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Research Article

Plant Diversity of a Fresh Water Swamp of Doon Valley, India.

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ABSTRACT: The present study was conducted in a highly degraded and fragmented swamp of Doon valley, India. A total of 162 plant species were recorded from the swamp. Dicotyledons contributed 71%, monocotyledons 23.5% and pteridophytes 5.6%. Poaceae with 15 genera and 17 species was the most represented family. Biological spectrum of the present study site shows that therophytes were the most dominating life-form of the swamp, representing high anthropogenic disturbance in the region and limited niche space for the vegetation. [Journal of American Science 2009: 5(1), 1-7] (ISSN: 1545-1003)

Key words: Dicotyledons, swamp

1. INTRODUCTION

Fresh water swamps are the unique ecosystems having very specific vegetation. These are sites of natural succession and therefore contain all the groups of plant kingdom in a single place. Water is the prime requisite of the vegetation of the swamp forests and any alteration in the availability of water affects their presence as well as distribution. Doon valley, situated at the foothills of the Himalaya between rivers the Yamuna and the Ganges, use to have a chain of swamps (Manhas et al., 2007). But due to anthropogenic activities these forests are disappearing at a very fast rate. Nakraunda is one of the most degraded swamps of Doon valley. Most of the area of the swamp has been converted to agriculture fields and residential colonies.

Taxonomic study of swamp forests of Doon valley was first carried out by Kanjilal in 1901, since then a number of studies have been conducted by various workers for floristic diversity (Dakshini, 1960a, 1960b, 1965, 1970 and 1974; Dhyani and Joshi, 2007; Sharma and Joshi, 2008), successional studies (Som Deva and Srivastava, 1978; Srivastava et al., 2000) and community dynamics (Manhas et al., 2007; Kandwal et al., 2007). In the present paper we have studied floristic and life-form diversity of Nakraunda swamp forest of Doon valley.

2. MATERIALS AND METHODS

Study Site

Nakraunda swamp is situated about 15 km east of Dehradun on Dehradun-Doiwala road at 30º 14′ 15″ N latitude and 78º 05′ 55″ E longitude. Most of the swamp is urbanized. A very few patches of swampy vegetation are present here and there along the river Dholani, a tributary of the Song river.

Methodology

Plant specimens were collected, dried, poisoned and mounted on the herbarium sheets. Standard methods given in Jain and Rao (1977) for collection, preservation and maintenance of specimen in herbarium were followed. Herbariums of Forest Research Institute and Botanical Survey of India, Northern Circle were consulted for the identification of each species. Floras written by Babu (1980) and Kanjilal (1901) were used for the nomenclature of the species. These plant species were further classified; first on the basis of habit and then on basis of life-forms as defined by Raunkiaer (1934).

3. RESULTS

A total of 162 plant species were found in the present study site (Table 1). The contribution of dicotyledons was 71.0%, monocotyledons 23.5% and pteridophytes 5.6%. Table 2 reveals that Poaceae (15 genera/ 17 species) was the most dominating family of Nakraunda swamp. The other important families were Asteraceae (11 genera/ 12 species), Acanthaceae (10 genera/ 11 species), Cyperaceae (6 genera/ 9 species) and Scrophulariaceae (4 genera/ 7 species). *Cyperus* and *Polygonum*, both having three species, were the most represented genera. Classification on the basis of habit (Figure 1) shows that herbs were the main vegetation form with 44.4% contribution followed by shrubs (15.4%) and grasses (10.5%).

