of effect of heavy metal pollution on human being. Heavy metal pollution is a major environmental problem threatening to biological system that is least studied in fast growing Amravati municipal area of Amravati District.

Materials and Method

The study was conducted in Amravati municipality which covers 36.26 square kilometer of Amravati District. Amravati is divided into 41 wards. Cotton is the main cultivation of this area. A total of 160 surface soil samples (0-5 cm) and 160 sub-surface soil sample (10-15 cm) were collected from industrial, agricultural, road side and residential area. The metal content were analyzed by Flame Atomic Absorption Spectrometry (FAAS) in the Environmental Impact And Risk Assessment (EIRA) Division, National Environmental Engineering Research Institute (NEERI), Nagpur.

Results and Discussion

For convenience of study Amravati Municipality was divided into industrial area, agricultural area, road side and residential area. In this

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municipality there was no major industries but a number of small scale industries are functioning in south-east side of city. The major part of municipality comprises residential area and agricultural area and main cultivation is cotton (*Gossypium hirsutum*), wheat (*Triticum astivum*), jowar (*Foeinocular valgure*), tur (*Cajanus cajun*), soyabeen (*Glycin max*), orange (*Citrus sp.*) garden and vegetable garden are common. The discussion here will be limited to six selected heavy metals namely lead (Pb), cadmium (Cd), zinc (Zn), chromium (Cr), copper (Cu) and cobalt (Co) (Table 1, 2, 3).

Table 1. Minimum, Maximum and Mean Levels (µg/g) of Metals of Various Study Areas:

Study Areas?		**Industrial Area**		**Agricultural Area**		**Road Side Area**		**Residential Area**	
		Top Soil	Sub-Soil	Top Soil	Sub-Soil	Top Soil	Sub-Soil	Top Soil	Sub-Soil
Pb	Min.	7.9	0.5	0.8	1.5	11.3	11.8	6.1	5.1
	Max.	190.7	135.9	23.8	21.1	83.0	36.4	19.4	16.7
	Mean	99.0	68.2	12.3	11.3	47.0	24.0	13.0	11.0
Cd	Min.	6.2	6.5	3.7	5.2	6.8	6.4	0.4	0.4
	Max.	8.6	8.5	7.1	6.7	8.7	8.6	8.4	9.2
	Mean	7.4	7.5	5.4	5.9	7.75	8.5	4.5	4.8
Zn	Min.	94.6	40.0	85.2	85.7	100.7	113.8	89.4	98.4
	Max.	539.6	621.9	183.9	208.3	187.9	201.6	178.8	164.5
	Mean	317.0	331.0	135.0	147.0	144.0	158.0	94.1	132.0
Cr	Min.	44.6	47.4	16.5	31.4	53.9	53.3	4.1	50.0
	Max.	109.3	122.1	105.0	100.3	87.0	111.1	115.0	87.9
	Mean	77.0	85.0	61.0	66.0	71.0	82.0	60.0	69.0
Cu	Min.	53.9	34.3	46.2	49.0	123.0	145.1	112.9	119.0
	Max.	144.4	181.4	161.9	159.5	163.0	215.4	137.9	199.1
	Mean	99.0	108.0	104.0	119.0	144.0	167.0	126.0	160.0
Co	Min.	35.9	40.9	6.7	2.7	30.6	30.6	3.2	11.0
	Max.	44.5	47.6	7.8	29.5	45.1	48.0	31.7	39.3
	Mean	40.0	44.0	08.0	16.0	38.0	39.0	17.45	25.0

Table 2: Metal variation in surface and subsurface soil in different study area:

	Industrial Area		Agricultural Area		Road Side Area		Residential Area	
	Surface	Sub-Surface	Surface	Sub- Surface	Surface	Sub- Surface	Surface	Sub- Surface
Pb	99.0±0.02	68.2±0.14	12.3±0.05	11.3±0.4	47.0±0.13	24.0±0.22	13.0±0.35	11.0±0.22
Cd	7.4±.050	7.5±0.13	5.4±0.09	5.9±0.25	7.75±1.25	8.5±0.42	4.5±1.3	4.8±1.9
Zn	317.0±12	331.0±14.4	135.0±10.2	147.0±16.5	144.0±14.9	158.0±12.6	94.1±5.6	132.0±8.9
Cr	77.0±2.3	85.0±1.3	61.0±5.2	66.0±8.5	71.0±1.5	82.0±6.5	60.0±4.2	69.0±5.3
Cu	99. 0±4.2	108.0±8.4	104.0±5.2	119.0±9.4	144.0±4.2	167.0±9.1	126.0±12.0	160.0±9.4
Co	40.0±1.2	44.0±4.6	08.0±3.2	16.0±8.7	38.0±5.2	39.0±10.9	17.45±1.5	25.0±0.9

Table 3: Surface/Subsurface soil ratio in different study area:

	Lead	Cadmium	Zinc	Cromium	Copper	Cobalt
Industrial Area	1.451613	0.986667	0.957704	0.905882	0.916667	0.909091
Agricultural Area	1.088456	0.915255	0.918367	0.924242	0.873950	0500000
Road-Side Area	1.958333	0.911765	0.911392	0.865854	0.852071	0.974359
Residential Area	1.181818	0.937500	0.712879	0.869565	0.787500	0.698000

Lead: More than 95% of lead now cycling in the biosphere is anthropogenic in origin. As lead has less mobility and persistence for long time, it enters in the biological system and will cause harm to living beings including humans (Fig.1).

The motor vehicles, paints and dyes, batteries, pesticides, fertilizers, explosives and metallurgical industries mainly release the lead to the soil. The highest level of lead was recorded (190.7µg/g) in soil sample from Agro-chemical and Engineering work Industries. Mechanical works with metals, unscientific measures or approach to motor vehicles, use of petrol, diesel and their release resulted serious lead pollution here.

Agricultural areas near major cities receive some

Pb

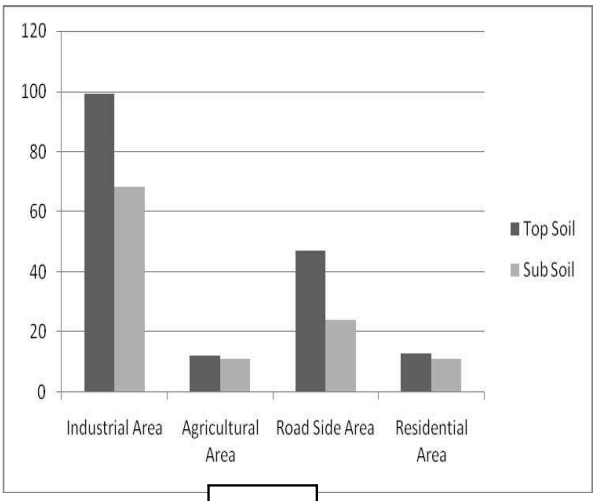

Fig. 1

Cd

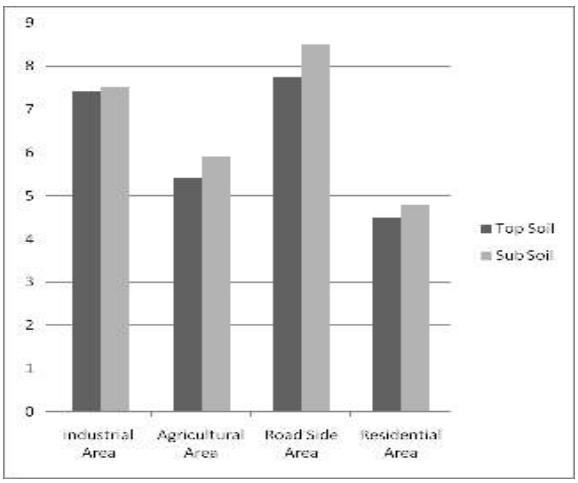

Fig. 2

lead from automotive emissions and metal industries emitting lead into the atmosphere.

Studies of a pastoral agro ecosystem near Adelaide showed that lead concentrations in surface soils had measurably increased up to about 50 km from the city boundary (Tiller *et al.*, 1987).

The roadside ecosystems are the natural targets of lead pollution. The roadside soils generally contain higher levels of heavy metals than other soils. The amount of Pb is higher in samples collected from the main roads and nearby soils of oil refinery (Smith and Flegal, 1995).

In the residential area though the value showed is little high most of the area is not polluted.

Cadmium: From the Fig. 2, it is clear that cadmium concentration is higher in subsurface soil samples, which indicates mobility and leakage of this metal. The study revealed that higher levels are strongly associated with human activities and automobile emissions. The levels of cadmium in both surface and sub-surface soil samples are higher than the permissible level. The wide use of metals paints and dyes in the industrial sites is the main reason for cadmium pollution in these soil samples.

Agricultural management practices that directly affect cadmium concentration and availability in the soil may influence the cadmium accumulation by crops. Specifically the addition of sludge or fertilizer having high cadmium concentration to agricultural land may cause significant increase in the uptake of cadmium by crops (Grant *et al.*, 1999). Soils treated with phosphate fertilizers showed an increase in concentration of cadmium.

High density traffic is pollutant for soil, water and environment (Koc *et al.*, 2004). The increasing concentration of heavy metals coincided with the increasing population and as a result increasing number of private and public vehicles in the city (Talebi and Bedi, 2004). The high value of cadmium in the subsurface of residential area has been noticed.

Zinc: The zinc content in surface soil samples varies from 85.2mg/g to 539.6mg/g with an average value of 172.53mg/g, whereas subsurface soil samples range from 40.0mg/g to 621.9mg/g with an average value of 192.0mg/g. High concentration of zinc was found to be associated more in the industrial locations than in residential areas (Claramma and Joseph, 2008). Even though, there was no sample showing zinc pollution, some subsurface and a few surface samples show higher levels, but never exceeded the lower limit or minimum level recommended for zinc concentration in soil shows a higher concentration of zinc in subsurface soil samples indicates continuous leaking of this metal (Fig. 3).

The higher concentration of zinc is present in Industrial area. In agriculture and farming there is extensive use of pesticides, fungicides and bio-solids along with fertilizers. These materials are good sources of heavy metals like zinc, lead, chromium, cobalt, cadmium and copper. The heavy metal concentration in soil is a result of soil forming processes, as well as agricultural and human activities.

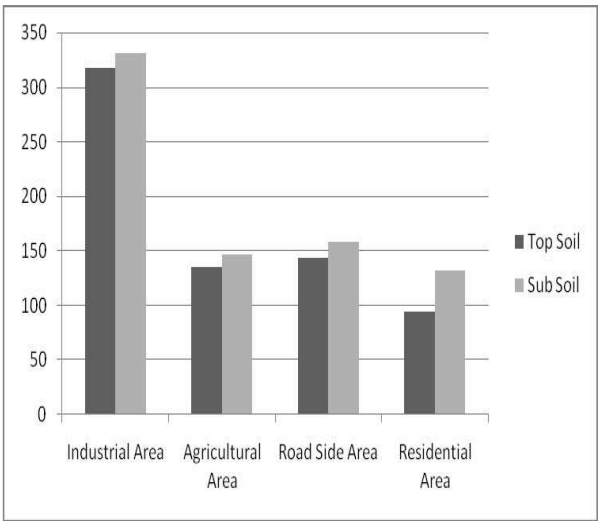

Fig. 3

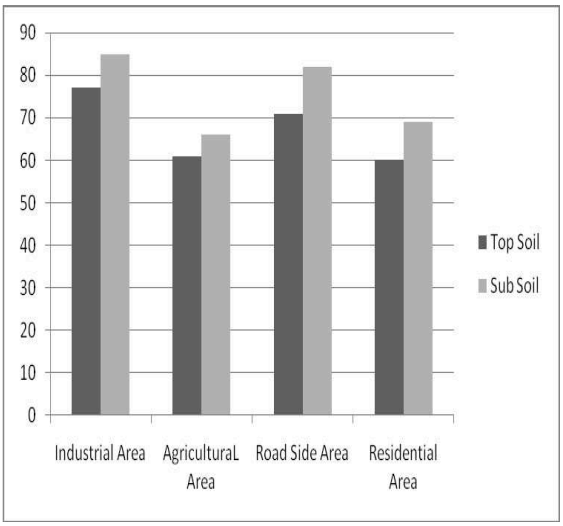

Fig. 4

Chromium: The chromium concentration is higher in subsurface soil samples, which indicates mobility and leakage of this metal. The chromium content in subsurface soil samples varies from 31.4mg/g to 121.1mg/g with an average value of 75.5mg/g, whereas surface soil samples range from 4.1mg/g to 115.0mg/g with an average value of 59.55mg/g. High concentration of chromium was found to be of subsurface soil sample in the industrial area. Chromium concentration in the agricultural area and residential area is near about same (Fig. 4).

Copper: The Fig. 5 shows a close average level in the roadside area and residential area but a higher accumulation of copper in the subsurface soil of roadside area probably continuous deposition and leaching. Roadside area is more-dry. The moisture content of the soil was important for Cu retention. Dry soil had higher Cu concentration than humid soil. One important peculiarity noted in Cu distribution is that when agricultural land is classified into different plantations/the mean concentration of Cu in the surface and subsurface soils are near about same or the ratio of cu in the surface to subsurface soil is unity. Extensive use of pesticides, fungicides and bio-solids along with certain fertilizers in agriculture and farming resulted in higher concentration of Cu in the soil. Application of animal manure and urban wastes on farmland and repeated application of copper containing pesticides concentrates copper in the soil (Van der Watt *et al.*, 1994).

Cobalt: The cobalt content in surface soil samples varies from 3.2g/g to 45.1mg/g with an average value of 24.15mg/g, whereas subsurface soil samples range from 2.7mg/g to 47.6mg/g with an average value of 25.15mg/g (Fig. 6). High concentration of cobalt was found to be of subsurface soil sample in the industrial area. The average concentration of cobalt in soils throughout the world is 8 parts per million (ppm) (MOEE 1993). The cobalt concentration is higher in subsurface soil samples, which indicates mobility and leakage of this metal. The cobalt concentration is found like above increasing order-

Residential Area > Road Side Area > Residential Area > Agricultural Area.

The concentration of heavy metals, lead, cadmium, copper, chromium, zinc and cobalt increases with increase in organic content in the soil (Wesley, 2004). The samples showing high levels of heavy metals had high organic content. A complication process occurs between heavy metals and organic carbon and results in the retention of heavy metals in the soil. The samples that showed highest levels of the heavy metals are collected from the soils of Industrial area. The organic carbon of this soil was 2.6 % and it was the highest value among other areas samples.

Increase in pH in the soil results in increase heavy metal concentration in the soil i.e. alkaline pH favors heavy metal accumulation in the soil (Claramma and Joseph, 2008). All the samples exhibited a positive correlation with pH (Table 4).

The highest value of pH 9.2 was noted in the sample from Industries. This sample had the highest concentration of heavy metals. Even though the higher pH favors heavy metal retention in soil it limits the heavy metal uptake by plants. The heavy metal uptake by plants decreases at higher pHvalued. But the acidic pH favors the uptake and causes harm to the living beings via, food chain.

Most of the samples analyzed had pH below 7, i.e., acidic in nature. This indicates that the uptake by plants was high and the biological system was already contaminated by the heavy metals. Soil pH and high organic content have a higher retention capacity of heavy metal in soil. The present study agrees with the findings of William and David (1976).

Cu

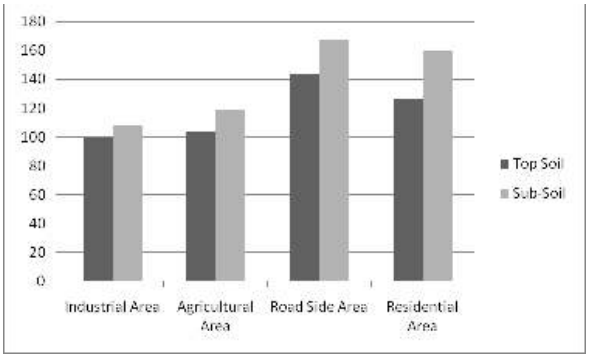

Fig. 5

Co

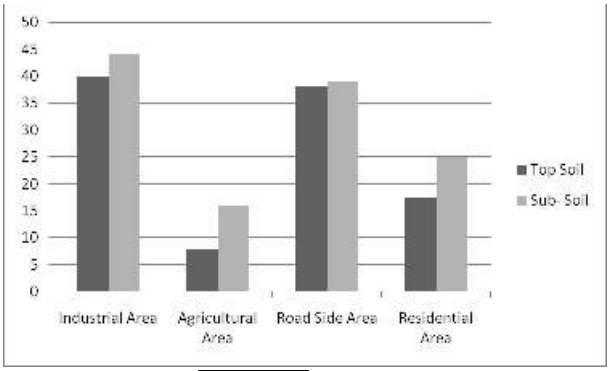

Fig. 6

Table 4: pH and OM variation in surface and subsurface soil in different study area:

	Industrial Area		Agricultural Area		Road Side Area		Residential Area	
	Surface	SubSurface	Surface	Sub- Surface	Surface	Sub- Surface	Surface	Sub- Surface
pH	9.22±0.4	8.25±0.3	7.10±0.2	7.21±0.3	5.91±0.5	5.84±0.4	8.42±0.2	8.11±0.5
OM(%)	1.53	1.68	10.31	11.10	3.46	3.59	5.20	5.68

Surface/subsurface ratios:

Table 3 records the ratios of surface mean metal content to mean subsoil content in the studied areas. Lead is markedly enriched in surface soil samples, general environmental contamination and arid conditions, particularly in the industrial area and along roadsides where the ratios 1.5 and 1.96 respectively. Al-Shayeb *et al*. (1995) reported that the lead content of Riyadh roadside was as much as 123.28μg g^{-1} in the top 5 cm layer and decreased to 39.56 μg g^{-1} at 10-15 cm. The most important reason for difference in these concentrations that lead is not very mobile, mostly accumulating in the top 5 cm of the soil profile. The ratio grades from lower value in the residential area to higher values in agricultural, roadside and industrial areas, with the highest values in the industrial areas.

Similarly copper enrichment was very obvious in industrial area soils. Soil samples show that all different metals were largely concentrated in the topsoil of industrial area soil. Cadmium, zinc and chromium have greater mobility and no significant difference between the top and sub soil levels. Cobalt enrichment was very obvious in roadside soils. Soil samples show that all-different metals were largely concentrated in the topsoil of roadside, confirming the automobile origin of these metals.

In the soils of Amravati the sources of large-scale pollution are not so much individual emitters as group of emission sources. Traffic volume, age of the road, prevailing wind etc. can affect roadside metal levels. Temperature is likely to influence the pattern of distribution of pollutants through its effects upon air movements.

Other major factors that affect heavy metal level in the soil are $CaCO_3$, cation exchange capacity (CEC) and clay content. Bansal (1997) reported that $CaCO_3$, acts as a strong adsorbent of cadmium, thereby affecting their availability. CEC has a negative co-relation with heavy metals. Increase in CEC of soil shows a decrease in heavy metal concentration (Bansal, 1997). Krishna Swami (2003) studied the cadmium adsorption capacity of Tamil Nadu soils and concluded that the cky present in it influenced Cd adsorption capacities of soil.

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Evaluation of Soil Microfauna under *Parthenium hysterophorus* (L.) Vegetation

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Abstract : Experiments were performed to evaluate the microfauna of soil under *Parthenium hysterophorus* vegetation. Soil samples were collected from a depth of 10-15cm, at an interval of 15 days for two months from dense vegetation of *Parthenium* and nearby normal vegetation from agro-ecosystem. Three types of bacterial colony viz. circular-entire, irregular-undulate and punctiform-entire were observed in normal soil while in *Parthenium* affected soil one additional filamentous type of colony was found. All types of colonies showed white pigmentation and Gram positive reaction. Quantitatively the bacterial population of affected soil sample was always significantly less than ($p < 0.001$) normal soil sample. Mean bacterial population of normal soil sample was $33.4\pm0.989\times10^9$, $52\pm0.866\times10^9$, $22.5\pm1.50\times10^9$ and $42.1\pm1.724\times10^9$ and in *Parthenium* affected soil it was $28.3\pm1.921\times10^9$, $49.4\pm1.40\times10^9$, $16.4\pm2.138\times10^9$ and $34.6\pm1.040\times10^9$. A similar trend of significant decline in wet weight (mg /g soil) and biomass (mg /g soil) of bacteria was observed between normal soil samples and affected one. The results indicated that *Parthenium hysterophorus* imparts adverse effect on soil bacteria. The Gram positive bacterial colony with punctiform-entire, shape and margin appeared to be resistant to *Parthenium* infestation in comparisons to other bacteria. Hence, bacteria colony having such type of colony morphology can be used as bioindicator for pollution caused by *Parthenium* .

Keywords : *Parthenium hysterophorus*, Microfauna, Parthenin, Bioindicator.

Introduction

Soil is the reservoir on which most of the life forms on earth depends, as the primary source of food, feed, forage, fiber and pharmaceuticals. Top soil is biologically the most diverse part of the earth and it plays a vital role in sustaining human welfare and assuring future agricultural productivity and environmental stability. An important factor in influencing the productivity of our planet is the nature of their soils. Parr *et al*. (1992) reviewed the different chemical, physical and biological properties of soils that interact to determine the fitness or capacity to produce healthy nutritious crops. It forms a dynamic and complex living system, comprise air, water mineral particles, dead organic matter and various types of living organisms. True soils are influenced, modified and supplemented by living organisms *viz*. micro, meso and macrofauna of soil. Soil microbes, invertebrates and minerals live and work together in an underground system that helps to improve soil and water quality and also to provide greater resistance of plants to pests and disease (Higa, 1994). Soil microorganisms are responsible for decomposition of residual agrochemicals in soil (Higa, 1993), greater mineralization of carbon (Daly and stewart, 1999), more efficient release of nutrients from organic matter (Sangakkara and Weerasekera, 2001) and improved resistant to adverse weather (Higa, 1993) etc. Hence any disturbance in soil microbial population results into adverse effect on the above process which ultimately alters the soil quality.

Quantitative and qualitative microbial activities are the key factors for productivity and sustainability of soils health for maintenance of crop production (Pankhurst *et al*., 1996; Nannipieri *et al*., 2003; Tilak *et al*., 2005). Analysis of structural and functional microbial diversity would, therefore, reveal their sustainability of both man powered and natural ecosystems. Apart from insecticides, pesticides and other various chemicals weed like *Parthenium hysterophorus* (L.) (Heliantheae : Asteraceae; 2n = 34), which is an exotic species of commonly found weed, adversely affect the soil subsystem.

Parthenium hysterophorus has been reported to affect earthworm population (Raipat, 2010), soil bacterial population under experimental condition (Saha *et al*., 2010), and human affairs (Towers and Subba Rao, 1992) etc. but its impact on soil microfauna under natural condition has not been studied.

Although microbial diversity of rice field soil has been nominally investigated (Das and Dangar, 2007a,

b), microbial population of habitats like cropland, botanical gardens, *Parthenium* infested cropland and fallow lands etc. remained unattained to date. The paper deals with the evaluation of soil bacteria (microfauna) under *Parthenium hysterophorus* vegetation to assess the impact of *Parthenium hysterophorus* (L.) on bacterial population.

Materials and Methods

The soil samples were collected with the help of sterilized spatula from a depth of 10-15 cm, at a interval of 15 days for two months from two fields, one having dense vegetation of *Parthenium* and other having normal vegetation. Soil sample of normal soil was labeled as sample A and soil sample collected from the area having dense vegetation of *Parthenium* was labeled as sample B. Enumeration of bacterial population of both the soil samples was done by pour plate technique or dilution plate method on nutrient agar medium (Thornton, 1922; Thom and Raper, 1945). 1 g of both the soil samples was taken and each was dissolved in 9 mL of autoclaved distilled water. Dilutions of 10^{-7} were prepared and 1 mL inoculums of the primary suspension were taken for pouring. Czapek Dox agar media (peptone - 10 g/L, NaCl - 5 g/L, beef extract – 10 g/L, agar – 15 g/L at pH - 7) was used for culture. The Petriplates (diameter 100 mm) were incubated at 37°C for 48 h. For each experiment, three replicates of Petriplates were incubated.