Table 1
Floristic diversity in the Nakraunda swamp and its comparison with other swamps of Doon valley

Plant Groups	Families	Genera	Species	References
Angiosperms	53	130	155	Dhyani and Joshi (2007)
Angiosperms	71	218	278	Sharma and Joshi (2008)
Total (1 + 2)	61	141	162	Present study
1. Angiosperms (i + ii)	55	135	153	
(i) Dicotyledons	45	103	115	
(ii) Monocotyledons	10	32	38	
2. Pteridophytes	6	6	9	

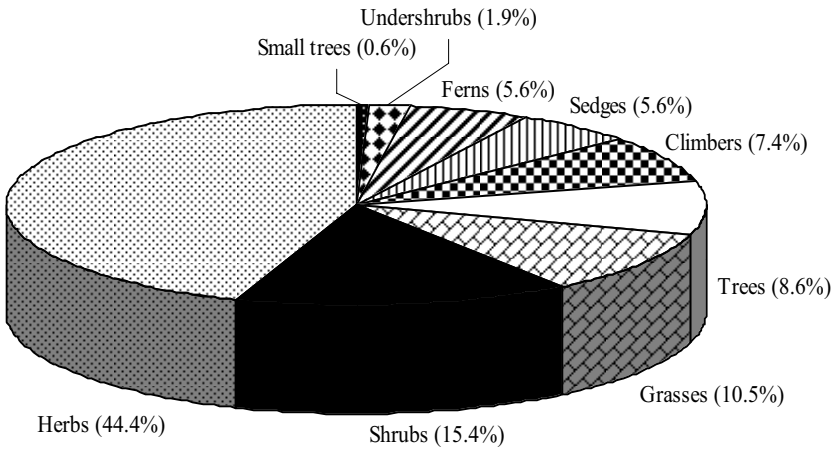

Fig 1: Pie diagram showing percentage contribution of various plant habits.

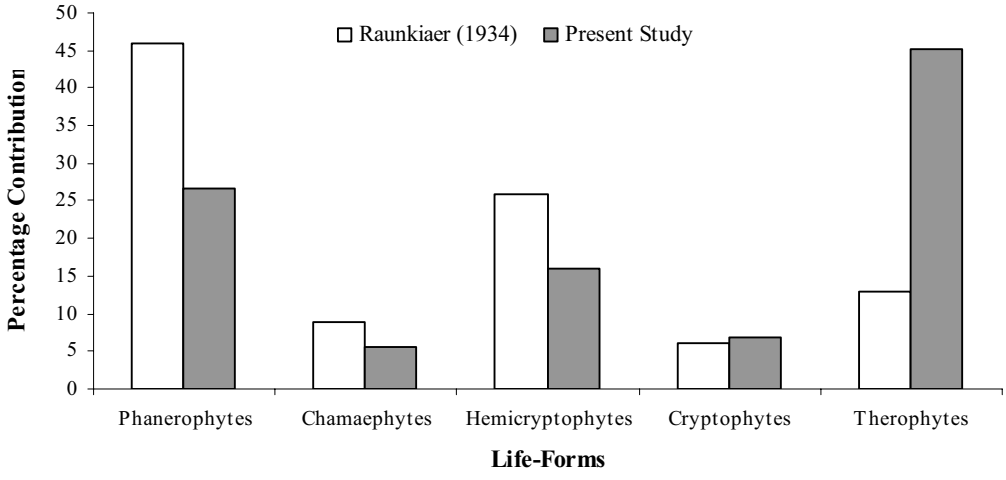

Fig 2: Biological spectrum of life-forms of present study and its comparison with the Raunkiaer's normal biological spectrum representing world flora**.**

Biological spectrum of the swamp was also studied (Figure 2) and compared with the Raunkiaer's normal biological spectrum (Raunkiaer, 1934) representing the world flora. Therophytes (45.1%) were the most characteristic life-form of the present study as compared to phanerophytes in Raunkiaer's normal biological spectrum.

Table 2
List of plant species present in the Nakraunda swamp along with family, habit and life-form. The life-forms mentioned in the table are: Ph = Phanerophytes; Ch = Chamaephytes; He = Hemicryptophytes; Cr = Cryptophytes; and Th = Therophytes (for definitions see Raunkiaer, 1934).