For qualitative analysis, colony morphology (shape-margin), elevation and pigmentation were studied. Quantitative analysis was done by counting number of bacterial colonies (cfu) by colony counter and the results were expressed as 1 g of soil (Table 2). Gram staining reaction of bacterial colony, wet weight and biomass of bacterial colonies of both the soil samples were also analyzed. The mean fresh weight of a bacterium cell was taken as 1.5×10^{-12}g (Toth and Hammer, 1977). This value, when multiplied with the number of bacterial colony, gave the fresh weight of bacteria. Assuming 80% of bacterial cell to be water (Clark and Paul, 1970) biomass of bacterial colony was calculated (Satpathy *et al.,* 1982). T-test was done to determine the significance of change in population and biomass of bacterial colony of both the soil samples.

Results

Qualitative analysis involved morphological details of different bacterial colonies grown on nutrient agar plates (Table 1). The developed bacterial colonies of normal soil (sample A) from shape and margin view point were of three types viz. circular-entire, irregular-undulate and punctiform-entire, while in *Parthenium* affected soil (sample B) one additional type of colony was observed i.e. filamentous. In sample A, 25% of the colonies were circular-entire with 50% flat and 50% convex elevation, 25% colonies were of irregular-undulate type with 50% flat and 50% raised elevation and remaining 50% of the colonies were of punctiform-entire type with flat elevation. All types of colonies showed white pigmentation and gram positive reaction. In sample B, 5% of the colonies were circular-entire, 20% were irregular-undulate, 70% were punctiform-entire and 5% of the colonies were of filamentous type.

Table 1: Morphological details of bacterial colonies in culture condition

Samples	Shape	Margin	Elevation	Pigmentation	Gram reaction
A	25% circular	25% entire	50% flat 50% convex	100% white	Positive
	25% irregular	25% undulate	50% flat 50% raised	100% white	positive
	50% punctiform	50% entire	100% flat	100% white	positive
B	5% circular	5% entire	80% flat 20% convex	100% white	Positive
	20% irregular	20% undulate	50% flat 50% raised	100% white	positive
	70% punctiform	70% entire	100% flat	100% white	positive
	5% filamentous		100% flat	100% white	positive

The elevation of circular-entire colonies were either flat (80%) or convex (20%) and pigmentation was white. 50% of irregular-undulate colonies were with flat elevation and rest 50% with raised elevation. While all the punctiform-entire and filamentous colonies showed flat elevation. Like sample A, bacterial colonies of soil sample B were also white in colour and were positive in gram staining reaction.

In quantitative analysis, bacterial population was observed and was represented as colony forming unit (cfu/g of soil) and has been presented in Fig. 1. It was observed that bacterial population of soil sample B was always less than soil sample A. In first observation, mean bacterial population in sample A was $33.4\pm0.989\times10^{9}$ and in sample B it was $28.3\pm1.921\times10^{9}$. The mean bacterial population of sample A after 2nd, 3rd and 4th observation was $52\pm0.866\times10^{9}$, $22.5\pm1.50\times10^{9}$ and $42.1\pm1.724\times10^{9}$ and for sample B it was $49.4\pm1.40\times10^{9}$, $16.4\pm2.138\times10^{9}$ and $34.6\pm1.040\times10^{9}$. The bacterial population gradually declined from first to last observation and the change in population was found to be significant ($p < 0.001$).

A similar trend of decline in wet weight (mg/g soil) and biomass (mg/g soil) was observed between sample A and sample B (Figs. 2 and 3). Wet weight for sample B was $42.5\pm2.88\times10^{-3}$, $74.1\pm2.1\times10^{-3}$, $24.7\pm3.20\times10^{-3}$ and $52\pm1.56\times10^{-3}$ which was always significantly ($p < 0.001$) less than $50.1\pm3.04\times10^{-3}$, $78\pm2.25\times10^{-3}$, $33.75\pm2.25\times10^{-3}$ and $63.2\pm2.88\times10^{-3}$ for sample A. The biomass of sample A recorded was $10.02\pm0.61\times10^{-3}$, $15.6\pm0.45\times10^{-3}$, $6.75\pm0.45\times10^{-3}$ and $12.64\pm0.52\times10^{-3}$ and of sample B was $8.5\pm0.57\times10^{-3}$, $14.82\pm0.42\times10^{-3}$, $4.94\pm0.64\times10^{-3}$ and $10.4\pm0.31\times10^{-3}$. The decline in biomass of sample B over sample A was also found to be significant ($p < 0.001$).

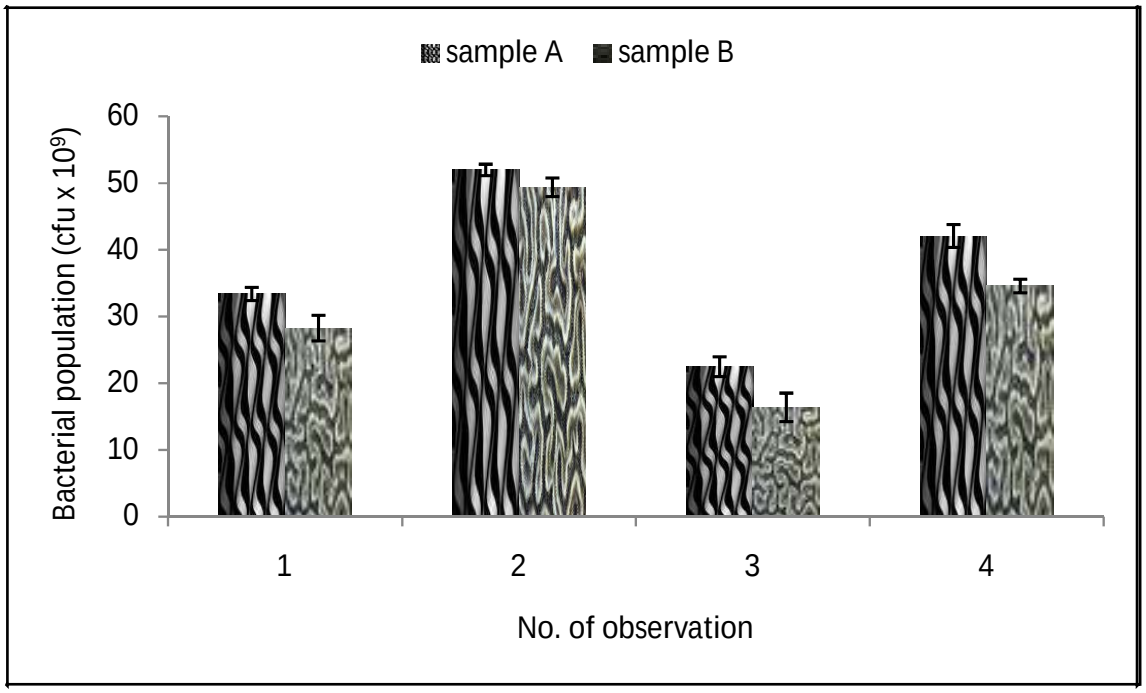

Figure 1 : Bacterial population (number per g soil) in sample A and sample B

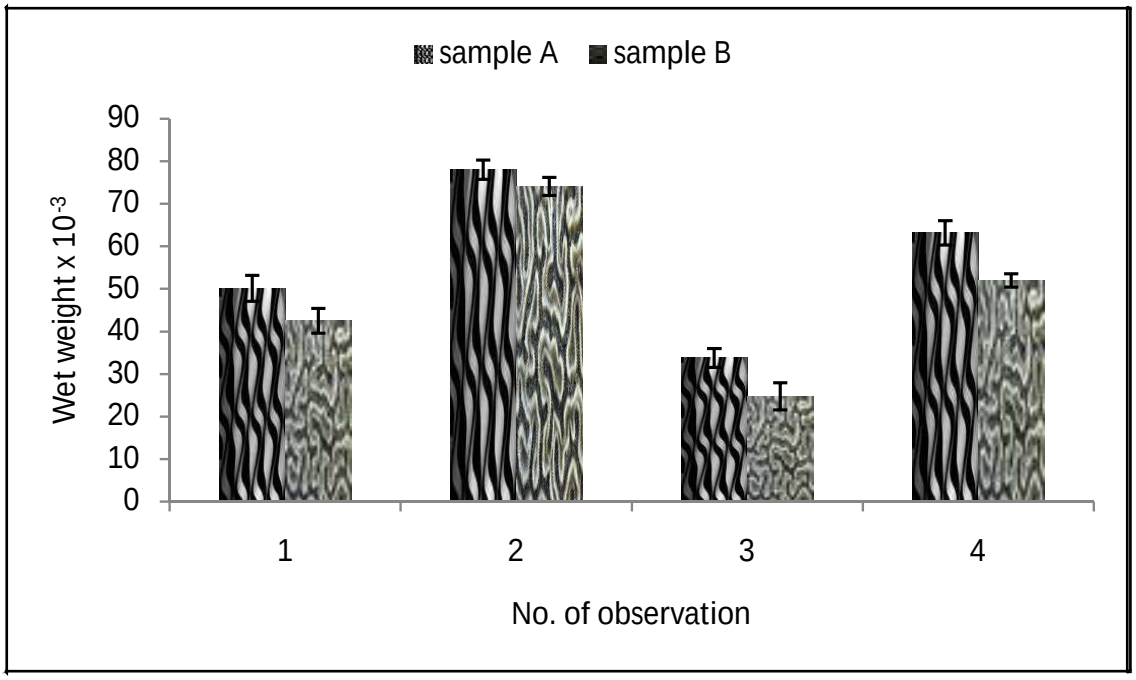

Figure 2 : Wet weight (mg/g soil) of bacterial population in normal soil and *Pathenium* affected soil

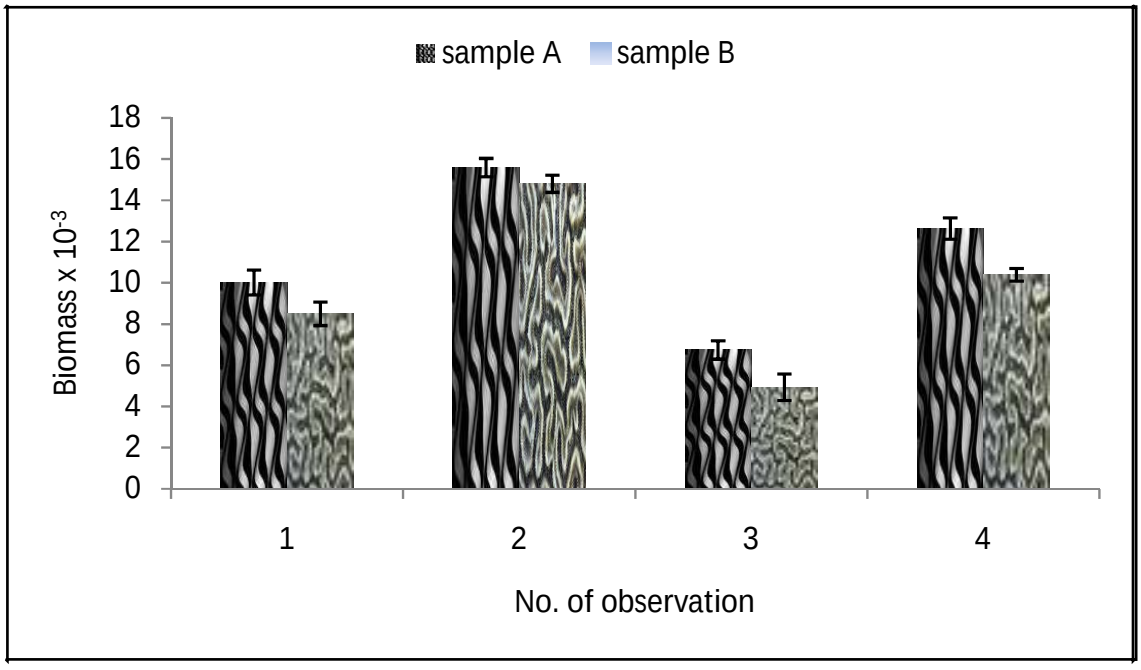

Figure 3 : Biomass (mg/g soil) of bacterial population in normal soil and *Parthenium* affected soil

Discussion

Parthenium hysterophorus L. is an aggressive and noxious weed, commonly known as congress weed has become a threat to the environment and biodiversity. The colony forming units of normal soil is always more than the *Parthenium* infested soil (Fig. 1). This could be attributed to the fertility of soil and fertile soil contains fantastic number of living microorganisms (Francis, 1951). The bacterial population of *Parthenium* infested soil showed decrease in colony forming units and this could be because of the difficulty in survival and multiplication of bacteria in soil containing decomposed material of weed or accumulation of toxic chemical, viz. parthenin, caffeic acid, vanillic acid, ansic acid etc. which are present in the weed. Chemical analysis has indicated that all plant parts including trichomes and pollen contains lethal toxin, parthenin from the chemical group of sesquiterpene lactones (Oudhia and Tripathi, 1998). Enumeration of bacterial population of soil samples showed variations in all observation and the variation could be due to seasonal changes. Large effect of seasonal changes in soil moisture, soil temperature and carbon input on soil microbial biomass and its activity has been reported by Ross (1987) which in turn, affect the ability of soil to supply nutrients to plants through soil organic matter turnover (Bonde and Roswall, 1987). Microbial biomass has been reported to vary seasonally in European soils (Patra *et al*., 1990). The resultant wet weight and biomass of bacterial colony revealed significant reduction (Figs. 2 and 3). Doran (1980) and Handrix *et al*. (1986) reported the microbial biomass and their activities in soil may fluctuate due to different soil management practices. Amendment of soil by FYM (field yard manure), sheep dung, green manure, rice straw and sewage sludge showed significant increase in available phosphorus content, microbial biomass and dehydrogenase activity in soil (Sindhu and Beri, 1986; Ghany *et al*., 1997; Tillak *et al*., 1986; Mukharjee *et al*., 1990; Harden *et al*., 1993 and Saha *et al*., 1995) while Miller (1973) and Kulkarni and Pushpa (1993) reported the adverse effect on soil microbial population and activities in sludge and weed amended soils respectively. Soil containing minerals and microorganisms are supportive for plant growth but if get contaminated becomes least supportive. Patrich *et al*. (1984) stated that decomposition of plant residue in soil may have either favourable or unfavourable effect on plant growth.

The results indicated that *Parthenium hysterophorus* imparts adverse effect on soil bacteria. The gram positive bacterial colony with punctiform-entire, shape and margin appeared to be resistant to *Parthenium* infestation in comparison to other bacteria. Hence, bacteria colony having such type of colony morphology can be used as bioindicator to indicate the level of pollution caused by weed *Parthenium* .

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Evidence for the Alteration of Plasma Calcium by Synthetic Glucocorticiod Dexamethasone

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Abstract : The present investigation deals with the effect of dexamethasone, a synthetic glucocorticoid on plasma calcium concentration in guinea pigs. The administration of dexamethasone @ 2.5 mg/kg body weight for seven consecutive days results to an elevation of plasma calcium concentration from a mean value of 4.51±0.106 mol eq/L to a mean value of 5.85±0.102 mol eq/L, an increase of about 30% which is statistically significant. The probable mode of action for dexamethasone is suggested.

Keywords : Dexamethasone, Guineapigs, Plasma calcium , Hypercalcemia.

Introduction

Calcium is one of the most important elements in the body of mammals including humans, involved in numerous physiological functions. It is the main structural component of bone and is also readily available to serve a variety of extracellular and intracellular activities e.g., its involvement in blood coagulation, nerve impulse conduction (Widdicombe, 1985), muscle contraction (Andrew, 1975), cell signaling (Clapham, 1995; Berridge *et al*., 2000; Carafoli *et al*., 2001), lymphocyte activation (Stefan, 2007), inhibition of renin secretion from kidney (Kurtz and Wagner, 1999) and mitochondrial functioning (Brini, 2003).

The homeostasis of calcium is classically maintained by parathormone [PTH], calcitonin, 1, 25 $(OH)_2$ D_3 and more recently discovered PTH related peptide [PTHrP] (Hadley and Levine, 2009). However, there exist a large number of non-classical modulators which also directly or indirectly influence the blood calcium concentration. One such modulator is glucocorticoid.

Effects of glucocorticoids in different mammals vary with species hence the results are often contradictory. Studies in rats have shown that glucocorticoids increase bone mineralization (Yasumura, 1976; Yasumura *et al*., 1976). The effects glucocorticoids on bone density in rats also varied with the dose of glucocorticoids administered (Jee, *et al*., 1970). On the other hand, rabbits lose bone rapidly with glucocorticoid therapy (Storey, 1961; Thompson and Urist, 1973). Limited reports are available on guinea pigs regarding glucocorticoid treatment and calcium regulation (Follis, 1951; Sobel, *et al*., 1960), although, guinea pigs and man both have cortisol is a predominant glucocorticoid. It seems that they would be a good animal model to use in such kind of studies. The present authors, therefore, took an attempt to investigate the effect of a synthetic glucocorticoid (dexamethasone, 9-fluoro-11β, 17, 21-trihydroxy-16a-methylpregna-1, 4-diene-3, 20- dione) on plasma calcium level in guinea pigs.

Materials and methods

For the present investigation, eighteen guinea pigs of both the sexes (Mammalia, Eutheria, Rodentia) weighing about 250-300 grams were procured from the local market of Ranchi and brought to the laboratory. They were acclimated to laboratory conditions for a period of fifteen days and fed with leaves of cauliflower and spinach *ad libitum*.

The animals then were divided into three groups. From the animals of Group 1 the blood was collected on day one and this group was treated as normal / control. Group 2 animals were injected with 2% normal saline alone for seven consecutive days, while group 3 individuals were treated with dexamethasone (Dexona, Cadila laboratories) intra-peritoneally @ 2.5 mg/kg body weight daily (Rao *et al.*, 1994) at 10 am for seven

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consecutive days. On the seventh day, one hour after the last injection, the blood was collected from individual each group. Group 2 treatment with saline was to assess the variation if any due to stress in blood parameters during the experimental phase.

All the samples were analyzed to measure the plasma calcium concentration together to minimize the inter assay variation if any, following the method of Tinder (1969).

The protocol is as follows :

Altogether 29 test tubes were taken and labeled as T_1-T_5 as blank, T_6-T_{11} as standard, T_{12} -T_{17} as group-1(1-6), T_{18}-T_{23} as group-2(7-12) and T_{24}-T_{29} as Group 3 (13-18).

InT_1-T_5, 0.2 ml of distilled water is taken and considered as blank.

In T_6-T_{11}, 0.2 ml of calcium solution is added in increased concentration; 1 mol eq/L, 2 mol eq/L, 4 mol eq/L, 6 mol eq/L, 8 mol eq/L and 10 mol eq/L respectively, these will help us to prepare a standard curve when required.

In T_{12} -T_{17}, 0.2 ml of samples of Group-1, in T_{18}-T_{23}, 0.2 ml of samples of Group-2 and in T_{24}-T_{29}, 0.2 ml of samples of Group-3 were added.

Now in each test tube 5 ml of calcium reagent 1 is added and incubated for half an hour at 37°C, cooled and centrifuged at 2000 rpm, the supernatant so formed were discarded.

To each test tube 1 ml of EDTA solution was added, the mixture is heated in water bath for one hour, cooled and in each test tube 3 ml of colour reagent was added. The colour so developed was measured by Systronic spectrocolorimeter at 450nm.

For measurement with the blank, zero was fixed and Optical Density (OD) values of different standard were measured followed by the samples. Considering the OD values the concentration was estimated by using the following formula:

$$\text{Concentration of Sample} = \frac{\text{OD value of sample}}{\text{OD value of standard}} \times 5 = \ldots\ldots\ldots \text{ mol eq/L}$$

The data obtained were statistically evaluated to paired sample t-test (Zar, 2006).

Results

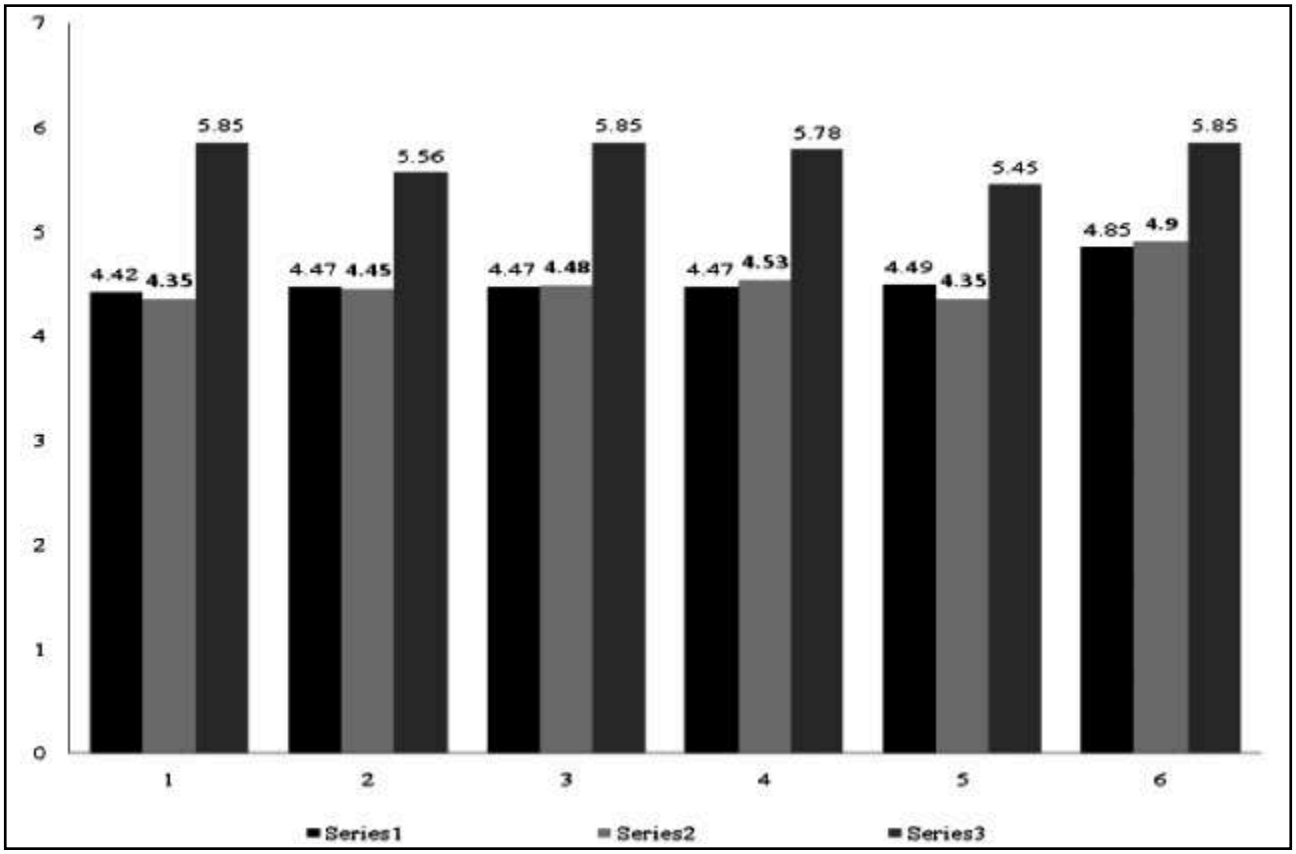
In normal/ control group of individuals, the plasma calcium concentration was found to be an average value of 4.52±0.104 mol eq/L on the first day while on last day it was 4.51±0.106 mol eq/L. However, the administration of dexamethasone for seven consecutive days@2.5 mg/ kg body weight caused a sharp increase in the level of plasma calcium from an average value of 4.51±0.106 mol eq/L to an average value of 5.85±0.102 mol eq/L an increase of about 30% (Table 1 & 2 and Fig. 1). The statistical analysis also indicates the elevation of plasma calcium due to the administration of dexamethasone @ 2.5 mg/kg body weight was a significant at less than 1% level (Table 2).