Plant Species	Family	Habit	Life-form
Achyranthes aspera Linn.	Acanthaceae	Herb	Th
Acorus calamus Linn.	Araceae	Herb	Cr
Adenostemma lavenia (Linn.) O. Kuntze.	Asteraceae	Shrub	Ch
Adhatoda vasica Nees	Acanthaceae	Shrub	Ch
Adiantum capillus-veneris Linn.	Adiantaceae	Fern	Cr
Adiantum incisum Forssk.	Adiantaceae	Fern	Cr
Aerva sanguinoleata (Linn.) DC.	Amaranthaceae	Herb	Th
Aerva scandens Wall.	Amaranthaceae	Herb	Th
Ageratum conyzoides Linn.	Asteraceae	Herb	Th
Alternanthera sessilis R. Br.	Amaranthaceae	Herb	Th
Amaranthus spinosus Linn.	Amaranthaceae	Herb	Th
Anagallis arvensis Linn.	Primulaceae	Herb	Th
Anisomelas indica Kuntze.	Lamiaceae	Herb	Th
Apium leptophyllum (Pers.) F. Muell. Ex Benth.	Apiaceae	Herb	Th
Arachne cordifolia (Decne) Hurusawa	Euphorbiaceae	Herb	Th
Argemone mexicana Linn.	Papavaraceae	Herb	Th
Arundo donax Linn.	Poaceae	Grass	He
Asclepias curassavica Linn.	Asclepiadaceae	Shrub	Ph
Asparagus racemosus Willd.	Liliaceae	Herb	Ph
Bacopa monniera (Linn.) Wettst.	Scrophulariaceae	Herb	Th
Bacopa procumbens (Mill.) Greenm.	Scrophulariaceae	Herb	Th
Bauhinia vahlii Wight and Arn.	Caesalpiniaceae	Climber	Ph
Bauhinia variegata Linn.	Caesalpiniaceae	Tree	Ph
Belamcanda chinensis (Linn.) DC.	Iridaceae	Herb	Cr
Bidens tripartite Linn.	Asteraceae	Herb	Th
Bischofia javanica Blume	Euphorbiaceae	Tree	Ph
Boehmeria platyphylla D. Don	Urticaceae	Herb	Th
Boerhavia diffusa Linn.	Nyctaginaceae	Herb	Th
Bombax ceiba Linn.	Bombaceae	Tree	Ph
Butea monosperma (Lamk.) Taub.	Fabaceae	Tree	Ph
Caesalpinia decapetala (Roxb.) Alston	Caesalpiniaceae	Climber	Ph
Calamus tenuis Roxb.	Palmaceae	Shrub	Ph
Capparis zeylanica Linn.	Capparidaceae	Climber	Ph
Capsella bursa-pastoris (Linn.) Medic	Brassicaceae	Herb	Th
Carissa opaca stapf.	Apocynaceae	Shrub	Ph
Cassia tora Linn.	Caesalpiniaceae	Herb	Th
Cheilanthes farinosa Blanford	Sinopteridaceae	Fern	Cr
Chenopodium album Linn.	Chenopodiaceae	Herb	Th
Chenopodium ambrosioides Linn.	Chenopodiaceae	Herb	Th
Chloris dolichostachya Lagasca	Poaceae	Grass	He
Clerodendron viscosum Vent.	Verbenaceae	Undershrub	Ph
Coccinea grandis (Linn.) Voigt.	Cucurbitaceae	Climber	Ph
Coix lachrymal-jobi Linn.	Poaceae	Grass	He

Commelina benghalensis Linn.	Commelinaceae	Herb	Th
Corchorus acutangular Lamk.	Tiliaceae	Herb	Th
Coronopus didymus (Linn.) J.E. Smith	Brassicaceae	Herb	Th
Crotalaria albida Heyne.	Fabaceae	Herb	Th
Curculigo orchioides Gaertn.	Hypoxidaceae	Herb	Cr
Cyathocline purpurea (D.Don) O. Kuntze	Acanthaceae	Herb	Th
Cynodon dactylon (Linn.) P. Beauv.	Poaceae	Grass	He
*Cyperus iria*Linn.	Cyperaceae	Sedge	He
Cyperus niveus Retz.	Cyperaceae	Sedge	He
Cyperus rotundus Linn.	Cyperaceae	Sedge	He
Desmodium trifolium DC.	Fabaceae	Herb	Th
Dicliptera roxburghiana Nees	Acanthaceae	Herb	Th
Digitaria adscendens (HBK) Henr.	Poaceae	Grass	He
Digitaria ischaemum	Poaceae	Grass	He
Dioscorea bulbifera Linn.	Dioscoreaceae	Climber	Ph
Dryopteris cochleata (D.Don) C. Chr.	Aspidaceae	Fern	Cr
Duchesnea indica Focke.	Rosaceae	Herb	Th
Echinochloa colonum (Linn.) Link.	Poaceae	Grass	He
Eclipta prostrata Linn.	Asteraceae	Herb	Th
Emilia sonchifolia DC.	Asteraceae	Herb	Th
Eranthemum nervosum (Vahl) R.Br.	Acanthaceae	Herb	Th
Eriophorum comosum Wall.	Cyperaceae	Sedge	He
Eupatorium adenophorum Spreng.	Asteraceae	Herb	Th
Euphorbia hirta Linn.	Euphorbiaceae	Herb	Th
Ficus palmata Forssk.	Moraceae	Tree	Ph
Ficus riligiosa Linn.	Moraceae	Tree	Ph
Fimbristylis dichotoma (Linn.) Vahl.	Cyperaceae	Sedge	He
Fimbristylis falcata (Vhl) Kunth	Cyperaceae	Sedge	He
Flacourtia indica (Burm. f.) Merr.	Flacourtiaceae	Shrub	Ph
Flemingia bracteata Wight	Fabaceae	Shrub	Ph
Floscopa scandens Lour.	Commelinaceae	Herb	Th
Galinsoga parviflora Cav.	Asteraceae	Herb	Th
Hemigraphis rupestris Heyne ex T. Anders.	Acanthaceae	Herb	Th
Holarrhena antidysentrica Wall.	Apocynaceae	Small tree	Ph
Holoptelia integrifolia (Roxb.) Planch.	Ulmaceae	Tree	Ph
Ichnocarpus frutescens (Linn.) R. Br.	Apocynaceae	Climber	Ph
Imperata cylindrical (Linn.) Beauv.	Poaceae	Grass	He
Ipomoea palmata Forsk.	Convolvulaceae	Climber	Ph
Ipomoea quamoclit Linn.	Convolvulaceae	Climber	Th
Isachne globosa (thumb.) O. Kuntze	Poaceae	Grass	He
Jasminum multiflorum (Burm. f.) Andr.	Oleaceae	Climber	Ph
Juncus bufonius Linn.	Cyperaceae	Sedge	He
Justicia gendarussa Linn.	Acanthaceae	Shrub	Ch
Justicia quinqueqngulqris Koenig ex Roxb.	Acanthaceae	Shrub	Ch
Kyllingia triceps Rottb.	Cyperaceae	Sedge	He
Lantana camara Linn.	Verbenaceae	Shrub	Ph
Lepidagathis cuspidate Nees	Acanthaceae	Shrub	Ph
Lindernia ciliate (Col.) Pennell	Scrophulariaceae	Herb	Th
Lindernia crustata (Linn.) F. Muell.	Scrophulariaceae	Herb	Th
Lygodium flexuosum(Linn.) Sw.	Schizaeaceae	Fern	Cr
Mallotus philippensis Muell. Arg.	Euphorbiaceae	Tree	Ph
Malvaviscus penduliflorus DC.	Malvaceae	Shrub	Ph
Martynia annua Linn	Martyniaceae	Undershrub	Th