Table: 1. showing the mean calcium concentration in different treatment groups

Experimental Groups	Group-1. Normal on 1st day	Group-2. Normal (Saline treated) on last day	Group-3. Dexamethasone treated
Mean value of plasma calcium concentration	4.52±0.104 mol eq/L	4.51±0.106 mol eq/L	5.85±0.102 mol eq/L

Table: 2.showing the paired sample t-test values between different treatment groups.

	Gr.1: Gr.2	Gr.1: Gr.3	Gr. 2: Gr.3
t-value	-0.58882 (insignificant)	-14.3878**	-14.8257**



Series 1 Individuals of Gr. 1. on first day

Series 2 Individuals of Gr.2. saline treated

Series 3 Individuals of Gr.3. dexamethasone treated

Figure: 1. showing the plasma calcium concentration in different experimental groups expressed in mol. eq/L (one to one comparison)

Discussion

It is well established that glucocorticoids are anabolic steroids for hepatic cells and catabolic steroids for skeletal muscles as well as for adipose tissue (Hadley and Levine, 2009). Glucocorticods are widely known for their gluconeogenic, anti-inflammatory and anti-allergic activity (Norris, 2006). In recent past, multiple functional roles for synthetic glucocorticod, dexamethasone (9-fluoro-11β,17,21-trihydroxy-16a-methylpregna-1,4-diene-3,20-dione) is ascertained as it is often used in the treatment of asthma (Walsh, 2005), rheumatoid arthitis (Boers , 2004) and tissue/organ transplantation (DuPont *et al.*, 1984) .It is also used in post dental surgery, in countering the side effects of anti tumor treatment, in carcinomatous metastatic spinal cord compression (Sørensen *et al.*,1994), in counteracting development of edema in brain tumors (Kaal and Vecht, 2004) and in the treatment of congenital adrenal hyperplasia (Rivkees and Crawford, 2000). Such wide spread use of glucocorticoids also influences the calcium balance in the body. Glade and Krook (1982) and Patschan *et. al.* (2001) have observed that long term administration of glucocorticods causes osteoporosis and osteonecrosis. On the other hand, Klein *et. al*. (1977) and Cosman *et al*. (1994) have reported the decreased calcium absorption form dietary source and increased urinary elimination of calcium after the administration of dexamethasone. Weinstein *et al.* (1998) and O'Brien *et al.* (2004) have found that glucocorticoids are involved in inhibition of osteoblastogenesis and in the promotion of apoptosis of osteoblasts and osteocytes. On the contrary, Hirsch *et al.* (1998) have observed calcitonin like anti-hypercalcemic property of glucocorticoid but in parathyrodectomised rats.

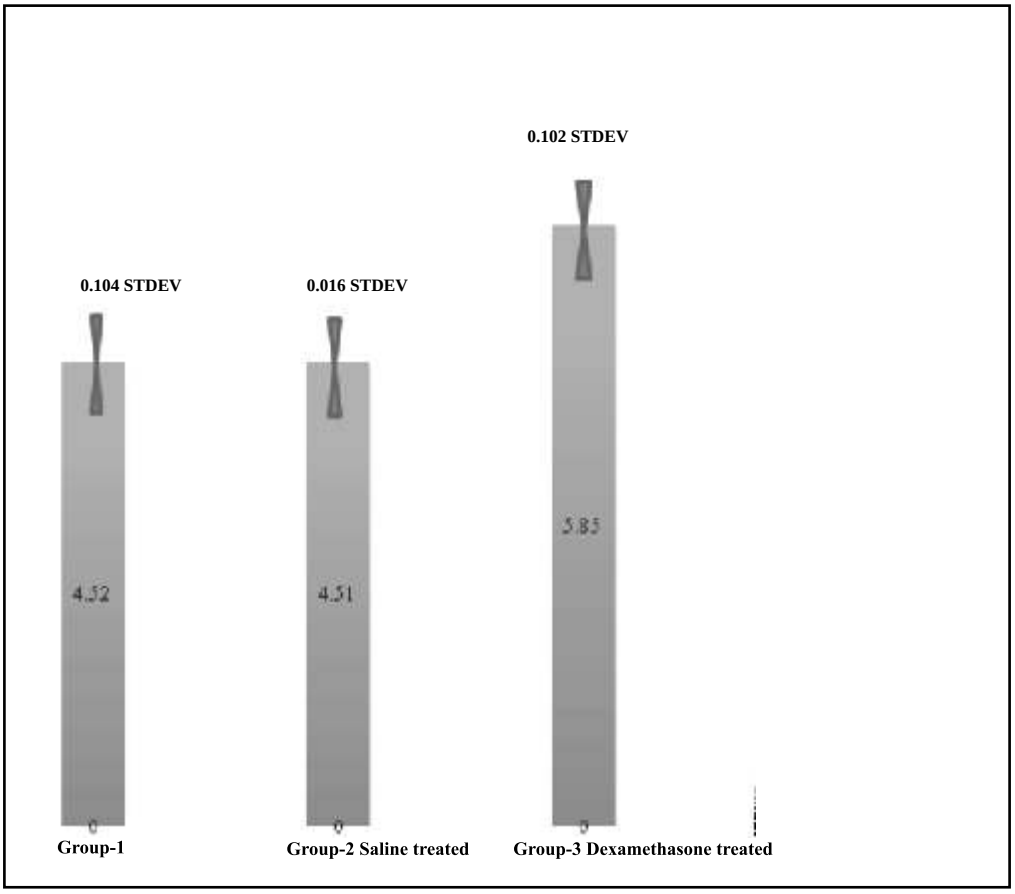


Figure: 2. indicating mean values of calcium in different experimental groups expressed in mol. eq/L

In the present investigation a significant increase in plasma calcium concentration has been observed after the administration of dexamethasone @ 2.5 mg/kg body weight for seven consecutive days. The probable action seems to be through demineralization of bone as dexamethasone is a potent apoptotic agent for osteoblasts and osteocytes. It can also be assumed that the action requires the presence of circulating parathyroid hormone hence in parathyrodectomised rats dexamethasone unable to cause hypercalcemic effect (Hirsch *et al.*, 1998) because parathormone and glucocorticoids act synergistically (Zhang *et al*., 1993).

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Community Based Disaster Management (CBDM) - A surveillance of Chhattisgarh.

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Abstract : The information about the disaster management program generated by the Government has not been disseminated in the communities properly, particularly in tribal population. The present study suggests that community disaster management education is essential and will develop capacity building amongst community in general and tribal population in particular to face disaster. The government should focus on the implementation of the programs at the grass root level through active community education. It is suggested that community level mitigation programs (CLMP) should also include structural and non-structural measures. The structural measures are safe construction, retrofitting, community shelters, legal framework while non-structural measures include financial resources in which Government(s) should actively involve Insurance companies, Municipalities, Corporations, Corporate houses Boards, Nigams , District Administration, Panchayats, Block level and Village level committees for providing sources of obtaining funds. Utilization of funds and awareness of proper construction of houses disaster free to avoid loses of the property and human life in any disaster. It is also suggested that survey through satellite be made for locating fresh underground water resources on account of draught due to short rain fall.

Key Words : Community, Disaster, Management, Technology, Information.

Introduction :

The disaster educated communities are defined as a means to assist communities in minimizing their vulnerability to natural hazards by maximizing the application of the principles and techniques of mitigation to their development and redevelopment decision-making process (Geis, 2000). Abolshams Asghari (2004) suggested that it is essential and helpful to educated to understand the community to face severe natural disasters. However, he also suggested that the disaster-resistant community model assumes a passive role for the community, as it is more relevant to the decision-making process than to engaging the communities. According to McEntire *et al.* (2002), the disaster-resistant community approach is more pertinent to disciplines such as geography, engineering, and urban planning. Consequently, it is not a holistic approach to disaster management, as it ignores the contribution of many academic disciplines, such as public administration, sociology, economics, political science, anthropology and psychology. Community education is essential in mitigation programs. In community education, even simple instructions play a vital role in disaster mitigation. Elliott *et al.* (2003) stated that community education, engagement, and awareness are the most important factors in improving warnings during disasters. Similarly, Davoodi *et al.* (2004) also stated that community education as the core of any disaster planning, because communities are the basic units. The present study is based on drought, flood (depends on Rainfall), industrial disasters, and manmade disasters and on field experiences during project with SAIL (Steel Authority Of India) and SARC (Society for Application Research and consultancy), a voluntary organization, working under corporate social responsibility in Dalli-Rajharra Mining area of Durg District.

Materials and Methods :

Data used in this investigation was primary, obtained from Government sources while the secondary data were collected by survey. LISS III Image of area is also used. The software used was Arc-GIS 9.1 and ERDAS Imagine 8.7.

Study Area :

Chhattisgarh, a 21st century State, came into being on November 1, 2000 by the Act of Parliament. Chhattisgarh takes its name from 36 (Chattis is thirty-six in Hindi and Garh is Fort) princely states in this region. Chhattisgarh is bordered by Bihar, Jharkhand and Uttar Pradesh in the north, Andhra Pradesh in the south, Orissa in the east and Madhya Pradesh in the west. The total geographical area of the state is 137,360 Sq. Kms. and nearly 44% of it is covered with forests. The main sources of water in the state are rivers, tanks and ground-water. The state has important rivers providing a lifeline to the socio-economic development of the state, such as Mahanadi, Sheonath, Indravati, Arpa, Hasdeo, Kelo, Son, Rehar, Kanhar etc.. The geographical area of the

state can be divided into five river basins, they are Mahanadi Basin, Godavari Basin, Ganga Basin., Narmada Basin and Brahmani Basin and Total Basin Area covered is about 137,360 Sq.Km. Since then the land has witnessed faulting, submergence, marine invasion and upliftment. The Physiography of Chhattisgarh can be divided into four physiographic provinces they are Chhotanagpur Plateau, Baghelkhand Plateau, Mahanadi Basin and Dandakaranya up-land **. T**he entire state is having high temperature (Table 1) and the area of the study is depicted in Fig.1.

Results and Discussion :

Disasters in Chhattisgarh:

The disasters has been divided into four categories they are drought, flood (depends on Rainfall), industrial disasters, and manmade disasters**.**

Regarding drought, Chhattisgarh was declared during 1960-2000 as drought state. On analyzing the data it has been noticed during last 40 years the rainfall in different months and years was not normal but occurred abnormal and often drought condition persist (Table 2). It is interesting to note that the drought (37.5%) conditions persist every five years of interval. The pre and post monsoon droughts were very regular phenomenon in the state, the maximum drought has been occurring mainly during the period of October-May and these months are registered for 97.88% drought months.

The main Districts which are drought prone were Raigrah, Bilaspur, Janjgir-Champa, Mahasamund and

Table 1. Average Max. &Min. temp.(in centigrade) **During different month in different station [Avearge From 2000 to 2008]**

Month	Bilaspur		Janjgir-champa		Korba		Raigarh		Jashpur		Ambikapur	
	Max.	Mim.	Max.	Mim.	Max	Mim.	Max.	Mim.	Max.	Mim.	Max.	Mim.
Jan.	24	10.9	27.2	13	23.4	11.3	19.7	9.4	28.3	13.2	23.2	7.9
Feb.	27.2	13.6	30.7	15.6	20.5	14.3	29	13	31.6	16	26	10.7
Mar.	31.9	18	35.5	19.6	26.6	16.5	32.9	12.4	36	20.4	30.8	15
Apr.	36.5	22.7	40.2	24.4	36.9	26.3	36.7	20.7	40.3	25.1	35	19.9
May	39.1	25.9	42.7	27.9	43.6	19	42.5	27.7	42.6	28	37	22.7
June	35.3	25	38.2	27	38	28.3	38.5	16.9	38	27.1	33.1	23
July	29	22.8	31.3	24.5	30.1	24.5	31.9	23.1	31.6	24.7	28.1	21.8
Aug.	28.4	22.5	30.7	22.5	27.4	22.1	29.2	20.8	31.1	24.7	27.6	21.5
Sep.	29	21.9	31.6	24.3	25	20.9	26.8	12	27.2	24.5	28.3	20.8
Oct.	29.2	18.7	31.7	21.4	20.3	15.2	22.5	18	23.4	22	19.4	20.2
Nov.	26.9	14.1	29.7	16.4	19.6	13.6	19.6	11.7	18.3	17.1	16	19.5
Dec.	24.3	10.9	27.3	13	17.9	10.6	16.9	7.7	14.2	13.3	13.7	5.9
Mean	30.1	18.9	33.1	20.8	27.44	18.6	28.9	16.117	30.217	21.34	26.52	17.41
SD	4.75	5.37	4.98	5.23	8.223	5.9	8.11	6.0751	8.5888	5.248	7.306	6.021

Source : Agriculture Department, Government of Chhattisgarh.

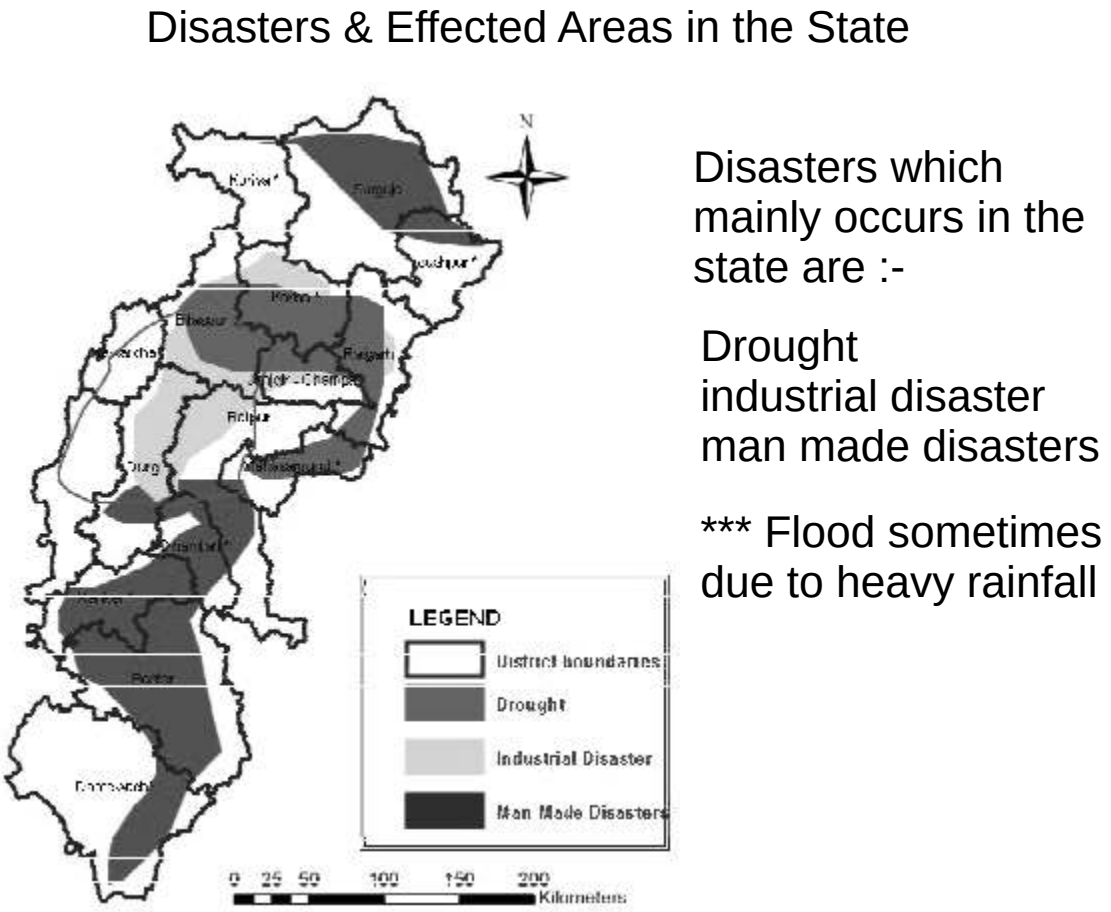

Figure 1 : The map showing disasters areas (as taken for the study) in the state..

Kwardha 66 blocks were declared drought prone in the year 2008-09. The drought like situation arises because of the lack of appropriate water management system. The water harvesting measures are still not implemented strictly neither in the rural nor in the urban area of Chattisgarh state. There were either little or no water in the seasonal rivers due to lesser amount of rainfall and also due to high temperature. The highest temperature was recorded in Raigarh and a part of Janjgir-Champa district, the area is having more than 27 degree C temperature annually. Lowest was recorded in Jashpur which is below than 23 degree C annually. It is apparent that the entire state is having high temperature (Table 1). The lack of water management and less of rainfall have been the main cause of drought, if properly handled drought could be as managed to a greater extent as also suggested by Singh (1995) and Elliott *et al*. (2003).

Asian development bank had already given a loan for participatory irrigation management in the state which has further slow down the problems of community for the water crisis. The water crisis is mainly due to drought and drought like situation. For this also the responsibilities should be properly taken by the each stakeholder for proper management of water resources as reported by Lohani, 1997.

Chhattisgarh have become first destination for industrial houses but the qualities of poor industrial constructions are leading to Industrial disasters. In addition to Industrial development, mining activities in mining area (coal, iron and others) are also responsible. Unfortunately, the lands are being utilized for mining activities finally leading to land degradation and deforestation leading to pollution (Table 3). The present study shows poor socioeconomic conditions persist amongst the villagers who are only employed as workers in the mining activities with poor wages without proper houses, food and poor health services. The above mentioned facts lead to man-made disaster of human suffering, loss of life and long-term damage to a country's economy and productive capacity.

Table 2. Annual rainfall distribution (in mm) in some districts of Chhattisgarh [2000-2008]

Year	Bilaspur [Pendra] (mm)	Janjgir-Champa (mm)	Korba (mm)	Raigarh (mm)	Jashpur (mm)	Ambikapur (mm)	Koriya (mm)
2000	674.5	1337.25	1308.5	1189.5	1152	1204.9	1181
2001	1028	1272.9	1410.5	815.1	1591.3	1651.7	1463.2
2002	857.6	930.8	1019.5	1261.6	1211.7	1664.7	926.3
2003	1242	1561.2	1778.8	1555	1647.4	1606.2	1785.9
2004	1028	1250	1266.7	1102.3	1172.6	1002.6	1271.5
2005	1308	1168.4	1510.3	1284.9	1211.8	996.2	1250.3
2006	1208	1147.41	1355.1	1178.03	1037.01	1356.8	1211.67
2007	1149	1012.22	1168.60	1099.12	984.28	1543.9	1345.98
2008	1021	901.25	1109.12	939.89	1011.05	987.7	925.45
Mean	**1057.3**	**1175.714**	**1352.25**	**1158.382**	**1224.349**	**1334.967**	**1262.367**
Standard deviation SD	**199.16**	**209.9271**	**228.1866**	**211.4118**	**239.8126**	**293.5735**	**263.9332**

Source : Agriculture Department, Government of Chhattisgarh.

Table 3. Forest Distribution in the Study area

S.No	Forest Type	Area Covered (Sq.km.)	%	Major species	Degradation in 10 years
1.	Dense Forest	2082.17	25.29	Sal (*Shorea robusta*) and Teak (*Tectona grandis*)	07.5%
2.	Non Forest	5801.66	70.48	NA	23.47%
3.	Open Forest	246.36	2.99	Saja (*Terminalia tomentosa*) bamboo (*Dendrocalamus strictus*)	00.94%
4.	Scrubland	9.99	0.121	NA	NIL
5.	Water Body	91.29	1.109	NA	0.06%

Source : The Level of Degradation of forest area is being calculated from the Map (Arc GIS 9.1)

Table: 4 A : Disaster and Vulnerability Assessments and Factors Determining the Community's coping Capacity in the state

Table 1.3A: Disaster Assessment in the state.		
Disaster **Assessment**	**1.Disaster type**	Flood, Drought(Natural Diaster), Industrial Disasters, Man Made Disasters (naxal Movement)
	2. Caution indication	Flood → Excessive rainfall, inadequate drainage system. Drought → No Rainfall, inadequate rainfall(rainfall less than required in an area). Industrial Disasters→ No such mechanism available as on date.(As the Korba Chimni disaster was Industrial disaster, open cast Mining and its Effect on the Environment specially on the forest resource is also Industrial Disaster, The water policy of Chhattisgarh state is not yet decided and the industrial housed are exploiting the water resources of the state which is finally leading to various type of water crisis)
	3. Forewarning	Rise in river level, Torrential rainfall related information dissemination from Emergency Flood Control Center (Bhuria), Raipur. For Drought There is no such system and Same for Industrial disasters.
	4. Speed of onset	Flood → Moderate, Drought→ Moderate, Industrial Disasters→ Mild to Extensive,
	5. Frequency	Flood → Once in every 2 years Effected Drought → Once in 5 years. Industrial Disasters→ Frequency of such hazard not yet measured.
	6. When	Flood → During monsoon (June-Sept) Drought →During (May-July) Industrial Disasters→Anytime, anywhere
	7. Duration	Flood → Days, (Seasonal) Drought → Months, Years. Industrial Disasters → Sudden & Momentary.
	8. Extent	Flood → Low lying area near Mahanadi Basin & surroundings may get flooded during monsoon, due to rise in river level. Drought → Rural and Agriculture Lands. Industrial Disasters → Applicable to all the industrials areas and peripheral areas.

***Vulnerability is the degree to which, the community is affected. Hence its assessment is very vital. **(Source: Based on field study analysis for disasters in Chhattisgarh)**

Man-made disasters are events which, either intentionally or by accident cause severe threats to public health and well-being.

The last decade has seen a marked increase in what are known as "complex emergencies**"** - complex because internal conflict lead to the breakdown and collapse of social, political and economic structures. Inevitably agriculture and food production are major casualties (Table 4 A, 4 B, 4C) showing the detail about the study for social and economic factors (Burton, 2004; Singh 1995, 2004 & Trim,2004). The present study suggests that Government should give main emphasis on basic need such as Insurance, Rehabilitation, Food and shelter, infrastructure with Expert Consultant, and Trained Village Resource Person should reach to Stake holders.

Table 4 B : Disaster Vulnerability Assessment in the state

Vulnerability Assessment	**1. Disaster type**	Flood, Drought , Industrial Disasters etc.
	2. Elements at risk: Infrastructure	Fly Ash bridge at Korba near Sarmangle , Arpa Bridge in Bilaspur near Sanichari ,Farmland, Roads, Crops, Hospitals, Schools, Industrial compound and under ground Mines areas inBilaspur,Surguja Korba, Raigrah,Dondi Lohara of Durg.
	Housing	Houses built by Mud mainly in rural areas.
	Livestock, etc.	Cows, Buffaloes, Oxen, Goats, Dogs etc.
	3. People at risk	Residing in weak structures all the villages have (Mud Hut) in Chhattisgarh , old age people, children, ladies, handicapped & isolated persons. Low-income groups: find it difficult to recover after disaster Women as a group are disproportionately affected by disasters Race / caste / ethnicity is closely related to their differential abilities for recovery Elderly people have limited coping strength In rural areas, vulnerable groups include smallholder agriculturalists, pastoralists, landless laborers, and the destitute
	4. Location of people at risk	Residing in villages near river Basin and remote forest side, areas of south Bastar Kanker are very remote many villages of study area does not have roads therefore it is difficult to communicate, alert & evacuate people from these areas.