Mazus pumilus (Burm. f.) Steenis	Scrophulariaceae	Herb	Th
Mentha piperita Linn.	Lamiaceae	Herb	Th
Mimosa pudica Linn.	Mimosaceae	Shrub	Ch
Monochoria vaginalis Presl.	Pontederiaceae	Herb	Th
Morus alba Linn	Moraceae	Tree	Ph
Murraya koenigii (Linn.) Spreng.	Rutaceae	Shrub	Ph
Narenga porphyrocoma (Hance ex Trim.) Bor	Poaceae	Grass	He
Nepeta hindostana (Roth.) Haines	Lamiaceae	Herb	Th
Ocimum gratissimum Linn.	Lamiaceae	Herb	Th
Oenanthe stolonifera DC.	Apiaceae	Herb	Th
Ophioglossum reticulatum Linn.	Ophioglossaceae	Fern	Th
Ophioglossum vulgatum Linn.	Ophioglossaceae	Fern	Th
Oplismenus compositus (Linn.) P. Beauv.	Poaceae	Grass	He
Panicum miliaceum Linn.	Poaceae	Grass	He
Parthenium hysterophorus Linn.	Asteraceae	Shrub	Th
Paspalum distichum Linn.	Poaceae	Grass	He
Passiflora incarnate Linn.	Passifloraceae	Climber	Ph
Phlogacanthus thyrsiformis (Hardw.) Mabb.	Acanthaceae	Shrub	Ch
Phoenix acaulis Buch.	Palmaceae	Tree	Ph
Phragmites karka Trin	Poaceae	Grass	He
Phyla nodiflora Linn.	Verbenaceae	Herb	Th
Phyllanthus niruri Linn.	Euphorbiaceae	Herb	Th
Pilea scripta (Buch.- Ham. ex D.Don) Wedd.	Urticaceae	Undershrub	Ph
Plantago major Linn.	Plantaginaceae	Herb	Th
Plumbago zeylanica Linn.	Plumbaginaceae	Herb	Th
Pogostemon plectranthoides Desf.	Lamiaceae	Shrub	Ch
Polygonum barbatum Linn.	Polygonaceae	Herb	Th
Polygonum hydropiper Linn.	Polygonaceae	Herb	Th
Polygonum plebejum R. Br.	Polygonaceae	Herb	Th
Pouzolzia pentandra (Roxb.) Benn.	Polygonaceae	Herb	Th
Pteris quadriaurita Retz.	Pteridaceae	Fern	Cr
Pteris villata Linn.	Pteridaceae	Fern	Cr
Pyrus pashia Buch.- Ham. ex D. Don	Rosaceae	Tree	Ph
Ranunculus sceleratus Linn.	Ranunculaceae	Herb	Th
Rorripa nasturtium-aquaticum (Linn.) Hayek.	Brassicaceae	Herb	Th
Rotula aquatica Lour.	Boraginaceae	Herb	Th
Rouvolfia serpentine (Linn.) Benth.- ex Kurz.	Apocynaceae	Shrub	Ch
Rubus niveus Thunb.	Rosaceae	Shrub	Ph
Rumex nepalensis Spreng.	Polygonaceae	Herb	Th
Rungia pectinata (Linn.) Nees	Acanthaceae	Herb	Th
Scirpus juncoides Roxb.	Cyperaceae	Sedge	He
Setaria glauca Baeuv.	Poaceae	Grass	He
Shorea robusta Gaertn. f.	Dipterocarpaceae	Tree	Ph
Sida cordifolia Linn.	Malvaceae	Herb	Th
Smilax zeylanica Linn.	Liliaceae	Climber	Ph
Solanum hispidum Pers.	Solanaceae	Shrub	Ph
Solanum torvum Swartz.	Solanaceae	Shrub	Ph
Sporobolus diander Beauv.	Poaceae	Grass	He
Sporobolus indicus R. Br.	Poaceae	Grass	He
Stellaria media Linn.	Caryophyllaceae	Herb	Th
Syzygium cumini (Linn.) Skeel	Myrtaceae	Tree	Ph
Tectona grandis Linn. f.	Verbenaceae	Tree	Ph
Trifolium repens Linn.	Fabaceae	Herb	Th