Source : Based on field study analysis for disasters in Chhattisgarh

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Table 4 C : Factors Affecting Vulnerability in the state

Factors	**No. of villages Affected**	**No. of people affected**	**Major cause**
Economic			
Income, employment, livelihood, etc.	1245	(355,116 persons)	Poverty, unemployment, illiteracy, there is no source of livelihood are main causes (The Unorganized Labours Sector is the Biggest sector in Chhattisgarh) during monsoon all construction sites are closed.
Gender			
Male including Male Child	12		People drowned due to severe flood
Female including Female Child	18		Couldn't survive during severe flood and lack of Immediate rescue operation
Age Groups	All age group people		Impact of Flood. (Most of the people evacuated to government camps in time). No. of Camps are **82 .**
Ethnicity	Tribal of Bastar & Farmer Community suffered huge tangible & intangible losses.		

Source : Disaster Management cell, government of Chhattisgarh

***This is the example of most credible vulnerability, in this area. Apart from it, if we consider other cases like Man Made disasters generally in South Bastar Kanker (Here reference is about Naxal Movement and threat to public Life), then the overall vulnerability may lead to higher figures/nos. The combined effect of worst scenario is hampering economics of more than 20,000 families these families are staying in relief camps, which is quite high. But one of the best practices of Community based Man made Disaster Management can be seen in relief camps and in the form of "SALWA-JUDUM" here communities are together against terror a new form of Man made Disaster in the present scenario.

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Substantion of the Parameters to Tilt the Camera Advanced Cleaning Seeds Pasture Plants

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Abstract : To improve performance, harvesting seed pasture plants we have developed and refined a promising new generation of camera tilt grain-harvesting machines. To study the optimal parameters improved feeding channel for harvesting seeds of pasture plants, in particular, methods of wheatgrass our experimental design, which consists in choosing the number and the experimental conditions, necessary and sufficient for the task with the required accuracy? Optimal parameters of the improved tilted camera have been discussed.

Key word : Tilt of camera, Cleaning seeds, Pasture plant

Introduction :

Due to the arid grassland and inconsistent usage is increasing degradation of vegetation and soil cover. In some regions of Kazakhstan and Central Asian republics of the processes of desertification in some cases give rise to dust storm sin crease the area of open sand.

In Kazakhstan, a significant proportion are areas of natural grassland, amounting to more than 180 million hectares, which provide cheap food and, there fore, appropriate animal products. However, their food supply is limited due to low productivity, which is due to aridity and irrational use of pasture, lack of proper care and improve the land. The main way to increase wield in arid rangelands is a radical improvement, establishment in their place, seeded hayfields and pastures by over seeding seeds of valuable food plants like wheat grass, adapted to local conditions. Currently, work is continuing on the development and improvement of machinery for cleaning seed pasture plants. However, development of scope of work to restore pasture by reseeding capacity feed seed pasture plants require accelerating the development, deployment and equipping of agriculture seed cleaning machines (Sadykov,1992, Toilybaev *et al*., 2006)

Analysis of the current status and trends of the world's leading harvester, theoretical and experimental work performed in the main job of the regulators and download show that to solve the most important economic task of improving the performance of combine harvesters is necessary to solve a scientific problem of intensifying the process of threshing and separation in combine harvesters.

In the Kazakh National Agrarian University developed a promising new generation of camera tilt (A.S. No.1687078, Sadykov *et al.*, 2008). In order to adapt the developed feeding channel for harvesting seeds of pasture plants we improved its structural scheme shown in Picture 1 (Sadykov *et al*., 2010).

Result and Discussion

To study the optimal parameters improved feeding channel for harvesting seeds of pasture plants, in particular, methods of wheatgrass our experimental design, which consists in choosing the number and the experimental conditions, necessary and sufficient for the task with the required accuracy? Using the general form of the quadratic model and evaluation of *b*-coefficients, we write the multiple regression equation in expanded form for each output measure $\mu = Z_1$, $\lambda = Z_2$ and $\nu = Z_3$, which characterizes the used method of destruction double ears wheatgrass. According to the model structure and obtained the following regression equation of second order :

Completeness of the destruction double spikes,%

$$Z_1 = 84{,}51 + 1{,}33333x_1^2 - 2{,}21667x_2 - 9{,}1625x_2^2 - 0{,}81111x_3 - 5{,}6125x_3^2 - 1{,}32222x_4 - 6{,}9125x_4^2 - 0{,}8x_1x_2 - 0{,}85x_1x_3 - 2{,}3875x_1x_4 - 2{,}2625x_2x_3 - 1{,}875x_2x_4 + 1{,}3x_3x_4 \quad (1)$$

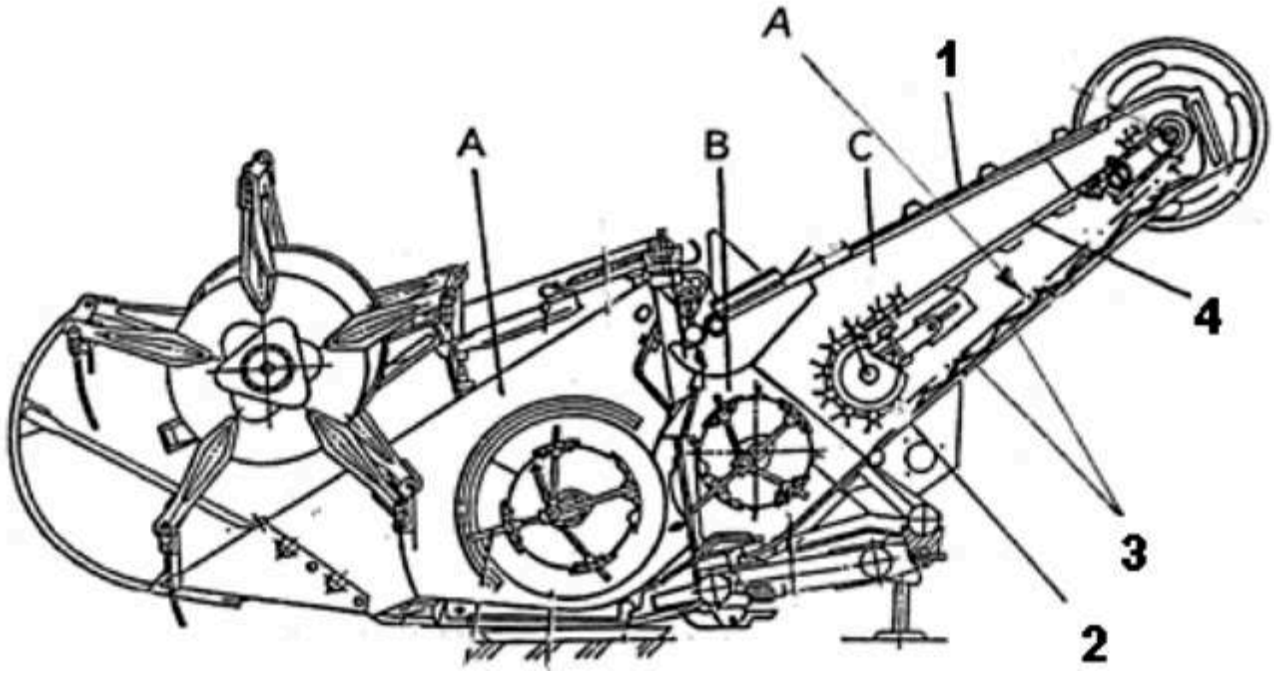
separation of wheat,%

$$Z_2 = 3{,}55 + 0{,}255556x_1 - 0{,}197917x_1^2 + 1{,}027778x_2 + 2{,}352083x_2^2 + 0{,}45x_3 + 1{,}6521x_3^2 + 0{,}34444x_4 + 1{,}40208x_4^2 - 0{,}28125x_1x_2 - 0{,}29375x_1x_3 + 0{,}66875x_1x_4 - 0{,}35625x_2x_3 + 0{,}15625x_2x_4 - 0{,}45625x_3x_4 \quad (2)$$

power leveling biomass,%

$$Z_3 = 82{,}14 + 1{,}05x_1 - 4{,}44375x_1^2 - 1{,}71111x_2 - 6{,}99375x_2^2 + 0{,}62778x_3 - 4{,}34375x_3^2 - x_4 - 5{,}29375x_4^2 - 0{,}60625x_1x_2 - 0{,}65625x_1x_3 - 1{,}84375x_1x_4 - 1{,}73125x_2x_3 - 1{,}44375x_2x_4 + 1{,}00625x_3x_4 \quad (3)$$

Equations (1) - (3) describe the relationship double ears completeness of destruction, separation, spikes and leveling wheatgrass biomass with independent parameters leveled the unit.



Picture 1 : The tilting camera for harvesting seeds of pasture plants
A - reaper, B - accelerator, C - tilt camera with the combine harvester, 1 – look out cap, 2 - lower shaft, 3 - a device for the destruction of corn double wheatgrass, 4 - transporter.

With a quadratic regression equation of four independent variables, we can convert it to canonical form and analyze multi-dimensional view of the response surface in the investigated region of the factor space, and find the zone settings in which the response is extreme.

In the next stage of regression analysis revealed statistically significant effects of factors. The significance of the obtained regression components are characterized by significantly influence the investigated parameters of the device on the completeness of the destruction of corn double $\mu = Z_1$, was determined from the calculated values of Student's t-test, absolute values are ordered by their descending and presented in a Pareto chart. Pareto chart is an effective means of determining what effects have the greatest contribution to the formation of interest on the dependent variable, for example - power leveling wheatgrass biomass Z_3.

The greatest influence on the completeness of destruction double ears have wheatgrass in the first place the squares (Q) variable x_2 (Q) - the length of the fracture and x_4 (Q) - the height of the corrugation. This is followed by the pair interaction x_1x_4 (1L by 4L) supply of biomass and height of the corrugation, linear (L), or the so-called main effect of x_2 - the length of the fracture, etc. The corresponding bands intersect the vertical line that represents 90% of the confidence level.

Table 1 : Analysis of variance of regression models for rates of destruction of ears wheatgrass

Source variation	Degrees of freedom *df*	Sum of squares *SS*	mean square *MS*	of the ratio of the mean square *F*	*p*-level of significance for *F*
The completeness of the destruction of corn double wheatgrass Z_1,%					
Regression (R)	14	2726.615	194.7582	8.399924	0.001504
The residue (E)	9	208.6714	23.18571		
The full amount (T)	23	2935.286			
Separation of spikes Z_2,%					
Regression (R)	14	126.874	9.276711	5.469177	0.007338
The residue (E)	9	15.26563	1.696181		
The full amount (T)	23	145.1396			
The degree of leveling biomass Z_3,%					
Regression (R)	14	1603.802	114.5573	8.526813	0.00142
The residue (E)	9	120.9145	13.43495		
The full amount (T)	23	1724.716			

Sum of squares due to regression (SS_R) to complete the destruction of corn double μ and the degree of leveling wheatgrass biomass ν, is about 93% of the total sum of squares (SS_T), and for the degree of separation of spikes λ - 89,5%.

Assessment of quality of regression models developed for performance threshing wheatgrass received by the laboratory-field data, the multiple correlation coefficients tested *R*, determination R^2 and *F*-test and Fisher's criterion for the Durbin-Watson *d*. These statistical characteristics and criteria for assessing the quality of the regression equations calculated by computer statistical programs *SPSS 16* and *Statistical 7.0* shown in Table 2.

Table 2 : Checking the quality of approximation of the regression models for performance threshing wheatgrass

Statistical	Index value for the criterion of threshing		
	$\mu = Z_1$	$? = Z_2$	$? = Z_3$
Multiple correlation *R*	0,964	0,946	0,964
The coefficient of determination R^2	0,929	0,895	0,930
Adjusted (for *df*) R^2	0,818	0,731	0,821
The standard error	4,815	1,302	3,665
The number of degrees of freedom *df:* k_1; k_2	14; 9	14; 10	14; 11
Fisher's criterion *F*	8,400	5,469	8,527
The level of significance of *p* to *F*	$1,5 \cdot 10^{-3}$	$7,3 \cdot 10^{-3}$	$1,4 \cdot 10^{-3}$
Durbin-Watson criterion *d*			
Serial correlation			
Note: k_1 and k_2 - the number of degrees of freedom for the numerator and denominator, respectively			

In Table 2 the coefficient of multiple correlation are significant, are quite high (0,964; 0,946; 0,964) and close to the limiting magnitude ($R \leq 1$), indicating that a high close relationship with the destruction of the investigated parameters and the separation of ears double wheatgrass and wheatgrass biomass leveling.

The calculated model allowed to define further the optimal area of adjustable parameters of the activator, outside of which the improvement in the completeness of destruction double ears wheatgrass will not bring proportionate effect.

The presence of negative coefficients (b_{11}, b_{22}, b_{33}, b_{44}) of the squares of the variables in the equation for the complete destruction of double ears wheatgrass $\mu = Z_1$ shows that for each of these variables there is an optimal level.

A similar type of response surfaces and lines of equal levels was obtained for the degree of separation of ears ($\lambda = Z_2$) and the degree of leveling the wheatgrass plant material ($v = Z_3$) improved oblique camera.

Investigation of response surfaces using the canonical transformation leads to the following equations :

$$Z_1 - 84,838 = -4,38166\xi_1^2 - 5,7873\xi_2^2 - 7,47413\xi_3^2 - 9,8569\xi_4^2;$$
$$Z_2 - 3,432 = 2,41328\xi_1^2 + 1,78493\xi_2^2 - 1,29105\xi_3^2 - 0,28803\xi_4^2;$$
$$Z_3 - 82,398 = -3,35959\xi_1^2 - 4,44959\xi_2^2 - 5,73273\xi_3^2 - 7,53309\xi_4^2$$

As follows from the first equation (4), response surface $\mu = Z_1$ to complete the destruction of corn double wheatgrass has a maximum equal to 84.8%, since the signs of all coefficients of the canonical equation is negative. Response surface for the separation of ears wheatgrass $\lambda = Z_2$ has a saddle point at which the response is equal to 3.4%, as coefficients of the second canonical equation (4) have different signs (three coefficients are positive, one negative). The response to the degree of leveling the wheatgrass plant mass $v = Z_3$ at a stationary point as a maximum, equal to 82.4%, since all the coefficients of the third equation (4) are negative.

Thus, all the coordinates of singular points of the response Z_1, Z_2, Z_3, lie in the experimental and slightly differ in magnitude for completeness of destruction of stalks double ears $\mu = Z_1$ and the degree of leveling wheatgrass biomass $v = Z_3$. Therefore, taking these coordinates for the optimal solution and converting them into natural scale, the following parameters improved feeding channel:

- supply of biomass $q = 2,57$ kg / pm;
- the length of the fracture $L = 58,73$ cm;
- angle of attack $\alpha = 25,76$ corrugation deg.;
- height of the corrugation $h = 19,62$ mm

at which the output quality of threshing wheatgrass the following values: complete destruction of ears double $\mu = Z_1 = 84,8\%$; degree separation ears $\lambda = Z_2 = 3,5\%$; degree of uniform distribution of plant mass wheatgrass $v = Z_3 = 82,4\%$.

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Role of Solvents in the Oxidation of Propane-1,2-Diol with Ditertiary Butyl Chromate and Ditertiary Amyl Chromate

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Abstract : Of late, ditertiary butyl chromate (TBC) and ditertiary amyl chromate (TAC) have attracted much attention of chemists as chromium (VI) based oxidants for organic synthetic processes. This is due to their stability, versatility, controllability of the reactions and, of course, due to better yield of the products. In the present work, we have carried out the oxidation of propane-1,2-diol with TBC and TAC in different solvents like tetrahydrofuran , acetonitrile, dioxane, dichloromethane etc. The products, thus, formed were purified, tested for purity, analysed spectroscopically and thermogravimetrically and formulated so as to make some generalization in regards to the role of solvents in such redox processes

Key words : Propane-1,2-diol, Dioxane, Tetrahydrofuran, Acetonitrile, TBC, TAC

Introduction

A large number of Chromium (VI) based oxidants like dipyridine chromium oxide (Collins and Frank, 1968) , Pyridinium chlorochromate, Chromium trioxide-3,5-dimethyl pyrazole complex (Corey and Fleet, 1973), Pyridinium dichromate , 2,2'-Bipyridinium chlorochromate (Guizice and Luzzio, 1980), Pyridinium flourochromate, Quinolinium flourochromate, Quinolinium chlorochromate-3,5-dimethyl pyrazolium flourochromate, 2,6- Dicarboxy pyridinium chlorochromate (Hosseinadeh *et al*., 2002), N-Methyl pyridinium chlorochromate, Tetramethyl ammonium flourochromate (Kassaie *et al*., 2003), N-Methyl benzyl ammonium flourochromate, TBC ,TAC etc have appeared in literature as potent oxidants for organic substrates. Among them, TBC and TAC ,though, less explored, have been found to be more useful in many respects like being less hazardous as compared to heterocyclic compound based oxidants, easy methods of preparation, stability at room temperature, controlled reaction and better yield. In the present paper, we report the detailed processes followed for the preparation of oxidants, the nature of products formed in different substrate : oxidant molar ratios and in different solvents used for the substrate. The solvents used were tetrahydrofuran, dichloromethane, dioxane, acetonitrile and dimethylsulphoxide which have different dielectric constants and other properties (Table 1). The solid products of different colour and compositions were obtained which were purified, tested for purity and analysed by elemental estimation, spectroscopic peaks and thermogravimetric loss pattern. The results were used to propose the mode of oxidative fragmentations of the substrate and the association/complexation of the same with chromium in different oxidation states. The observations of the results in different aprotic solvents chosen for the purpose were used to make some generalization in regards to the role of solvents in such redox processes.

Table 1 (Constants of Solvents)

Solvents	Formula	Mol. Mass	B.P ^{o}C	M.P ^{o}C	density	Dielectric Const	Flash Point
Tetrahydrofuran	C_4H_8O	72.11	66	-108	0.886	7.6	-21
Dichloromethane	CH_2Cl_2	84.93	39.8	-96.7	1.326	9.08	1.6
Dioxane	$C_4H_8O_2$	88.11	101.1	11.8	1.033	24.6	13
Acetonitrile	C_2H_3N	41.05	81.6	-46	0.786	37.5	6
Dimethylsulphoxide	C_2H_6OS	78.13	189	18.4	1.092	47	95

Materials and Methods

TBC was prepared by dissolving 0.5 g., 1.0 g and 2.0 g of pure AR grade chromium trioxide (CrO_3) separately in 10 ml of tertiary butyl alcohol. (TBA) (Mishra and Lakra, 2008) solution. The deep red transparent solution were tested for purity and homogeneity. Three solutions of TAC were prepared in the same way by taking calculated quantities of pure AR grade of chromium trioxide in tertiary amyl alcohol (TAA) in place of TBA.

The substrate solutions were made by dissolving 1.52 g of pure A.R. grade propan-1,2-diol in 10 ml of different solvents as in Table 1. Solid products of different colour and composition were obtained when the solutions of the substrate and that of oxidant were mixed and heated with constant stirring on a magnetic stirror . The samples were collected as PTF-121, …… etc and analysed for suitable formulations.

Results and Discussion

Commercially available propane-1, 2-diol also called α-propylene glycol, is a racemic mixture of the active forms and is miscible in all proportion with water, acetone, dioxane, diethyl ether, dichloromethane, ethyl alcohol etc. It is mainly used as antifreeze and humectants. Its 52% mixture with water freezes at as low temperature as -34° C. It is metabolised in human body into pyruvic acid, acetic acid, lactic acid (Miller and Bazanno, 1965; Ruddick, 1972) and propanaldehyde which are mostly its oxidation products expected in chemical oxidations too. In the present work, the authors have carried out the chemical oxidations of 1,2-propanediol under different conditions of molar ratio and in different solvents in order to trap degradative oxidation products as compounds or complexes of chromium in different oxidation states. The evidences of fragments like $CH_3CH(OH)CHO$, CH_3COCHO, $CH_3COCOOH$ as well as CH_3CHO, CH_3COOH and HCOOH were obtained. Table 2 and Table 3 contain the details of the reactants and products obtained by the oxidation of propane-1,2-diol by TBC in tetrahydrofuran, acetonitrile and dichloromethane as solvents whereas Table 4 and Table 5 contain the same when oxidant is TAC and the solvents are tetrahydrofuran, acetonitrile and dioxane. The emperical formula in the brackets in Table 3 and Table 5 correspond exactly to the formulations done on the basis of experimental values. No pure compound or complex could be isolated in dimethylsulphoxide (DMSO) as solvent. Formulations of the samples have been proposed on the basis of elemental estimation, FTIR peaks and thermo-gravimetric loss patterns. Manygeneralizations could be made in regards to mode of fragmentation of the substrate as a result of degradative oxidation and their subsequent association /co-ordination with the metal in different oxidation states.

- Formic acid appears essentially when the ratio of oxidant is higher in all the products except in oxidation of propane-1,2-diol with TAC in dioxane as solvent.
- Acetic acid is essentially present in all the oxidation products except in PDM 121, PAA 121, PAA 122 and PAD 121
- Oxidised but undegraded products like $CH_3CH(OH)CHO$, CH_3CO-CHO and CH_3CO-COOH appear only in cases of lower ratios of oxidant.
- The oxidation states of chromium in the products are lower when the ratio of oxidant is

Table-2 (Propane-1,2-diol + TBC)

Product Code	S:O Ratio	Solvent	Colour	Solubility
PTF-121	4:1	Tetrahydrofuran	Light green	Insoluble
PTF-122	2:1	"	Green	Partially soluble
PTF-123	1:1	"	Dark green	Insoluble
PDM-121	4:1	Dichloromethane	Light green	"
PDM-122	2:1	"	Green	Partially soluble
PDM-123	1:1	"	Grey	"
PAN-121	4:1	Acetonitrile	Light grey	Partially soluble
PAN-122	2:1	"	Dark green	Insoluble
PAN-123	1:1	"	Dark green	"

Table-3 (Formulations of the products)

Product Code	Emperical Formula	Formulations
PTF-121	$Cr_2C_5H_{20}O_{12}(Cr_2C_5H_{18}O_{12})$	$Cr_2O_3.CH_3COOH.CH_3CHO.HCOOH.4H_2O$
PTF-122	$CrC_2H_9O_6$ ($CrC_2H_{10}O_6$)	$CrO.\ CH_3COOH.3H_2O$
PTF-123	$Cr_2C_3H_{12}O_7$ ($Cr_2C_3H_{10}O_7$)	$Cr_2O.CH_3COOH.HCOOH.2H_2O$
PDM-121	$Cr_2C_6H_{21}O_{11}(Cr_2C_6H_{20}O_{11})$	$Cr_2O_3.2CH_3CH(OH)CHO.4H_2O$
PDM-122	$Cr_2C_5H_{15}O_8(Cr_2C_5H_{12}O_8)$	$Cr_2O_3.CH_3CH(OH)CHO.CH_3COOH.H_2O$
PDM-123	$Cr_2C_3H_{12}O_7(Cr_2C_3H_{10}O_7)$	$Cr_2O.CH_3COOH.HCOOH.2H_2O$
PAN-121	$Cr_2C_5H_{19}O_{11}(Cr_2C_5H_{18}O_{12})$	$Cr_2O_3.2CH_3COOH.HCOOH.2H_2O$
PAN-122	$Cr_2C_5H_{19}O_{11}(Cr_2C_5H_{18}O_{12})$	$Cr_2O.CH_3CO\text{-}COOH.CH_3COOH.2H_2O$
PAN-123	$Cr_2C_3H_{14}O_7(Cr_2C_3H_{10}O_7)$	$Cr_2O.CH_3COOH.HCOOH.2H_2O$

Table-4 (Propane-1,2-diol + TAC)

Product Code	S:O Ratio	Solvent	Colour	Solubility
PTA-121	4:1	Tetrahydrofuran	Light green	Insoluble
PTA-122	2:1	"	Bottle green	Partially soluble
PTA-123	1:1	"	Dark green	Insoluble
PAD-121	4:1	Dioxane	Light green	"
PAD-122	2:1	"	Light green	Partially soluble
PAD-123	1:1	"	Green	"
PAA-121	4:1	Acetonitrile	Light green	Partially soluble
PAA-122	2:1	"	Greyish green	Insoluble
PAA-123	1:1	"	Yellowish green	"

Table-5 (Formulations of the products)

Product Code	Emperical Formula	Formulations
PTA-121	$Cr_2C_7H_{16}O_{14}(Cr_2C_7H_{18}O_{14})$	$2CrO_2.2CH_3COOH.2HCHO.HCOOH.2H_2O$
PTA-122	$Cr_2C_4H_{14}O_7$ ($CrC_2H_{12}O_6$)	$Cr_2O.\ 2CH_3COOH.2H_2O$
PTA-123	$Cr_2C_4H_{12}O_8$ ($Cr_2C_4H_{10}O_8$)	$Cr_2O.CH_3COOH.2HCOOH.H_2O$
PAD-121	$Cr_2C_6H_{12}O_9(Cr_2C_6H_{16}O_9)$	$Cr_2O_3.2CH_3CH(OH)CHO.2H_2O$
PAD-122	$Cr_2C_5H_{14}O_7(Cr_2C_5H_{12}O_7)$	$Cr_2O.CH_3CO\text{-}CHO.CH_3COOH.2H_2O$
PAD-123	$Cr_2C_4H_{10}O_6(Cr_2C_4H_{12}O_6)$	$Cr_2O.CH_3CHO.CH_3COOH.2H_2O$
PAA-121	$CrC_4H_{11}O_9(CrC_4H_{12}O_9)$	$CrO_2.CH_3CO\text{-}COOH.HCHO.3H_2O$
PAA-122	$CrC_4H_8O_8(CrC_4H_{10}O_8)$	$CrO_2.CH_3CO\text{-}COOH.HCHO.2H_2O$
PAA-123	$Cr_2C_3H_{10}O_{10}(Cr_2C_3H_{12}O_{10})$	$Cr_2O_3.CH_3COOH.HCOOH.3H_2O$

higher. It decreases with increasing ratio of oxidant invariably in the two cases of oxidant and all the cases of solvents.

- Water is formed and incorporated as lattice water or as ligand in all the cases.
- Aldehydic groups as HCHO and $CH_3CH(OH)CHO$ appear in the lower ratio of oxidant except CH_3CHO which is present in higher ratio of oxidant also in dioxane as solvent i.e. sample PAD-123.
- The compounds are mostly green which shows that chromium is reduced to lower states (Bhattacharjee *et al*., 1987). The colour deepens as the ratio of oxidant increases.
- The oxidation products obtained with TBC and TAC as oxidants are almost same in colour and composition if the solvent is THF.
- In case of acetonitrile as solvent, TBC seems to be more efficient in lower ratio of oxidants as PAN-121 has fragments like CH_3COOH and HCOOH whereas in case of TAC, PAA-121, undegraded fragment $CH_3COCOOH$ and less oxidised fragment HCHO are formed.
- The extent of oxidation is less in case of TBC as well as in case of TAC when the solvents are dioxane and dichloromethane if the ratio of oxidant is less. This is substantiated by the presence of less oxidised fragment $CH_3CH(OH)CHO$ in PDM-121, PDM-122 and PAD-121

As Table 1 shows, the dielectric constant decreases in the series - DMSO > Acetonitrile > Dioxane > Dichloromethane > THF. The similarities in colour and composition of the products in both the oxidants, TBC and TAC accompanied with better yield and greater extent of oxidation in case of THF as solvent clearly indicates the non-ionic path of reaction which is favoured in solvent of low polarity. This is also supported by the fact that no meaningful product could be obtained in DMSO which has maximum value of dielectric constant among the solvents used. In addition to having high value of dielectric constant, DMSO is also vulnerable towards oxidation leading to its competition with the substrate for its oxidation. DMSO has also been reported to be an active participant in the redox processes at low temperature as in Swern reaction (Omura and Swern , 1978 ; Mancuro *et al*., 1978). The slow reaction in case of dioxane may also be due to its greater value of dielectric constant which may be preferred for controlled oxidation of organic substrate with powerful oxidant like Cr(VI) and Mn(VII). The formation of water and oxidation of alcohol to acid is less likely in nonaqueous medium and at higher temperature. The presence of alcoholic groups in the substrate which on dehydration catalysed by oxides of chromium present , might have produced water. Similarly the better ligating power of carboxylic acid as compared to aldehydes may be a factor which drives the oxidation of alcohol to acid stage.

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Report on Heteroceran Lepidoptera diversity of Harmu, Ranchi, Jharkhand

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Abstract : Heteroceran lepidoptera (moth) are common pests over plants and damage leaves, stems, flowers and fruits. A study was carried in Harmu, Ranchi between January 2010 and December 2010. A total 11 species belonging to 4 families (Tortricidae, Pyralididae, Noctuidae and Sphingidae) were collected. The statistical interpretations were carried out by using Shannon-Wiener diversity index and Shannon's equitability. Biodiversity of moth with host plants was observed and species richness was observed 2.37. The species richness and abundance are indicative of that the Harmu area has good representation of moth diversity.

Key Words : Heteroceran lepidoptera, Shannon-Wiener diversity index, Harmu, Ranchi

Introduction

The Ranchi district is known for its rich biodiversity and remains as merely explored especially in the area of the study of Heteroceran lepidoptera. Insects of this class are mainly pests (Metcalf and Flint, 1973; Mathur and Singh, 1961) high economic importance. Scientific work has enriched the field of biodiversity as commercial exploitation of silk moth belonging to the family Saturniidae and Bombycidae (Grimaldi, 2005) have been done since the establishment of the Central Tasar Research Institute (CTRI) at Ranchi. On the other hand, other families of heteroceran lepidoptera have not been studied properly till date in the newly carved out State of Jharkhand or in undivided Bihar. Due to the continuous change in the landscape of Ranchi district by urbanization the species requires immediate attention. Many of the species may be endangered or may be on the verge of extinction.

In view of this, a survey work was carried out to map the biodiversity of the heteroceran lepidoptera profile from January 2010 to December 2010. The moth diversity index (Shannon, 1948) of this report was 2.37 which is lower to the Peshawar town of Pakistan was 3.14 (Aslam, 2009) and higher to the Karaikal region of Pondicherry, India was about 1.71 (Adiroubane and Kappummal, 2010).

Materials And Methods

The present study was conducted in Harmu, Ranchi located at 23°22´52´´ N latitude to 85°18´05´´ E longitude covering an area of nine km^2. The Harmu municipal area is dotted with several man made constructions, Harmu filed, Harmu River. The area is divided into localities by a network of roads. The annual mean temperature value of Harmu area was maximum 29.24 °C and minimum 18.08 °C. The maximum monthly mean temperature was 36.9 °C (May) and minimum monthly mean temperature was 9.9° C (January). The annual mean rain fall was 121.05 mm. The annual mean value of average relative humidity was 68.17%. The annual mean speed of wind was 7.76 km/h. Regular visits during year January 2010 to December 2010 were made to different areas of Harmu municipal area especially around the Harmu field, residential area, and Harmu River. The collection work was made monthly by photophilic trap method between 6pm to 9pm by quadrate method (With a caution not to disturb the dispersal and movement of species in order to observe the ethics and movement of species) and a comparative data sheet were prepared (Table 2). The specimens were identified up to species level with the help of keys from the Richard and Davies (1934), Bell and Scott (1937), Metcalf and Flint (1939) and Pradhan (1994).

Vegetation profile of Harmu, Ranchi

Harmu field is outlined by rich plantation of Sesum (*Delbergia latifolia*), Amaltas (*Cassia fistula*), Potato (*Solanum tuberosum*), Tomato (*Lycopersicum esculentum*), *Nerium oleander* and man made flower gardens, Grasses (family poaceae), and bushy shrubs (Graph 1). Harmu housing colony is planned with good number of exotic and endogenous trees and shrubs around the fields and roads. Citizens also planted fruit trees like mango (*Mangifera indica*), litchi (*Litchi chinensis*), Guava (*Pasidium guajava*), Citrus (*C. limon)*, Papaya (*Carica papaya*) along with herbs and shrubs.

Specimens have been deposited in the Department of Zoology, Ranchi Women's College, Ranchi.

Following statistical tools were applied for the biodiversity measurement of the area :

Shannon-Weiner (1948) diversity index was used to calculate Diversity Index:

$H' = -pi \ln pi$

Here, $pi = ni/N$

ni = number of individuals of a species

N = total number of individuals of all species

ln = natural logarithm (to base)

H´ = diversity index

The maximum possible diversity consisting X categories (no. of species here) was calculated by using the formula

$H'max = \ln.X$

Another parameter evenness (J) was calculated by

$J' = H' / H'max$

Richness Index:

This was a measure of number of species in a community.

$D = (S-1)/ \ln(N)$

Here, D = Margalef index (1948-51)

S = No. of species

N = Total number of species.

Results And Discussion

Species richness is the simplest diversity measure to count the number of species in an area. Species diversity, on the other hand takes into account the relative abundance of a species and not just its occurrence. The first index used in the present study is Shannon-Weiner diversity index (1948), which comes from information-statistics. Information statistics

TABLE 1 : Heteroceran lepidoptera (moth) diversity of Harmu, Ranchi collected between January 2010 and December 2011 by photophilic trap method.

Sl. No.	Sample No.	*Name of moth species*	Common Name	Family	Host Plant
1	SPH001	*Acherontia atropos,* Linn.	Death's head Hawk moth	Sphingidae	Solanaceae, Lantana
2	SPH002	*Acherontia styx,* Westwood	Death's head Hawk moth	Sphingidae	Sesamum indicum, Solnaceae (Potato)
3	SPH003	*Deilephila nerii,* Linn.	Sphinx moth or oleander hawk worm moth	Sphingidae	Nerium oleander
4	SPH004	*Deilephila lineate,* Linn.	Melonworm moth	Sphingidae	Poaceae family
5	PYR001	*Diaphania nitidalis,* Stoll	Pickleworm moth	Pyralididae	Cucumber & melon
6	PYR002	*Diaphania hyalinata,* Linn.	Melonworm moth	Pyralididae	Poaceae family
7	PYR003	*Chilo partellus,* Swinhoe	spotted stem borer moth	Pyralididae	Sorghum
8	NOC001	*Alabama argillacea,* Hubner	Cotton bollworm moth	Noctuidae	Cotton
9	NOC002	*Heliothis zea,* Boddie	Cotton bollworm moth	Noctuidae	Cotton, corn, tomato
10	NOC003	*Catocala fraxini,* Schrank	cutworm moth	Noctuidae	Leaves of herbs & shrubs
11	TOR001	*Cydia pomonella,* Linn.	Codling moth	Tortricidae & Olethruridae (Metcalf & Flint)	Tree fruits

indices are based on the rationale that diversity in a natural system can be measured in a way that is similar to the way information contained in a code or message is measured.

All the observations of field survey have been recorded in a Table number 1, 2 and 3 along with graph 1 and 2 have been plotted. In the Table 1, taxonomy of collected moths is listed indicating scientific name, common name, family and host plants. In the table 2, the monthly collection record from January 2010 to December 2010 is arranged and on this basis a comparative line graph 2 was sketched out. It clearly outlines the species richness of collected samples during the above mentioned time period. In the Table 3, diversity index (Shannon-Weiner, 1948) of collected species and Dominance of all the 11 species of Heteroceran lepidoptera has been calculated. From the present study it is concluded that *Diaphania nitidalis* belongs to class Pyralididae is the most abundant species (0.2554) belonging to this area. Next to *Diaphania nitidalis* is *Cydia pomonella* or Codling moth (0.2502), *Alabama argillacea* or cotton leaf moth (0.2332), *Catocala fraxini* or cutworm moth (0.2332), *Deilephila nerii* or sphinx moth (0.2076) and *Heliothis zea* or bollworm moth (0.2209) are evenly distributed over this region. The number of the above mentioned species varies between 10-15 individuals per species. However, the species richness value of *Diaphania hyalinata* or Melon worm moth (0.1610)) is lowest but *Acherontia atropos* or Death's head hawk moth (0.1778), *Acherontia styx* or Death's head hawk moth (0.1857) ranked low.

The result of Table 3 indicates the value of *Pi ln Pi* is 2.373. The Shannon diversity index for real communities are often found to fall between 1.5 and 3.5. It indicates that the diversity richness of collected moths is still good in the Harmu area of Ranchi. But due to the lack of sufficient data of the diversity of moths (except silk moth) in Ranchi the comparison and analysis of the mode of loss or gain in diversity of them is little difficult. The moth diversity index (Shannon, 1948) of Peshawar town of Pakistan was 3.14 (Aslam, 2009) which is certainly much higher than the value obtained from Ranchi in this report while the Shannon index of Karaikal region of Pondicherry, India was about 1.71 (Adiroubane and Kappummal, 2010), which is much lower than obtained data in this report (2.37).

The author reports that the present study is probably the median study of this type of this region. Therefore, we suggest that the area requires taxonomic further rigorous morpho-taxonomic study of heteroceran lepidoptera.

TABLE 2 : Monthly collection data of heteroceran lepidoptera by light trap method from Harmu, Ranchi during January 2010 to December 2010 of one year.

Sl. No.	Sam.No.	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Tot
1	SPH001	0	0	0	1	2	2	3	2	1	2	0	1	14
2	SPH002	0	1	1	0	0	2	2	2	3	2	1	1	15
3	SPH003	1	3	1	2	2	1	2	1	2	1	2	0	18
4	SPH004	0	0	3	2	4	5	1	3	1	1	0	0	20
5	PYR001	3	7	4	1	1	0	0	1	2	1	2	4	26
6	PYR002	1	2	1	0	0	0	1	0	0	2	2	3	12
7	PYR003	2	2	3	4	1	1	0	0	1	1	3	2	20
8	NOC001	5	6	3	3	1	0	0	0	0	0	2	2	22
9	NOC002	3	2	3	4	5	1	0	0	1	0	1	1	21
10	NOC003	0	5	2	3	2	2	4	1	1	0	1	1	22
11	TOR001	0	3	2	1	4	6	2	0	3	4	0	0	25
	TOT	15	31	23	21	22	20	15	10	15	14	14	15	215

TABLE 3 : Heteroceran lepidoptera (moth) diversity of the collected samples with species richness index (Shannon-Weiner diversity index, 1948) of Harmu, Ranchi.

Sl. No.	*Codes number*	No. of individuals sampled	Relative abundance R.A. = ni/100	Pi = ni/N	Pi Log Pi
1	SPH001	14	0.065	-2.732	-0.177870371
2	SPH002	15	0.070	-2.663	-0.185761941
3	SPH003	18	0.084	-2.480	-0.207650199
4	SPH004	20	0.093	-2.375	-0.220921466
5	PYR001	26	0.121	-2.113	-0.255470134
6	PYR002	12	0.056	-2.886	-0.161064077
7	PYR003	20	0.093	-2.375	-0.220921466
8	NOC001	22	0.102	-2.280	-0.233260943
9	NOC002	21	0.098	-2.326	-0.227201988
10	NOC003	22	0.102	-2.280	-0.233260943
11	TOR001	25	0.116	-2.152	-0.250204907
		N = 215	1.000	-26.660	2.373588434

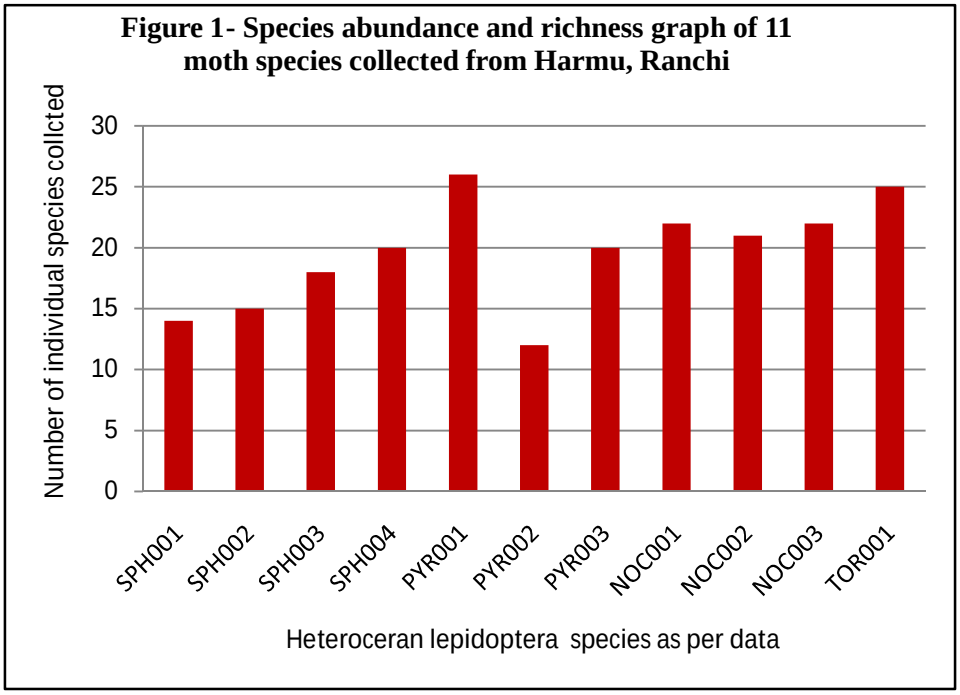
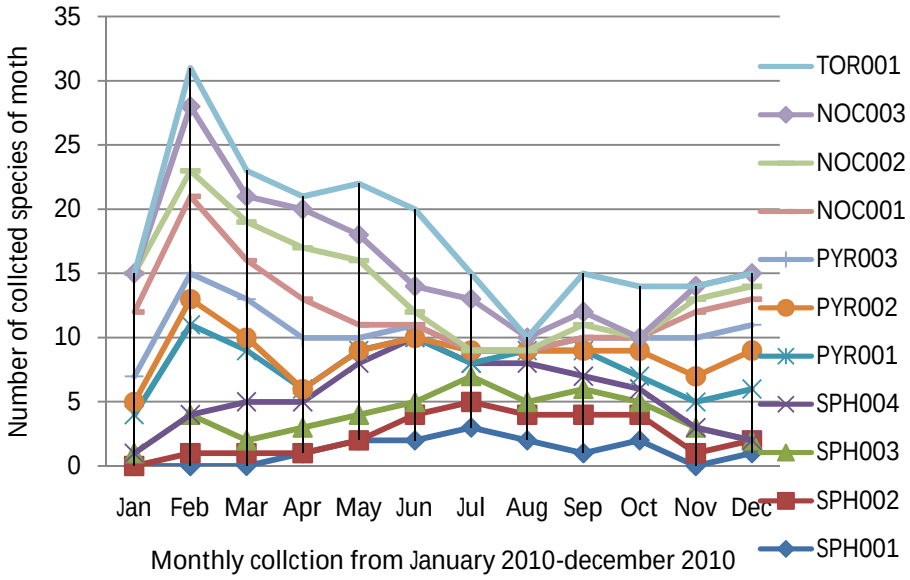


Figure 1- Species abundance and richness graph of 11 moth species collected from Harmu, Ranchi

Figure 2: Monthly collection data of heteroceran lepidoptera by light trap method from Harmu, Ranchi during January 2010 to December 2010 base on table 2.



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Limitation:

The filed studies were not done during mid summer days. The following data is a rough sketch.

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Web site: www.watherforecastmap.com

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A Report on Butterfly Diversity of Reclaimed OBDs of Kathara Coalmine Area, Jharkhand

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Abstract : Butterflies are one of the largest groups of insects and are most noticeable due to their conspicuous nature in a landscape. A study was carried out of four different aged reclaimed over burden dumps (ROBDs) of Kathara coalmine area of Jharkhand from 2007 to early 2009 and the present paper is a report on the diversity of the recorded butterflies. About 327 specimens of butterflies were collected from 10 randomized quadrates of 10 m 10 m along transact. Out of total observed butterflies twenty seven species belonged to four families: Pieridae, Lycaenidae Nymphalidae and Papilionidae of the supper family Papilionoidea. The family Pieridae dominated the ROBDs with their highest occurrence (44 per cent). It was observed that the newly ROBDs are having higher diversity index than the older ROBDs.

Keywords : Butterfly, Reclaimed over burden dumps (ROBDs), Diversity index, Habitat fragmentation

Introduction

Butterflies are economically important winged hexapods (Mani, 1973; Campbell and O'Toole, 1987; Zahradnik and Chvala, 1989). They are beautiful, attractive and have lured zoologists, especially entomologists throughout the world since time immemorial. Hence, there are good number of scientific, literary and jungle stories available (Marshell and De Niceville, 1882; Watson, 1984; Hall, 2005; Tej Kumar, 2009; Mathew and Kumar, http://wiienvis.nic.in/ran_forest/chapter6.htm). The caterpillars of many butterflies are phytophagous and cause damage to agricultural as well as horticultural products. Hence, are economically dear too. There are number of scientific work on ecology and functions of butterfly (Mathew, 1990; Luis-Martinez *et al.*, 2003; Tangah *et al.*, 2004; Hall, 2005; Winarni, 2007; Chandra *et al.*, 2007; Hamback *et al.* 2007; Pozo, *et al.*, 2008; Verma, 2009) and have been referred as 'flagships' and 'honorary birds'. They are valuable pollinators, important food chain components of the birds, reptiles, spiders and predatory insects; and indicators of environmental quality as they are sensitive to the changes in the environment (Hamback *et al.*, 2007). They are good candidate materials for the study of genetics, insect-plant interactions and co-evolution. Therefore, their diversity becomes an index for status of a habitat and landscape.

There are good number of literature on butterflies of India (Moore, 1881; Marshell and de Niceville, 1883; de Niceville, 1886; 1890; Moore and Swinhoe, 1890-1913; Bingham, 1905; Bell, 1909-1927; Ormiston, 1924; Evans, 1932; Yates, 1935, 1946; Wynter-Blyth, 1957; Laithwaite *et at.*, 1975; Smart, 1975; Larsen, 1987a; Kunte, 1997; Anu, 2006; Anu *et al.*, 2009; Shanti *et al.*, 2009; Tiple and Kuhrad, 2009; Verma, 2009; Rajgopal *et al.*, 2011; Ramesh *et al.*, 2010; Singh, 2010 and Hussain *et al.*, 2010). Most of the studies have been carried out in the southern part of the country and there are a few studies of Jharkhand and Bihar (Verma, 2009; Singh, 2010).

There are between 15,000 and 20,000 species of butterflies worldwide and about 1501 species have been reported from India (Gaonkar, 1996). There are a few serious workers (Verma, 2009 and Singh, 2010) who have reported butterflies diversity from Jharkhand and works on the reclaimed OBDs are equivocal. There are several scientific papers on habitat fragmentation and its impact on the species diversity. Previous studies have shown that the response of species richness to habitat change is not instantaneous, but usually occurs after a time delay (Diamond, 1972, Tilman *et al.*, 1994). Such delays vary from years to centuries depending on the taxon and the severity of fragmentation (Brooks and Balmford 1996, Brooks *et al.*, 1999, Ferraz *et al.*, 2003). But there has been lack of information on ROBDs. The present paper is an effort to report the diversity of the butterflies of ROBDs, and the impact of habitat fragmentation on the diversity of butterflies and its consequences on the ecological process.