Triumfetta rhomboidea Jacq.	Tiliaceae	Herb	Th
Vallaris solanacea O. Kuntze	Apocynaceae	Climber	Ph
Vernonia anthelmintica Willd.	Asteraceae	Herb	Th
Vernonia cineria (Linn.) Lees.	Asteraceae	Herb	Th
Veronica agrestis H.K.f.	Scrophulariaceae	Herb	Th
Veronica anagallis Linn.	Scrophulariaceae	Herb	Th
Vicia sativa Linn.	Fabaceae	Herb	Th
Vitex negundo Linn.	Verbenaceae	Shrub	Ph
Woodfordia fruticosa (Linn.) Kurtz.	Lythraceae	Shrub	Ph
Xanthium strumarium Linn.	Asteraceae	Shrub	Th
Youngia japonica DC.	Asteraceae	Herb	Th
Zeuxine strateumatica (Linn.) Schltr.	Orchidaceae	Herb	Cr
Ziziphus mauritiana Lam.	Rhamnaceae	Shrub	Ch

4. DISCUSSION

The floristic diversity (162 plant species) is very less as compared to Mothronwala swamp (Sharma and Joshi, 2008), and higher than Karwapani swamp (Dhyani and Joshi, 2007). The possible reason for less floristic diversity may be closeness of swamp to human habitations and dependence of human population on these swamp forests for fuelwood, fodder, food, medicinal plants etc. Sharma and Joshi (2008) have also given similar reasons for the dwindling diversity and degradation of Mothronwala swamp of Doon valley. We found that herbs were the most dominant habit followed by shrubs among all the plant forms. Sharma and Joshi (2008) have also reported similar results from Mothronwala swamp of Doon valley. Dominance of herbs and shrubs again signify high rate of anthropogenic disturbances.

High percentage of therophytes in the present study is an indicator of the amount of influence such as grazing (Tiwari, 2005) and anthropogenic activities like catching of fishes and other eatable fauna, collection of vegetables etc. (Manhas et al., 2007), which maintain the vegetation open for further invasion of therophytes. The dominance of therophytes also point towards the harsh environmental conditions of the swamp, which provide very limited niche space to vegetation of these marshy areas.

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HISTOLOGICAL INTERACTIONS OF PAECILOMYCES LILACINUS AND MELOIDOGYNE INCOGNITA ON BITTER GOURD

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ABSTRACT: Momordica charantia roots were histologically examined for the interaction of Meloidogyne incognita and the fungus Paecilomyces lilacinus which was applied at different time intervals. Meloidogyne incognita induced large sized galls on the plants which were treated with P.lilacinus. Fully mature females were found associated with giant cells. All the mature females on the roots of untreated, Meloidogyne incoginta infected plants laid egg masses. The xylem and the phloem exhibited abnormalities in structure near the giant cells.Abnormal vessel elements were occupying larger area near giant cells. The fungus P.lilacinus. Soon after the application, entered the roots and spread through the lumen of the vessel elements. The plants that were treated with fungus either one weak before nematode inoculation or simultaneously, produced significantly (P= 0.01) small sized galls in comparison to untreated plants. The size of galls remained unchanged after completion of one life cycle by the nematode. In fungus treated plants the giant cells were small sized and the abnormality of vascular plants was less. Paecilomyces lilacinus entered the giant cells and also into the body of mature females. It destroyed the eggs and egg masses in and out side females. The fungus by destroying eggs checked the possibility of secondary infection that ultimately arrested increase of gall size.
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Key words: Abnormality, gall, giant cell, histology, vascular tissue.

1. INTRODUCTION

The fungus Paecilomyces lilacinus (Thom) Samson has been reported as a potential biological control agent for root –knot nematode and other plant parasitic nematodes (jatala, et al., 1979, 1982, 1986; Adiko,1984;Cardona and Leguizamon,1997 and Khan and Williams ,1998).P. lilacinus is common soil hypomycete closely related to Penicillium (Samson,1975).The sedentary stages of the root - knot and cyst nematode are most vulnerable to P. lilacinus. The fungus is capable of colonising nematode reproductive structures thus causing destruction of females, cysts and eggs. (Franco, et al,. 1981, Jatala, 1982, 1986; Gintis, et al., 1983 and Cardona and Leguizamon,1997).