Materials and Methods

Present study was carried out in the Kathara Coalfield area (Bokaro district) in the State of Jharkhand (Map 1). It is situated at 23° 47' N latitude and 85° 57' E longitudes, above 210 meter above sea level. The experimental area experiences average annual rainfall between 157 cm - 195 cm and the temperature oscillates between 2°C in winter to 45°C in summer. The average

Map 1 : Location map of Kathara coalmine area (study area), Jharkhand, India

pH of the soil ranges from 4.8 to 7. Four sites were selected with different age of plantation as+ a reclamation process. Site I was having five years old plantation, site II had 15 years old plantation, site III had 30 years old plantation and the site IV had more than fifty years plus (approximately) of plantation.

The methods for collection of data was observation, sighting, photography, ground net sweeping and aerial net sweeping of the butterflies from all the four sites. Butterflies were sampled by recording them from randomized quadrates of 10 m X 10 m on the either side of the laid transect (Manakadan and Rahmani, 1977; Anon 2000).

In the present paper authors have followed Mani (1973); different relevant websites, appended after the reference, and Zahradnik and Chvala (1989) for the purpose of field identification and classification.

Results and Discussion

A list of collected and observed butterflies of the ROBDs has been presented in the Table 1. There were 327 butterflies collected during the study period and were identified and classified to four different families. It was noticed that the percentage of the four butterfly families observed /sighted varied greatly: Pieridae family was represented by 44 per cent, Nymphalidae family was represented by 34 per cent, Lycaenidae was represented by 15 per cent, and Papilionidae had 7 per cent representation only (Fig. 1). The Shannon-Weaver (1949) diversity index for these families was calculated and has been presented in table 2. It was observed that the family Lycainidae had highest diversity index (1.9596) and the family Papilionidae had lowest (1.3261). There is no obvious reason why there are differences in diversity index of these four families. Further, it can be noticed from the table that there is formation of two groups among these four observed

Table: 1. A list of specimen of butterflies collected/observed from all the four mine spoils.

SN	Family	Sp. Name	C Name	Collection Method	Wing Colour
1	Pieridae	*Catopsilia Sp.*	Mottled Emigrant	Sighting/ Observation, Sweeping, Hand picking, and Photography	Light Green
2	Pieridae	*Catopsilia Sp.*	Common Emigrant	Sighting/ Observation, Sweeping, Hand picking, and Photography	Light Yellow
3	Pieridae	*Catopsilia Sp.*	Common Emigrant	Sighting/Observation and Photography	Off white
4	Pieridae	*Catopsilia Sp.*	Common Grass Yellow	Sighting/Observation and Photography	Yellow
5	Pieridae	*?*	Common Orange Butterfly	Sighting/Observation	Orange
6	Pieridae	*?*	Common Blue Butterfly	Sighting/Observation	Blue
7	Lycaenidae	*Freyeria sps*	Grass Jewel	Sighting/ Observation, Sweeping, Hand picking, and Photography	light Brown
8	Lycaenidae	*Rapala Sp.*	Indigo Flash	Sighting/Observation and Photography	Steel Grey
9	Lycaenidae	*Castalius rosimon*	Common Pierrot	Sighting/Observation and Photography	White background with black spots
10	Lycaenidae	?	?	Sighting/Observation and Photography	Yellowish at thorax, white wings with grey at the end
11	Lycaenidae	*Catochrysops Sp.*	Silver Forget-me-not I	Sighting/Observation and Photography	Whitish Brown
12	Lycaenidae	*Catochrysops Sp.*	Silver Forget-me-not II	Sighting/Observation and Photography	
13	Lycaenidae	*Actytolepis Sp.*	Common Hedge Blue I	Sighting/Observation and Photography	Light Brown with black spots
14	Lycaenidae	*Actytolepis Sp.*	Common Hedge Blue II	Sighting/Observation and Photography	Bluish white
15	Lycaenidae	*Tajuria Sp.*	Peacock Royal	Sighting/Observation and Photography	Ashy Brown
16	Lycaenidae	*Curetis Sp.*	Indian Sunbean	Sighting/Observation and Photography	Ashy White
17	Nymphalidae	*Precis almana*	Peacock Pansy	Sighting/Observation and Photography	Orange-Yellow
18	Nymphalidae	*Precis orithya*	Blue Pansy	Sighting/Observation and Photography	Black, followed by Peacock blue and grey on the margin

19	Nymphalidae	*Pantoporia Sp.*	Common Lascer	Sighting/Observation and Photography	Yellow with black tiger motif
20	Nymphalidae	*Euploea core*	Common Indian Crow	Sighting/Observation and Photography	Chocolate Brown white spots along the outer margin
21	Nymphalidae	*Tirumala sps*	Blue Tiger	Sighting/Observation and Photography	Black to Brown with bluish-white semi hyaline spots and streaks
22	Nymphalidae	*Danaus Sp*	Monarch Butterfly	Sighting/Observation and Photography	Orange wing with dark brown and white markings
23	Nymphalidae	*Limenitis Sp*	Viceroy Butterfly	Sighting/Observation and Photography	
24	Papilionidae	*Papillio Sp.*	Lime Butterfly	Observation	Black with white patches
25	Papilionidae	*Papillio Sp.*	Common Mormon I	Sighting/Observation	Dark Chocolate Brown
26	Papilionidae	*Papillio Sp.*	Common Mormon II	Sighting/Observation	Black with small whitish patches on the edge of the wing
27	Papilionidae	*Graphium Sp.*	Common Jay	Sighting/Observation	Black with Whitish patches

Fig. 1. Percentage occurrence of four butterfly families observed/sighted in mine spoils of Kathara coalfield area

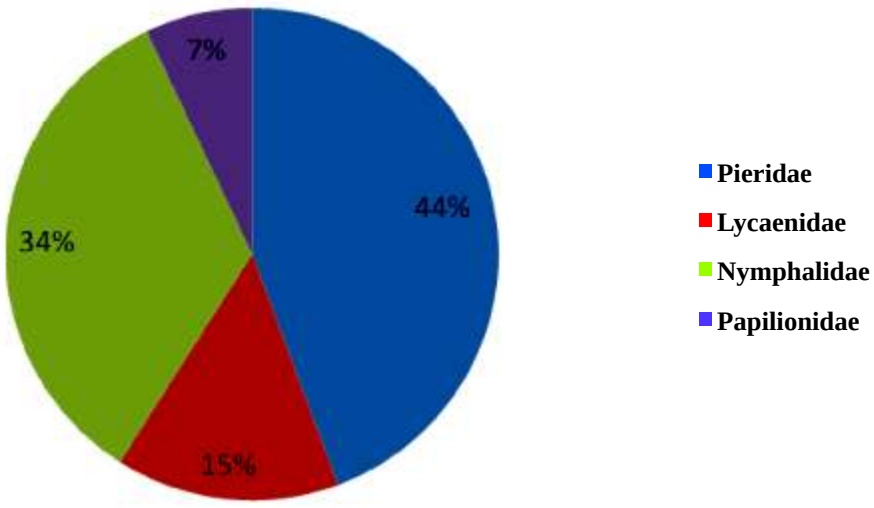

Table 2 : Shannon diversity index of the four families in four sites

SN	Family	Total Observation	Diversity Index
1	Pieridae	145	1.6781
2	Lycaenidae	48	1.9596
3	Nymphalidae	102	1.3291
4	Papilionidae	23	1.3261

families of the butterfly. Pieridae and Nymphalidae are the dominant group, where as the Lycaenidae and Papilionidae forms the other group.

According to Benedic *et al*. (2007) populations within habitat fragments are expected to have lower genetic diversity than those in continuous habitats, due to restricted gene flow, genetic drift and increased inbreeding (Frankham *et al*., 2002). Further, Hanski's (1999) metapopulation model designed for butterflies': extinction rate depends on patch area and colonisation rate depends on size of and distance to neighbouring patches. In the present study, we observed fewer families of butterflies. The study area is in patches and is distant from the neighboring forest. Hence, the butterflies show a different pattern of distribution and there is lower variety at the family level. In the opinion of the authors it may be due to the habitat fragmentation and anthropogenic activities going in the experimental area.

Family Pieridae :

Out of the total collection of 327 specimens from all the four experimental sites 145 individuals were classified to family Pieridae. Shannon-Weaver diversity index for this family (community) was 1.6781 (Table 2). Butterflies from this family are predominantly white or yellow in colour along with black markings. Their flight was rapid and they move erratically from plant to plant (Benedick *et al*., 2007)

Mottled Emigrant: Collected from all the four sites in different months. Wing span varied from 50-65 mm. The mottled emigrants are greenish white butterfly with a black apical border on the upper side of the wings (Mani,1973; Zahrdnik and Chvala 1989)

Common Emigrant I & II: Collected from all four sites in different months. Colour of the butterfly is pale yellow. Wing span varied from 50-65 mm.

Common grass yellow: Collected from all the four sites (site I, II, III and IV). These are very common butterflies and are found in all seasons. Wing colour is yellow and wing span is of 35-45 mm.

Common grass orange: Collected from Site I, II and IV. Wing span is 30-40 mm.

Common grass blue: Collected from site IV only. Wing span varied from 30-35 mm.

Family Lycaenidae :

Butterflies of this family are small, mostly under 5 cm. Their flight is rapid and erratic and very close to the ground. Subfamilies include the Blues, Coppers, Hairstreaks and Harverstes. We were able to collect only forty eight individuals for this family. They were identified and classified into seven species and we were not able to identify one species up to the genus level. Shannon-Weaver diversity index for the family was recorded to 1.9596.

Grass Jewel: Collected from site IV only. Wing span varied from 40-50 mm.

Indigo Flash: Collected from site I. Wing span of 30-35 mm.

Common Pierrot : Collected from site IV in the month of September 2008. The Common Pierrot is a small pied butterfly, flies close to the ground and settles down often. Body colour is white with black patch. It has a distinct unmarked gap in the centre of its hind wing. Wing span is about 35 mm.

Lycaenidae, Genus: Collected from site I. Colour of wing is pale gray to pale white. Wing span is of 60-70 mm.

Forget-me-not I: Collected from site III. Forget-me-not II: Collected from site III and IV. Common Hedge Blue**:** Collected from site II. Common Hedge Blue**:** Collected from site III. Peacock Royal**:** Collected from site I, II and III. Indian Sunbean**:** Collected from site II.

Family Nymphalidae :

The Brush-footed family is the largest butterfly family in the world, consisting of several thousand species. The butterflies are medium to large sized and can be extremely diverse in nature. In India there are about 480 species from this family. This family includes

the subfamily Danainae, the milkweed butterflies. Total number of collection/observation was 102 recorded for this family, in which seven species were indentified and it had the Shannon diversity index of 1.3291.

Peacock Pansy: Collected from site II in the month of Jan. 2009. Butterflies are orange to yellow in colour. Wing span varied from 70-75 mm. It has a very plain underside and looks like a dry leaf when its wings are closed. This butterfly gets its name from the prominent eye spots on its wings. These 'eyes' can be suddenly displayed by opening its wings to startle predators.

Blue Pansy: Collected from site IV in the month of Aug 2008. One of the prettiest butterflies, it has a very plain underside and looks like a dry leaf when its wings are closed. Blue Pansy (*Precis species*), is a sexually dimorphic butterfly. They have unique color combination of blue, black and brown. Wing span varied from 40-60 mm.

Upper side of the male fore wings basal with two thirds black and apex has pale brown with white transverse bands. Hind wing predominantly bright blue (hence the common name). Both wings are with 2 orange-ringed ocelli on each. Female is larger, pale almost light brown. Blue marking in the hind wing is slight and orange-ringed spots bigger than in male. Underside is grayish brown with white markings and there are wavy lines. Ocelli are visible in forewing only. Wing span was about 40-60mm.

Common Lascer : Collected from site II in the month of July 2009. Upper side of the wing was yellow to orange with black bands. Wing span varied from 24-32 mm.

Common Indian Crow : Collected from site II in month of Aug. 2008. The Common Crow (*Euploea core*) is a glossy black butterfly or glossy chocolate brown butterfly with white marks along the outer margins of the wing. The body also has prominent white spots along its wing margins. Wing span varied from 8-9 cm.

Blue Tiger : Collected from site I, II and III in the month of September 2009. Most are large brightly coloured butterflies usually brownish with black and white markings and white spotted body segments. Upper side was black, with bluish-white semi hyaline spots and streaks. Wing spans about 75 mm.

Monarch Butterfly : Very common butterfly in the study sites, collected from all the four sites. There are white spots on outer margin and orange patches near the top of the forewings. The hind wings are round, and are lighter in color than the forewing. The body is black with white spots. Wing span is about 70-75 mm.

Viceroy Butterfly : Collected from all the four sites. Its wings feature an orange and black pattern, and over most of its range it is Monarch butterfly. Wingspan is between 53 and 81 mm.

Family Papilionidae :

Swallowtail butterfly family consists of about 550 species of which 84 are found in India. Most swallowtails are medium to large, brilliantly coloured and extremely beautiful. Butterflies from this family are commonly found in both tropical and temperate habitats. They are called swallowtails because some of species have tailed hind wings. However, not all family members have tails. Total number of observation was twenty three; they were identified in two species. Shannon-Weaver diversity index of this family was 1.3261. Five different species were collected of this family. Lime Butterfly**:** Collected from site III. Common Mormon I: Collected from site II. Common Mormon II: Collected from site III. Common Jay: Collected from site I and III.

Conclusions :

The variation in the Shannon-Weaver diversity indices (Table 2) of the butterfly and percentage occurrence (Fig 1), the authors opine, are due to mining – landscape of the area. The change in the land use pattern has consequent impact on habitat and finally the number and density of the butterflies. The fragmentation of habitat has affected the life-cycle of butterflies. Further, it appears that the habitat fragmentation has impact on the distribution pattern as the newer ROBDs have higher DI than the older one. The natural food chain is broken. This can be obvious from the results that the sites, which are having newer plantations (Site I and II) had more Shannon diversity index (1949), implying that the species are more rare, rudrals rather than the sites having more than 30 years of plantation (Site III and IV) had lower species diversity, implicitly presence of K-select species, established to their habitats (Magurran, 1988; Krebs, 1999 and Bitzer*et al.*, 2005).

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Modulation of radiation induced oxidative damage in brain of Swiss albino mice by flaxseed oil.

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Abstract : Study was done to evaluate the biochemical and histopathological changes after 5 Gy whole body irradiation and its modulation by supplementation of flaxseed oil on male Swiss albino mice brain up to 30 days. For this, healthy mice from an inbred colony were divided into four groups: (i) control (vehicle treated) (ii) flaxseed oil treated : mice in this group were orally supplemented with flaxseed oil (100 µl/mice/day) once daily for 15 consecutive days; (iii) irradiated mice (iv) FO + irradiated : mice in this group were orally supplemented with FO for 15 days (once a day) prior to irradiation. Marked radiation induced changes in the amount of whole brain lipid peroxidation (LPO), Glutathione (GSH), protein, nucleic acids and histopathological changes could be significantly ($p < 0.001$) ameliorated specially at later intervals by supplementation of FO prior to irradiation. The results of present study showed that prior supplementation of FO has radioprotective potential as well as neuroprotective properties against the radiation.

Key Words : Whole brain, Cerebellum, Oxidative stress, Lipid peroxidation, Glutathione, Nucleic acids, Purkinje cells.

Introduction

Humans have always been exposed to natural background radiation. During oxidative stress and exposure to radiation, excessive free radicals are produced (Sonntag, 1987; Halliwell and Gutteridge, 1993). The free radicals disturb cellular homeostasis by enhancing the peroxidation of membrane lipids, oxidation of proteins, base damage and adduct formation in DNA which ultimately leads to cell death if the damage is beyond repair. The endogenous antioxidant defense systems of the cell competitively counteract the oxidative stress (Szumiel, 1994). However, free radical insult surpassing the endogenous cellular defense mechanism warrants external antioxidant supplementation. Naturally occurring antioxidants may also provide an extended window of protection against low dose- rate irradiation, and may have therapeutic potential when administered after irradiation. Several plant constituents have been proven to possess considerable free radical scavenging or antioxidant activity (Weiss and Landauer, 2003; Bhatia *et al.*,2001; Bhatia *et al.*,2006; Sharma and Sisodia, 2000; Bhatia and Jain, 2004; Yadav *et al.*,2004; Verma *et al.*,2002; Sisodia *et al.*,2011; Sisodia and Singh, 2009; Sharma and Sisodia, 2010; Jagetia *et al.*,2005).

Flaxseed is also known as flax and linseed. Its oil is derived from the seeds of the plant *Linium usitatissimum.* Flaxseed oil is a very rich source of Alpha- linolenic acid (ALA) which is approximately 40 to 60%. Lower amounts of linoleic acid and oleic acid (each about 15%) are also present in flaxseed oil. In addition, flaxseed oil is the richest source of the lignan secoisolariciresinol diglucoside (SDG). The level of SDG in flaxseed varies between 0.6 to 1.8g /100g or 1-4% by weight (Johnsson *et al.*,2000; Eliasson *et al.*,2003) 60-700 times greater (Prasad, 1999) than any other edible plant (Thompson *et al.*,1991) with the variability in components depending on the cultivar and the growing location and year (Westcott and Muir, 1996). It also has smaller quantities of other lignans; matairesinol, isolariciresinol, lariciresinol, demethoxysecoisolariciresinol and pinoresinol (Sicilia *et al.*,2003). It has been suggested that the lignans within flaxseed are the beneficial components within flax (Hemmings and Barker, 2004). Flaxseed has been demonstrated as excellent anticarcinogenic (Chen *et al.*,2002, 2003), antiatherogenic and anti cholesterolic effects (Lin *et al.*,2002). An antioxidant activity of the flaxseed lignan was also demonstrated by Chun and Collaborators (Chun *et al.*,2007). Dietary flaxseed also prevents radiation- induced oxidative lung damage, inflammation and fibrosis in a mouse model (Lee *et al.*,2009). Flaxseed has been shown to protect against harmful effects of radiation and is crucial for the development of brain and nervous system. In this study, it was found that SDG (secoisolariciresinol diglucoside), which is believed to confer potent antioxidant properties of flaxseed, regulates the transcription of antioxidant enzymes that protects and detoxify carcinogens, free radicals and other damaging agents and also flaxseed when given to mice not only protected tissues before radiation exposure occurred, it reduced damage after exposure as well (Christofidou-

Solomidou *et al*.,2011).

In order to provide a novel insight into radioprotection, we tried to evaluate the effect of oral administration of flaxseed oil before irradiation, on brain dysfunction with reference to biochemical, qualitative and quantitative histopathological changes in the cerebellum.

Materials and methods

Animals

The Departmental Animal Ethical Committee (DAEC) approved the study. Animal care and handling was done according the guidelines of INSA (Indian National Science Academy new Delhi, India). Swiss albino mice (*Mus musculus*) (6-8 weeks old, weighing 25±2 gm) from an inbred colony were used for the present study. These animals were maintained under controlled conditions of temperature (25±2 C) and light (light: dark, 14: 10 hrs). Four animals were housed in a poly propylene cage containing sterile paddy husk (procured locally) as bedding through the experiment. They were provided standard mice feed (procured from Hindustan Lever Ltd. Mumbai) and water *ad libitum*.

Chemicals

Thiobarbituric acid was purchased from Sigma, USA and Flaxseed oil (*Linum usittatisimum*) family Linaceae derived through a cold pressure method, procured from commercial shop Mulkusha Natural Oiltak, Ganpati Plaza, Jaipur, India. All other chemicals used were of analytical grade.

Source of Irradiation

The cobalt teletherapy unit (ATC-C9) at the Cancer Treatment Center, Radiotherapy Department, SMS Medical College and Hospital, Jaipur, Rajasthan, India, was used for irradiation. Unanesethesized animals were restrained in well- ventilated Perspex box and whole body exposed to gamma radiation.

Dose Selection of Flaxseed Oil

Dose selection was performed on the basis of a drug tolerance study in our laboratory (Bhatia *et al.*,2007). Various doses of Flaxseed oil (50 µl, 100 µl, 150 µl, and 200 µl/mice/day) were tested against 10 Gy of gamma radiation. 100 µl / mice /day was obtained as optimum dose based on survivability of mice. This dose was used for further experiments.

Experimental Design

For studying modification of radiation induced oxidative stress by flaxseed oil 40 animals were divided into four groups having ten animals in each group:

Group I. Control (vehicle treated) : Mice of this group did not receive any treatment.

Group II. FO treated- mice: In this group mice were orally supplemented with flaxseed oil (100 µl/mice/day) once daily for 15 consecutive days.

Group III. Irradiated mice (IR): The animals were exposed whole body to gamma radiation (5Gy) at a distance (SSD) of 77.5 cm from the source to deliver the dose rate 1.07 Gy/min.

Group IV. FO + irradiation**:** Mice were orally administered FO (100 µl/mice/day) for fifteen consecutive days then exposed to a single dose of whole body to gamma radiation (5Gy) one hour after last treatment with FO.

Removal of Brain Tissue

Animals of each group were humanly sacrificed by cervical dislocation and autopsied at various post irradiation intervals at different time points between 1 to 30 days post irradiation. To remove brain an incision was given at the sides of the jaws to separate the upper and the lower palates. The upper palate was cut in the middle and after having cleared the surrounding tissues; brain was excised and separated from the spinal cord at the decussation of pyramids. Whole brain was used to estimate various changes in biochemical parameters and cerebellum was fixed in Bouin's fluid for paraffin sectioning and stained with hematoxyin, eosin for histopathological studies.

Biochemical assay

Lipid peroxidation (LPO) assay. LPO was measured by the method of Buege and Aust (1978). Briefly, 1.2 ml solution of TCA-TBA-HCl prepared in 1:1:1 was added to tissue homogenate (0.8 ml). This final mixture was heated on a water bath for 30 min at 80°C and cooled. After centrifugation the absorbance was recorded at 532 nm using a UV (ultraviolet)-Visible double beam spectrophotometer. The LPO has been expressed as malondialdehyde (MDA) in n mole/gm tissue.

Reduced glutathione (GSH) assay. The reduced glutathione (GSH) content of tissue samples as determined by the method of Moron *et al.* (1979). Tissue sample was homogenised in the sodium phosphate-EDTA (ethylenediaminetetraacetic acid) buffer then 0.6 M DTNB [5, 5_dithiobis-(2-nitrobenzoic acid)] was added. The optical density of the yellow coloured complex developed by the reaction of GSH and DTNB was measured at 412 nm using a ultraviolet (UV)- vis spectrophotometer. The results were expressed as nano mol/100 mg of tissue.

Protein assay. Estimation of protein was based on the method proposed by Bradford (1976) and 10%

homogenate was prepared (1 gm of tissue in 9 ml of NaCl) and 0.1 ml of the sample was taken for the Bradford assay. Three repeats of the assay from each animal were carried out. The absorbance was read at 595 nm.

Deoxyribonucleic acid (DNA) estimation assay. The quantification of DNA is based on determination of deoxypentoses described by Ceriotti (1952). Under extreme conditions, DNA is initially depurinated quantitatively followed by the dehydration of deoxyribose sugar to ω- hydroxylevulinic aldehyde, this ω- hydroxylevulinic aldehyde condenses, in acidic medium, with diphenylamine, which produces a deep blue colored condensation products with maximum absorption at 595nm. As in DNA only deoxyribose sugar of purine nucleotides is released, so the value obtained represents only half of the total deoxyribose in the sample. So after calculation values results were multiplied by the factor of two and expressed in µ gm/mg tissue.

Ribonucleic acid (RNA) estimation assay. Orcinol method described by Ceriotti (1955) is general method for determination of pentoses. Acid hydrolysis of RNA releases ribose sugar, which in presence of strong acid undergoes dehydration to form furfural. This furfural in the presence of ferric chloride as catalyst, reacts with orcinol and produces green coloured compound, which has maximum absorbance at 665 nm. The colour formed largely depends on the concentration of HCl, ferric chloride, orcinol and the timing of heating at 100°C etc. The results were expressed in µ gm/mg tissue.

Histopathological study. Blind examination of the stained sections of cerebellum were done using a light microscope, at a magnification of 40x. Counts of Purkinje cells were made for a total length of 2500 µ m in the lobule simplex VI B . Larsell (1952) in ansiform lobule using an occularmeter in the eyepiece, in mice cerebellum. Volumes of Purkinje cells were measured using the formula $4/3\pi r^3$ where 'r' is the radius of the cell. In order to determine the radii of the Purkinje cells, measurement at their major and minor axes was taken and the average obtained by dividing them by '2'. Thickness of molecular and granular layers were also observed at a magnification of 40x and expressed in terms of µm.

Statistical analysis

The results obtained in the present study were expressed as mean ± Standard error of mean. Statistical difference between various groups were analyzed by student t-test and the significance was observed at $P<0.001$, $P<0.005$ levels.

Results

Irradiation raised the LPO levels at all the autopsy intervals. Initially sharp increase was noted (approximately 57%) in comparison to later autopsy intervals. Oral supplementation of flaxseed oil prior to irradiation succeeded to bring the LPO level near normalcy (95.70%) (Table-1). Oral Supplementation of only flaxseed oil (Group-II) was also able to reduce the base line values approximately by 12 %.

Table 1. Variations in lipid peroxidation (LPO) and glutathione level (GSH) in whole brain at various post-treatment days, in the presence or absence of Flaxseed Oil (+SEM).

Parameters	**Groups**	**Days post treatment**					**Control** no treatment	**FO treated** (100 µl / mice /day)
		1	3	7	15	30		
LPO (nmol/gm)	Irradiated	222.50±2.06 (157.07 %) b ##	184.50±1.64 (130.25 %) b ##	170.68±1.90 (120.49 %) b ##	167.20± 1.21 (118.03 %) b ##	160.34±1.13 (113.19 %) b ##	141.65 ± 2.11 (100 %)	92.50±0.59 (87.87 %) a ##
	FO+IR	153.75±1.28 (73.24 %) c ## d ##	152.50±1.03 (107.65 %) c ## d ##	165.00±1.00 (116.48 %) c ## d ##	133.70± 0.77 (94.38 %) c ## d ##	135.56 ±1.11 (95.70 %) c ## d ##		
GSH (nmol/100 gm)	Irradiated	8.25 ± 0.50 (45.05%) b # #	7.382±0.99 (40.31%) b # #	7.49 ± 0.65 (40.90%) b # #	12.08 ± 0.72 (65.97%) b # #	13.246±0.74 (72.34%) b # #	18.31 ± 0.98 (100%)	19.55±0.505 (106.77%) a ##
	FO+IR	12.07±0.32 (65.92%) c# # d # #	12.48±0.42 (68.15%) c# # d # #	12.95 ± 0.68 (70.72%) c# # d # #	14.086±0.22 (76.93%) c# # d # #	16.088±0.88 (87.86%) c# # d # #		

Control vs. FO treated = a, Control Vs. Irradiated = b, Control vs. FO+IR. = c, Irradiated vs. FO+IR = d.
Significance levels: # # p<0.001, # p<0.005

Glutathione (GSH) content decreased sharply after radiation exposure (Group-III) till 7th day, thereafter slight recovery was noted. In Group-IV GSH estimated was raised at all the corresponding intervals compared to Group-III. Magnitude of such a recovery from oxidative damage was significantly higher ($p<$ 0.001, $p<0.005$) in FO+IR group as compared to irradiated mice. Only FO treated mice also showed significant increase ($p<$ 0.001) in GSH content as compared to control (Table 1).

Protein estimated also showed statistically significant increase ($p<$ 0.001) in FO+IR treated mice brain in comparison to irradiated group at all the autopsy intervals. Only FO treated (Group-II) also showed significant increase ($p<0.001$) in protein content as compared to the control (Table 2).

DNA content declined continuously up to 15th day after irradiation (Group-III), but on day 30 a slight recovery was noticed as evident by increased DNA content. In the FO+IR group values of DNA content increased significantly ($p<0.001$), continuously after day 3 and attained the normal level of DNA by 30 days post- irradiation (Table-2).

Radiation induced continuous deficit ($p<0.001$) in whole brain RNA was noted in comparison to Group I (Control). In FO pre treated irradiated group (Group-IV) at all the corresponding intervals, RNA levels were noted at higher concentration compared to only irradiated (Group-III) but the values were still lower by approximately 7% from the control at the end of the experiment. Non- significant differences existed between group I and II (Table-2).

Histopathological observations

Histopathological observations in cerebellar folia in both irradiated and experimental groups in terms of alteration of volume and number of Purkinje cells indicates a statistically significant ($p<0.001$) deficit, which showed slight recovery after 15 days in Group- III and after 7 days in Group-IV, indicating early recovery. In mice pre-treated with flaxseed oil, however, the deficit was less than irradiated group mice (Group-III), indicating protection offered by flaxseed against irradiation induced oxidative stress which resulted in cyto- architectural damage.

Alterations in the thickness of molecular and granular layer in mice cerebellum, control and FO treated mice depicted similar pattern of changes, though

Table 2. Variations in Protein, DNA and RNA in Whole brain at various post -treatment days, in the presence or absence of Flaxseed Oil (+SEM).

Parameters	**Groups**	**Days post treatment**					**Control** no treatment	**FO treated** (100 µl / mice /day)
		1	3	7	15	30		
Protein (mg/gm)	Irradiated	76.19±2.91 (72.38%) b # #	85.00 ± 3.44 (80.75%) b # #	73.33 ± 3.05 (67.66%) b # #	58.00 ± 2.497 (55.10%) b # #	48.90 ± 3.27 (46.45%) b # #	105.263 ±1.84 (100%)	112.15±2.25 (106.54%) a# #
	FO+IR	80.68 ± 3.16 (76.64 %) c# # d# #	92.85±3.24 (88.20%) c# # d# #	101.11 ± 3.32 (96.05 %) c# # d# #	110.227± 2.59 (104.71 %) c# # d# #	111.28± 2.10 (105.566 %) c# # d# #		
DNA (µg/mg)	Irradiated	1.325± 0.46 (73.36%) b # #	1.292 ± 0.07 (71.53%) b # #	1.107±0.06 (61.29%) b # #	0.994 ± 0.03 (55.03%) b # #	1.128±0.12 (62.45%) b # #	1.806 ± 0.031 (100%)	1.798±0.09 (99.55%) aNS
	FO+IR	1.402±0.07 (77.65%) c# # d # #	1.392 ± 0.08 (77.07%) c# # d # #	1.475 ± 0.04 (81.67%) c# # d # #	1.782± 0.094 (98.67%) c# # d # #	1.801± 0.153 (99.72%) cNS d # #		
RNA (µg/mg)	Irradiated	1.59± 0.17 (89.32%) b # #	1.42 ± 0.078 (79.77%) b # #	1.36 ±0.16 (76.40%) b # #	1.28 ± 0.02 (71.91%) b # #	1.19±0.12 (66.85%) b # #	1.78 ± 0.18 (100%)	1.72±0.09 (96.62%) a # #
	FO+IR	1.62±0.07 (91.01%) c# # d # #	1.50 ± 0.08 (84.26%) c# # d # #	1.54 ± 0.04 (86.51%) c# # d # #	1.60± 0.095 (89.88%) c# # d # #	1.67± 0.15 (93.82%) c# # d # #		

Control vs. FO treated = a, Control Vs. Irradiated = b, Control vs. FO+IR. = c, Irradiated vs. FO+IR = d.
Significance levels: # # p<0.001, # p<0.005

Table-3 Variations in thickness of granular and molecular layer and counts and volume of Purkinje Cells in cerebellum at various post-treatment days, in the presence or absence of Flaxseed Oil (±SEM).

Parameters	**Groups**	**Days post treatment**					**Control** no treatment	**FO treated** (100 µl / mice /day)
		1	3	7	15	30		
Thickness of granular layer (µm)	Irradiated	88.45±1.77 (93.43%) b # #	80.12±1.48 (84.63%) b # #	75.28±1.98 (79.52%) b # #	60.55±0.92 (63.96%) b # #	62.92±0.86 (66.46%) b # #	94.66± 0.787 (100%)	92.54 ± 2.40 (102.29%) a# #
	FO+IR	89.92±1.87 (94.99%) c# # d# #	85.66±1.99 (90.49%) c# # d# #	82.74± 1.34 (87.40%) c# # d# #	88.15±1.20 (93.12%) c# # d# #	90.2± 0.76 (95.28%) c# # d# #		
Thickness of molecular layer (µm)	Irradiated	88.54±0.73 (81.79%) b # #	85.09±1.56 (78.60%) b # #	82.75±0.87 (76.44%) b # #	74.06 ±0.37 (68.41%) b # #	77.82 ±0.64 (71.88%) b # #	108.25± 1.76 (100%)	105.59±1.89 (97.54%) a# #
	FO+IR	98.29±1.84 (90.79%) c# # d# #	95.22±1.720 (87.96%) c# # d# #	98.56 ±0.99 (91.04%) c# # d# #	102.62±1.02 (94.79%) c# # d# #	106.75±0.74 (98.61%) c# # d# #		
Count of Purkinje cells/2500 m lobule	Irradiated	84.09± 2.55 (85.80%) b # #	77.24 ±4.20 (78.81%) b # #	70.00 ±4.20 (71.42%) b # #	66.08 ±3.57 (67.42%) b # #	69.11 ±4.01 (70.52%) b # #	98.00 ± 1.109 (100%)	108.00±4.256 (110.20%) a# #
	FO+IR	90.58 ±3.20 (92.42%) c# # d# #	87.97 ±2.54 (89.76%) c# # d# #	85.22±2.27 (86.95%) c# # d# #	86.11 ±3.05 (87.86%) c# # d# #	90.12 ±3.19 (91.95%) cNS d# #		
Volume of Purkinje Cells (μm^3)	Irradiated	408.23 ± 1.33 (87.08%) b # #	399.01 ± 2.32 (85.11%) b # #	382.89±2.44 (81.67%) b # #	392.91±2.74 (83.81%) b # #	409.80±2.33 (87.41%) b # #	468.79 ± 3.224 (100%)	469.02 ± 3.425 (100.04%) aNS
	FO+IR	422.69 ± 4.373 (90.16%) c# # d# #	415.82 ± 4.56 ((88.70%) c# # d# #	410.54±4.00 (87.57%) c# # d# #	426.83±3.067 ((91.04%) c# # d# #	449.49±2.991 (95.88%) c# # d# #		

Control vs. FO treated = a, Control Vs. Irradiated = b, Control vs. FO+IR. = c, Irradiated vs. FO+IR = d.
Significance levels: # # p<0.001, # p<0.005

molecular layer showed more damage till 7th day but on 15 and 30th day granular layer showed more damage. Maximum reduction (68.41% and 63.96%) in the thickness of molecular layer and granular layer respectively occurred at day 15th from the control. The thickness of molecular and granular layer was more at all the post irradiation intervals in FO+ IR group compared to irradiated group (Table-3).

Histopathological observations in the irradiated mice indicated an extensive damage in the cerebellar folia. The damage included necrosis, pyknosis of Purkinje cells and granular cells together with cytoarchitectural disturbances. Migration of Purkinje cells into granular layer leading to the formation of empty baskets and dissolution of connective tissue could be seen. Reduction in number of Purkinje cells was also noted (Fig. 1). Similar pattern of damage was noticed in FO pretreated irradiated group, though it was of relatively lesser degree (Fig. 2). The results indicate protective efficacy of flaxseed oil against radiation damage (Fig. 1 and 2).

Discussion

Results obtained from this study indicate that FO may act as a prophylactic agent and render protection against radiation induced oxidative stress. Oxidative stress leads to lipid peroxidation, protein and carbohydrate oxidation and metabolic disorders (Sies,1985; Pryor and Godber, 1991). The product of LPO such as MDA (malondialdehyde) and 4-hydroxynonenal are toxic to cells (Raleigh,1985). LPO within the membrane has a devastating effect on the functional state of the membrane because it alters membrane fluidity, typically decreasing it and thereby allowing ions such as Ca^{+2} to leak into the cell. The peroxyl radical formed through lipid peroxidation

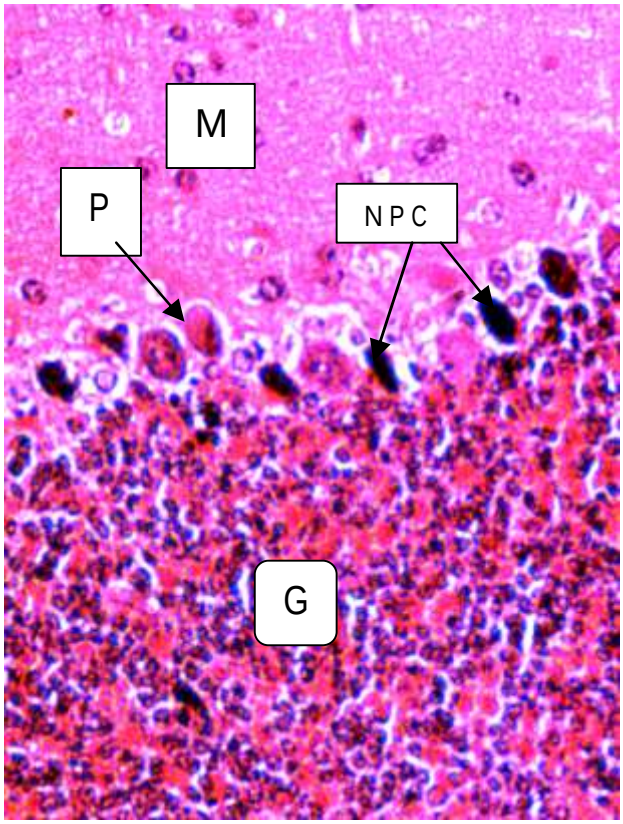

Fig. 1. Photomicrograph of cerebellar folia of Swiss albino mice at 3rd day, after irradiation to 5 Gy of gamma radiation (group-3) (20X).

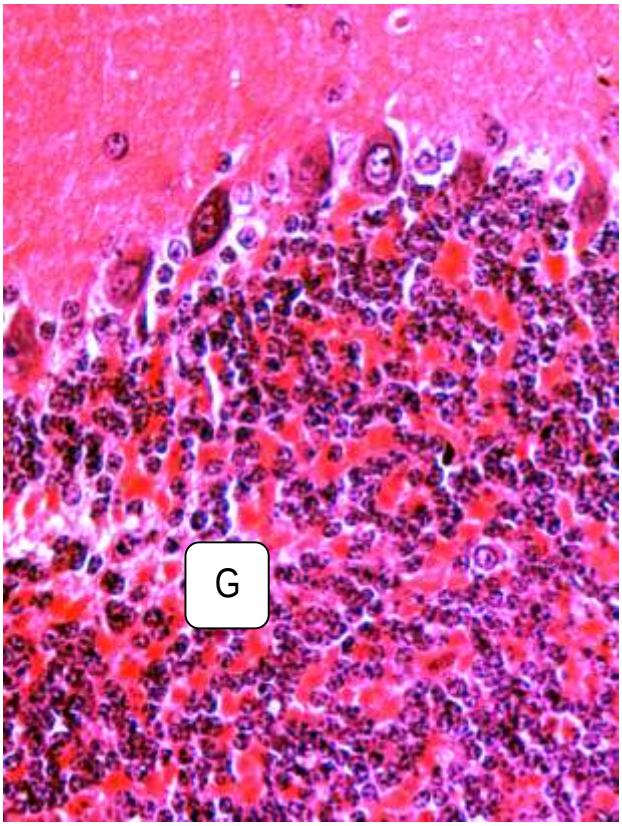

Fig. 2. Photomicrograph of cerebellar folia of flaxseed oil pretreated mice (FO+I R) at 3rd day post irradiation (20X).

Abbreviations: G = granular layer, M = molecular layer, P = Purkinje cell, NPC = necrotic Purkinje cells

attacks the protein membrane and enzymes and reinitiates lipid peroxidation. The preservation of cellular integrity of the cellular membrane depends on protection or repair mechanisms capable of neutralizing oxidative reactions. Inhibition of LPO in cell membrane can be caused by antioxidants (Konings and Osterloo, 1980). Shimoi *et al*. (1996) concluded that plant flavonoids, which show antioxidants activity *in vitro* also, function as antioxidants *in vivo*, and their radioprotective effect may be attributed to their radical scavenging activity. Flaxseed oil has very high content of alpha linolenic acid (c 18: 3n-3 omega-3 (n-3) fatty acid) together with small amount of phytoestrogen/ lignan. Lignan are platelets activating factor receptor antagonists and have antioxidant activity (Prasad, 1999).

In the present study the reduction in the amount of TBARS or MDA equivalents and elevation in the GSH level in the FO treated animals suggests that FO may scavenge the free radicals generated during oxidative stress. GSH, with its sulfhydryl group, functions in the maintenance of the sulfhydryl group of the other molecule (especially protein), acts as a catalyst for disulfide exchange reactions and in the detoxification of foreign compounds like hydrogen peroxide and free radicals. The lesser depletion of brain GSH content in the FO+IR treated group compared to the irradiated group may be an indication of higher availability of GSH, which increases the ability to cope with the free radicals produced by radiation. Decreased brain GSH levels have been reported in neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, in which oxidative processes contribute to the pathology (Reiter, 1995; Wu *et al*., 2004. Previous studies of our lab also suggest that elevated levels of glutathione by FO pre-treatment in irradiated mice liver may facilitate the reduction of

oxidative free radicals by H^+ donation. This allows the restoration of glutathione by glutathione reductase activity (Bhatia *et al.,* 2007).

In the present study, supplementation of only FO has also resulted in statistically significant ($p<0.001$) increase in protein content in comparison to control mice. Decrease in the protein content after exposure to irradiation might be due to either decline in the rate of protein synthesis or increase in the consumption of protein. It may also be the result of the depression of enzyme involved in the activation of amino acid and transferring to t-RNA or by the inhibition of release of synthesised polypeptides from polysomes (Bhatia *et al.,* 2007). Some studies have indicated that oxidative stress diminishes and the levels of some proteins vary during the progression of Alzheimer's disease (AD) (Nunomura *et al.,*2001; Lee *et al.,*2005). Increased protein concentration in the present study may be due to improved ribosomal activities, which enhance protein synthesis.

DNA damage induced by ROS is involved in mutagenesis and generation of mutation related diseases, including cancer. In the present investigation nucleic acids (DNA+ RNA) concentration showed depletion after radiation exposure which could be elevated by FO supplementation at all intervals indicating a significant protection. Reduced DNA noticed post- irradiation may be due to acute cell death leading to loss of DNA in excess than is normally eliminated from the tissue. The prolonged interphase or delayed onset of DNA synthesis after irradiation also could lead to decreased content of DNA. Egana *et al.* (1983) corroborated present finding that 60 Co gamma irradiation had more effect in DNA, RNA and protein function (in developing CNS). A correlation seems to exist between DNA degradation and cell pyknosis. The RNA in the transcription of genetic information (mRNA) as components of ribosomes and as an intermediate in the activation of amino acids (acy1 t-RNA) is responsible for active synthesis of new proteins. Although new protein synthesis ceases at maturation, RNA synthesis continues at a diminished rate and a rapid turnover persists throughout the life span of the organisms. The maximum activity of RNA polymerase at birth decreased upto the age of 30 days and remained at the minimal level in adult brain. This is the enzyme responsible for protein synthesis.

Results indicated continuous decline in the number and volume of Purkinje cells up to 15 days post irradiation, then recovery starts in both FO+IR and irradiated group. The reduction in volume and number of Purkinje cells may be due to the radiosensitive nature of the cells. The potent antioxidative action of FO may make it a significant factor on reducing damage to the brain from oxidative stress. Gueneau *et al.* (1979) also reported that granular cells were the most radiosensitive. Sharma (2001) also reported that granular layer was more radiosensitive compared to molecular layer. Similar results have been obtained in our study at later intervals.

Mostly neural proliferation ends soon after birth, in some regions of the brain the cell proliferation continues, leaving the brain radiosensitive. The most common histological changes induced by X-rays were dilation of vascular endothelial cells, perivasculitis and edema. The permeability of blood barrier significantly increased within few hours after exposure (Fogarty *et al.,*1988).

Qualitative analysis in our study revealed damage present in the granular cells and Purkinje cells of brain cerebellum, could be modulated by flaxseed oil. Dose reduction factor (DRF) was calculated to be 1.41 for FO in earlier studies in our laboratory (Bhatia *et al.,* 2007). If FO is to produce an antioxidative effect, its antioxidant constituents must be absorbed by the body and available to the tissue exposed to oxidative stress.

When flaxseed oil is administered for a period of 15 days of time just before irradiation it confronts the oxidative load generated by irradiation. The damage is prevented by the pre- existing load of antioxidants. The antioxidative load combats and balances by lignan SDG and 3-omega fatty acids. SDG and its metabolites enterolactone (EL) and enterodiol (ED) have a number of antioxidant activities, including inhibition of lipid peroxidation and scavenging of hydroxyl radicals. The free radicals are not only quenched and scavenged by SDG but also it suppresses the oxidant load. In this manner the ultimate mode of action becomes prophylactic. The results of the present investigation demonstrate that FO treatment protects the mice whole brain and cerebellum against radiation-induced damage by inhibiting the glutathione, protein depletion and nucleic acids and ameliorating lipid peroxidation levels and it also reduced radiation induced histopathological damage in terms of quality and quantity. This may be due to the synergistic effect of the lignan and Alpha

linolenic acid (ALA) present in the FO.

Conclusion

The radioprotective activity of FO may be mediated through several mechanisms. The presence of lignan and ALA in FO elevates the cellular antioxidants and enables it to scavenge free radicals in the irradiated system, which could be a leading mechanism for radioprotection. Reduction in lipid peroxidation and elevation in non-protein sulphydryl groups may also contribute to some extent to their radioprotective activity. Supplementation with flaxseed may be a useful adjuvant treatment mitigating adverse effects of radiation.

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Neuronal morphology of dorsal cerebral cortex of the Indian wall lizard, *H. flaviviridis* (Rüppell)

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Abstract : The cerebral hemisphere of lizards is divided into pallium (cerebral cortex) and subpallium. The structural organization of dorsal cerebral cortex of the *H. flaviviridis* has been studied with the help of neurohistological techniques. The dorsal cortex shows three neuronal layers viz. outer plexiform layer, middle cellular layer and inner plexiform layer. Using characteristics such as criteria of location, soma shape and size, dendritic tree pattern/dendritic field shape, and dendritic spine covering four types of neurons have been distinguished in the cellular layer of dorsal cortex: monotufted neurons (18.14 %), bitufted neurons (28.43 %), multipolar neurons (22.55 %) and pyramidal neurons (30.88 %). The comparative study of the morphology of these neurons represents their similarities with other animal groups.

Key words: lateral cortex /bitufted neuron/ spinous/Golgi study/lizards.

Introduction :

The reptiles are interesting class of vertebrates in many respects and exhibit the highest degree of diversification because they were the first vertebrates to inhabit the terrestrial mode of life. Consequently a lot of physiological, morphological and behavioral changes have occurred in them including the modifications in brain. The cerebral cortex of lizards represent a laminar structure in which most of the neuronal cell bodies are grouped forming a principal cell layer sandwiched between the inner and outer plexiform layers (Luis de la Iglesia & Lopez-Garcia, 1997; Lopez-Garcia *et al*., 2002; Srivastava *et al*., 2007, Maurya and Srivastava, 2006; Srivastava *et al*., 2009; Srivastava and Maurya, 2009a, 2009b; Maurya *et al*., 2011), which are populated by scarce interneurons and where the afferent connections terminate in a highly laminated fashion (Lopez Garcia *et al.*, 2002).

By using Golgi-impregnation method different workers described various numbers of the neuronal types in all the four cortical areas of the lizards (Srivastava *et al*., 2012). In the medial cortex only one type of neurons in snake genera *Natrix* and *Boa* (Ulinski, 1977), five types in the lizard *L. pityusensis* (Berbel *et al.*, 1987), *P. hispanica* (Luis de la Iglesia & Lopez-Garcia, 1997), *M. carinata* (Srivastava *et al.*, 2007) and seven types in *H. flaviviridis* (Maurya and Srivastava, 2006) have been reported. In case of dorsomedial cortex one type of neurons in the lizard *A. agama* (Wouterlood, 1981), three types in each layer of snake's dorsomedial cortex (Ulinski, 1979), six types in *H. flaviviridis* (Srivastava *et al.*, 2007) and seven types in *C. versicolor* (Sakal *et al*., 2010) have been reported. In the dorsal cortex four types of neurons in the lizard *P. algirus* (Guirado *et al.*, 1987), five types in *M. carinata* (Srivastava *et al.*, 2007) and seven types in *C. versicolor* (Sakal et al., 2010) have been reported. In lateral cortex four types of neurons are present in all the three layers of snakes (Ulinski and Rainey 1980), three types were observed in the *M. carinata* (Srivastava *et al*., 2007) and *C. versicolor* (Sakal *et al.*, 2010).

Since, there exists a wide variation in the composition of different parts of brain in different reptilian orders and also among various species of lizards due to behavioural and environmental factors. Hence, in order to increase our knowledge the present study provides the morphology of the neurons of the dorsal cerebral cortex of the telencephalon of the Indian wall lizard, *H. flaviviridis.*

Materials & Methods :

A total of 32 male lizards (*Hemidactylus flaviviridis*) were used in the present study (5 for Nissl staining and 27 for Golgi study). The adult lizards with 60 – 85 mm snout to vent size have been taken for the experiment. Animals were captured from the surroundings of Allahabad (Uttar Pradesh, India), and kept in terrarium prior to the experiments. Five anaesthetized adult lizards (*H. flaviviridis*) were perfused with 100 ml of physiological saline followed by 10% formalin solution for 1 hour. The brain was immediately removed out from the skull and fixed in 10% formalin. The brain was processed for Cresyl violet staining to study the Cyto-architecture of the cerebral cortex of the lizard (Srivastava *et al*., 2009). Under ether anesthesia, animals were perfused with 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer, pH 7.2-7.4 by immersion in the fixative. The brain was immediately removed out from

the skull and kept overnight in the same fixative at 4°C. For staining, the Golgi-colonnier method was used (Colonnier, 1964) with some improvements (Luis de la Iglesia and Lopez-Garcia, 1997) adapted to the present material (i.e., 3 to 5 days of indurations at 4°C in a mixture of 2.4% potassium dichromate and 5% glutaraldehyde followed by 1 to 2 days of impregnation in 0.75% silver nitrate). After impregnation the brain was dehydrated in different grades of alcohol, cleared in xylene and embedded in paraffin wax (m. p. 52-56°C). 60 to 100 µm thick transverse sections were cut by the rotatory microtome. Sections were cleared in xylene and mounted in DPX. Photomicrographs were taken with computer aided microscope, Nikon eclipse 80i (Software, ACT-1).

Results :

The cerebral hemisphere of *Hemidactylus flaviviridis*, is composed of a roof (pallium) and a floor (subpallium). In the pallium (cerebral cortex) four cortical regions were present medial cortex (MCx), dorsomedial cortex (DMCx), dorsal cortex (DCx) and lateral cortex (LCx) (Fig. 1A). The septal area occupied the medial portion of the subpallium, whereas the lateral portion organized by the dorsal ventricular ridge (DVR) and the striatum (Str).

The dorsal cortex of *Hemidactylus flaviviridis* displayed three distinct neuronal layers and an ependymal layer. The basic pattern of three layers: outer plexiform layer (opl), cellular layer (cl) and inner plexiform layer (ipl) could be seen in the cresyl violet stained transverse sections through the cerebral hemisphere. The thickness of outermost layer-I ranged from 89 – 200 µm. Layer-II was characterized by densely packed neuronal cell bodies. The thickness of layer-II ranged from 61 – 93 µm. Layer-III was 65 - 104 µm thick having loosely packed neuronal cell bodies (Fig. 1B).

The four cortical regions of cerebral cortex were not continuous with each other and the sheet of somas in the cell layer-II was interrupted by a discontinuity from rostral to caudal regions. The dorsomedial cortex was overlapped with the medial extreme of the dorsal cortex whereas the lateral portion of the dorsal cortex overlapped by the lateral cortex (Fig. 1A). The result of this discontinuity and overlap is that the basic trilaminar pattern have been replaced by five-layered cortex within the annulus of overlap.

A Golgi analysis of the cell types present in the dorsal cerebral cortex of the lizard *Hemidactylus flaviviridis* gave highly variable results depending on individual animals or unknown experimental factors. The study of dorsal cortex has been carried out in the sections, in which the maximum number of impregnated neurons was achieved. The neurons were carefully selected for the study that showed well-developed dendritic tree pattern and clear dendritic branching. The axonal branching pattern was also traced for exploring the connections.

Using characteristics such as criteria of location, soma shape and size, dendritic tree pattern/dendritic field shape, and dendritic spine covering, four types (Table 1) of neurons have been distinguished in the cellular layer of dorsal cortex: monotufted, bitufted, multipolar and pyramidal neurons.

Monotufted neurons :

The monotufted neurons are of medium size and located at the upper border of the cellular layer. Their cell bodies are spherical or polygonal shape and measures on average 15 µm × 10 µm. The upper half of the soma gives rise to one or two primary dendrites, which usually gives off secondary dendritic branches in the outer plexiform layer (Fig. 1C). These dendrites are smooth or covered by few sparsely distributed spines on their surface (Fig. 2A). The dendrites arise from apical part, go directly towards the pial surface and then run parallel to the pial surface in the outer plexiform layer. The length of the dendrites of these neurons ranges from 75 µm to 110 µm. Axon and axon collaterals are not observed in these types of neurons in the present study.

Bitufted neurons :

The bitufted neurons are usually located in the

Table 1. Characteristic features of the projection neurons of the dorsal cerebral cortex of the lizard, *H. flaviviridis*.

Type	Descriptive name	Layer	n	%age	Mean soma size (µm)
1.	Monotufted neurons	cl	37	18.14 %	15 × 10 µm
2.	Bitufted neurons	cl	58	28.43 %	15 × 10 µm
3.	Multipolar neurons	cl	46	22.55 %	16 × 10 µm
4.	Pyramidal neurons	cl	63	30.88 %	16 × 11 µm

Percentage is given as n.100/204, 204 is the number of neurons examined in this study.

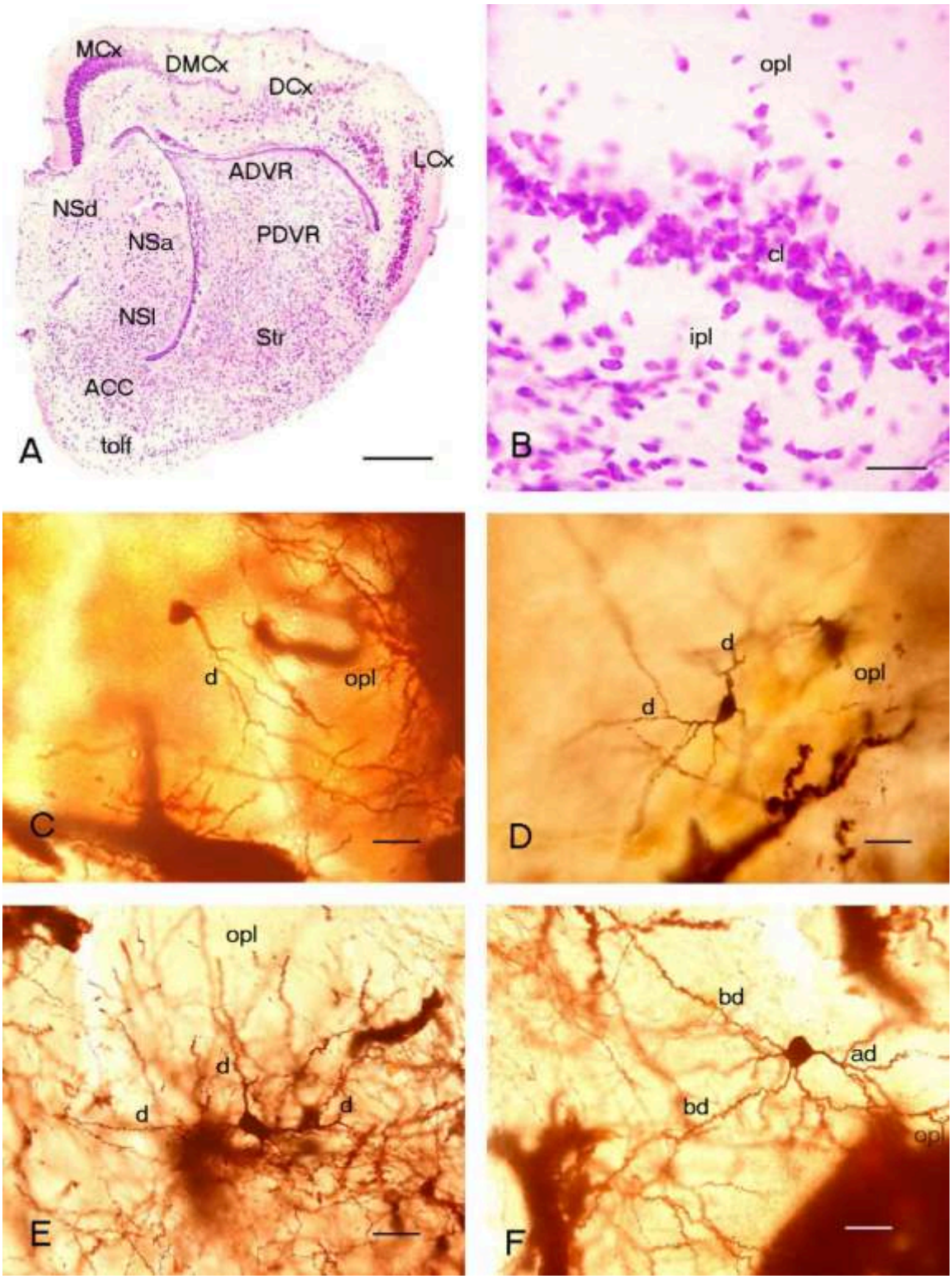

Fig. 1. (A) Cytoarchitecture of the *H. flaviviridis* cerebral cortex. (B) Nissl stained section of dorsal cerebral cortex. Neuronal types in the dorsal cerebral cortex of *H. flaviviridis*: monotufted (C), bitufted (D), multipolar (E), and pyramidal (F). Bars 100µm (A), 25µm (B-F). ADVR – Anterior dorso ventricular region, MCx – Medial cortex, DMCx – Dorsomedial cortex, DCx – Dorsal cortex, V – Ventricle, cl – cell layer, SE – Septum, LCx – Lateral cortex, opl – outrér plexiform layer, ipl – inner plexiform layer, d- dendrite, ad- apical dendrite, bd- basal dendrite, cl- cell layer.

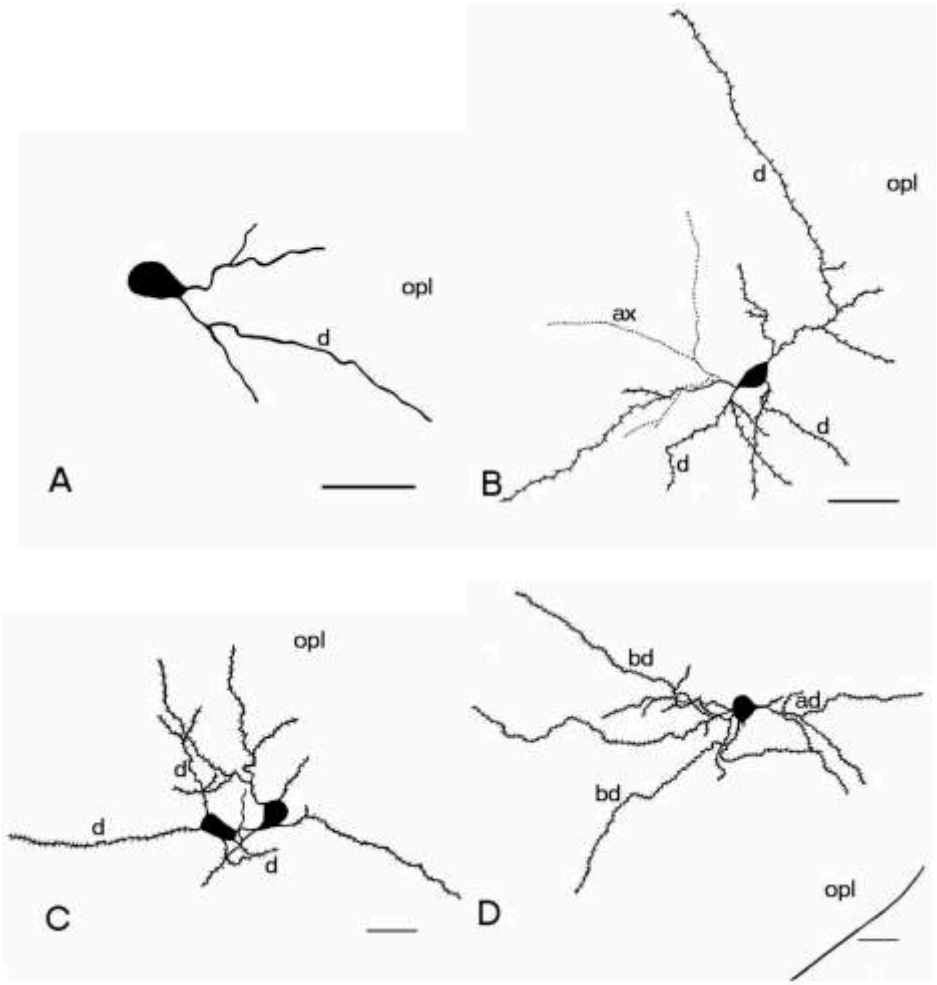

Fig. 2. Camera lucida drawing of the dorsal cerebral cortex neurons: monotufted (A), bitufted (B), multipolar (C), and pyramidal (D). Bars 25µm (A-D).
opl- outer plexiform layer, d- dendrite, ad- apical dendrite, bd- basal dendrite, ax- axon

middle region of the cellular layer. These neurons have somata of elongated or superficial shape which measures on average 15 µm × 10 µm. Primary apical and basal dendrites arise from the two poles of the soma and, after extending for only short distances, they branched to produce apical and basal dendritic tufts (Fig. 1D). The dendritic branches of these neurons have spinous covering on their surface. The apical dendritic tuft, which extends towards the outer plexiform layer, is larger than the basal dendritic tuft (Fig. 2B). The dendritic extent of apical tuft ranges from 64 µm to 169 µm, while the extension of the basal dendritic tuft ranges from 36 µm to 119 µm. The axon usually arises from the basal pole of the soma or from a basal dendrite. It enters to the inner plexiform layer and run parallel to the ependymal layer. It gives off a number of thin collaterals, some of which curved back towards the cell body. The range of axon extension is 41 µm to 82 µm.

Multipolar neurons:

The multipolar neurons have oval or rectangular cell body present in the cellular layer. The somata measures on average 16 µm × 10 µm. The spinous dendrites are originated from the cell body and radiates in all direction towards the outer and inner plexiform layers (Fig. 1E). The length of dendrites in these neurons ranges from 32 µm to 151 µm. The mean length of dendrites is 67.8 µm. The axon of these neurons arises from the upper half of the cell body and reaches the outer plexiform layer (Fig. 2C). The axon length in these neurons ranges from 96 µm to 164 µm.

Pyramidal neurons :

These neurons have conical to pear shape cell body, on average 16 µm × 11 µm. From the apices of these neurons arise one or two smooth primary apical dendrites, branching in the secondary and tertiary dendrites in the outer plexiform layer. These secondary and tertiary dendrites normally reach the pial surface and bear many spines (Fig. 1F). The length of apical

dendrites ranges from 82 µm to 171 µm. The pyramidal neurons have few primary dendrites emerging from the lower part of the cell body and extending their secondary branches in the inner plexiform layer forming the basal dendritic tuft. The length of basal dendrites ranges from 69 µm to 164 µm. The axon of these neurons arises from the basal pole of the cell body and bifurcates near the soma usually emitting two branches (Fig. 2D). These branches enter the inner plexiform layer where it gives off collaterals, which run parallel to the inner plexiform layer. The axon length ranges from the 69 µm to 124 µm.

Discussion :

Dorsal cortex was present on the dorsolateral surface of the cerebral hemisphere in all snakes and lizards. In the lizard *Hemidactylus flaviviridis* the dorsal cortex was present at the dorsolateral surface of the pallium and consist of three layers viz. from the pia to the ependyma, a superficial plexiform layer, a cellular layer and a deep plexiform layer. It could be divisible into two subdivisions, the dorsal cortex medialis and dorsal cortex lateralis. The medial and lateral edges of the dorsal cortex overlaped with the dorsomedial and lateral cortices giving rise to the "superposito medialis" and "superposito lateralis" respectively. In snakes such as *Eryx* and in turtles (Northcutt, 1970; Platel *et al.*, 1973) little overlapping was observed while crocodilians show no overlapping (Crosby, 1917; Platel *et al*., 1973). Thus the five layered pattern at the edges due to overlapping is a lacertilian character. The dorsal cortex of the lizards *Iguana* (Northcutt, 1967) and *Psammodromus algirus* (Guirado *et al*., 1987) had been divided into two subdivisions medialis and lateralis. The subdivisions in the dorsal cortex of *M. carinata* had not been observed (Srivastava *et al*., 2007) which shows its less developed condition.

Using various characteristics four types of neurons in the cellular layer of *H. flaviviridis* namely monotufted, bitufted, multipolar and pyramidal neurons have been described. In the *Lacerta galloti*, while considering the size and location within the dorsal cortex only single type of neuron had been described in the granular stratum (Garcia-Verdugo *et al.,* 1983). This neuron showed large neuronal somata which received axo-somatic synapses and covered by ependymoglial ramifications. This neuron represents 60% of all somata of the dorsal cortex observed in the *Lacerta galloti* (Garcia-Verdugo *et al.,* 1983) and seven types have been observed in *C. versicolor* (Sakal *et al.,* 2010). The additional criteria such as dendritic tree pattern and spine covering allowed to describe three types of neurons namely bitufted, multipolar and pyramidal neurons in the lizard, *Psammodromus algirus* (Guirado *et al.,* 1987). Five types' monotufted, bitufted, candelabra, pyramidal and bipyramidal neurons have been differentiated in the cellular layer of dorsal cortex in *M. carinata* (Srivastava et al., 2007). Recently De Carvalho Pimentel et al. (2011) described ten neuronal types in the dorsal cortex of the lizard *Tropidurus hispidus*. Most of these neuronal somata were dispersed without forming a conspicuous cell layer. Thus the classification criteria used in considering the types of neurons may play a major role. Due to this reason two or more previously reported types match with the single type in latter studies or vice versa. In addition difference in the morphology of the neurons between the different species can be expected since there is a considerable variation between them.

All the neurons of the dorsal cortex cell layer of *H. flaviviridis* showed homologous large size somata averaging 15 x 10 µm. This observation resembled to that of large neuronal somata (type B) of granular stratum of the dorsal cortex of *Lacerta galloti* (Garcia Verdugo *et al*., 1983). The projection neurons of the cell layer of dorsal cortex in *H. flaviviridis* were found to be uniformly distributed. The monotufted neurons observed in the *H. flaviviridis* had not been reported in any lizard except in *M. carinata* (Srivastava *et al.,* 2007). The bitufted neurons, multipolar neurons and pyramidal neurons observed in the *H. flaviviridis* have clear matching types reported in the *Psammodromus algirus* (Guirado *et al.,* 1987), *M. carinata* (Srivastava *et al.,* 2007) and *C. versicolor* (Sakal *et al*., 2010) on the basis of their similar morphology.

Anatomically, the three neurons of reptilian dorsal cortex namely bitufted neurons, multipolar neurons and pyramidal neurons have formed the basis for comparing the medial aspect of dorsal cortex being the homologous to the mammalian hippocampal formation (Lacey, 1978; Guirado et al., 1998), whereas the lateral aspect of the dorsal cortex had been compared to the mammalian isocortex, or at least to part of it (Ulinski, 1990; Ten Donkeelar, 1998). These three neurons are found to be dominant neuronal types in both reptiles and mammals. The dorsal cortex is a structure unique to reptiles, and its relationship to structures in mammalian brain is of great theoretical interest such as the dorsal cortex of lizards differ from the isocortex in being three layered instead of six layered and in lacking columnar organization (Reiner, 1993).

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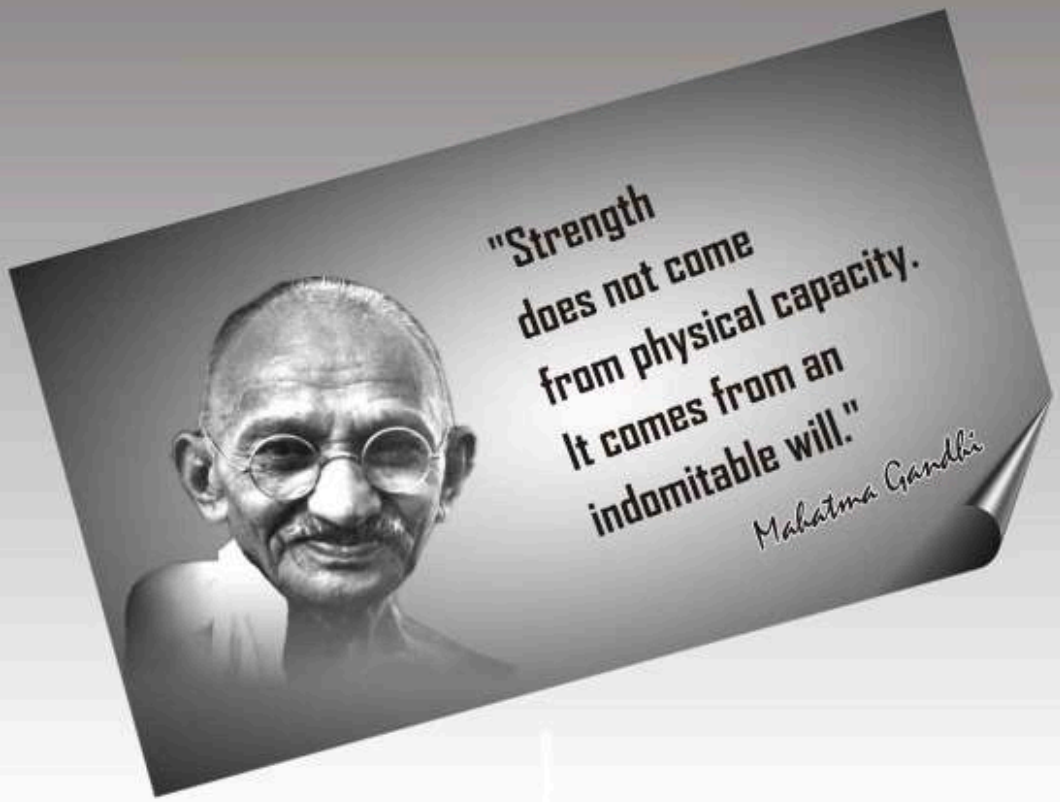

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