Paecilomyces lilacinus increased the yield of tomato and okra and lowered the population of M. incognita juveniles, at the mid and at the beginning of the next season in treated pots than in untreated pots(Neo and Sasser 1984). M.incognita juveniles when exposed to fungus resulted in reduced gall formation and egg mass production.

The association of P.lilacinus with the eggs of M.incognita is well documented but the exact mode of its parasitism is unknown. Root galling and giant cell formation were absent in tomato roots inoculated with fungus infected eggs of M.incognita. P. lilacinus colonised surface of epidermal cells as well as the internal cells of cortex of tomato roots (Cabanillas et al.,1988).The effects of fungus on M.incognita parasitizing the roots of Momordica charantia has not yet been reported.The objectives of this study were : (i) to examine the effect of P.lilacinus on M.incognita infected plant tissues.(ii) to know the effect of P.lilacinus on nematode development, (iii) to determine the effect of P.lilacinus on eggs and egg masses,(iv) to examine the effect of P.lilacinus on the giant cells and (v) to evaluate the efficacy of P.lilacinus in controlling the disease development, on applying the fungus at varying time intervals, before or after nematode inoculation.

2. MATERIALS AND METHODS

Nematode cultures of Meloidogyne incognita was maintained from single egg mass on egg plant (Solanum melongena L.)grown in green house in 15 cm diameter earthen pots containing a mixture of steam sterilized soil and sand (3:1). M.incognita originally was isolated from vegetable crop fields of Aligarh. The root-knot nematode was identified on the basis of characteristic perineal pattern and North Carolina differential host test. Freshly hatched second stage juvenile inoculum was prepared by hatching egg masses picked from egg plant roots, maintained as pure culture in green house. Paecilomyces lilacinus, used in the experiment, was obtained from international potato centre, Lima,

Peru. The fungus was cultured on PDA for 15 days at then inoculated to Richards Medium (Riker and Riker, 1936) for en-masse propagation .The mycelia (100 gm) were blended in distilled water (100 ml) in warring blender to make mycelial suspension for soil application (10 ml of suspension containing 1gm mycelia).The fungus was applied into the rhizosphere zone by making three or four holes around the plant.

Plant Materials : Seeds of Momordica charantia L. variety NSC were surface sterilised with 1% sodium hypochlorite (NaOCl) for five minutes and rinsed three times with sterile distilled water. 100-150 axenised seeds were placed on a moist sterilised filter paper kept in sterilised petri dishes. Seeds were allowed to germinate. The germinated seeds were transferred to 15 cm diameter clay pots filled with autoclaved soil, sand and farmyard manure in the ratio of 7:3:1. Two week old seedlings were inoculated with a suspension of 1000 J2 pipetted into root zone via the holes in the rhizosphere zone around plant in each pot. To achieve the aim and objective the experiment was designed as per the following treatment schedule

1) T_1- un-inoculated control
2) T_2- inoculated with 1,000 J2 only
3) T_3- inoculated with 1,000 J2 and treated with fungus one week before inoculation
4) T_4- inoculated with 1,000 J2 and treated with fungus simultaneously
5) T_5- inoculated with 1,000 J2 and treated with fungus two week after inoculation

Each treatment was replicated five times, arranged in randomised block design. Each set of plants was uprooted carefully after 45days . The roots were cut into small pieces and processed for histopathological studies. Root pieces were fixed in formalin- aceto - alcohol (F.A.A) and then dehydrated through tertiary-butyl-alcohol (T.B.A.) schedule as given by Johansen (1940). Infiltration and embedding of root pieces in paraffin wax was followed and sections of 12µm thickness were taken with the help of rotary microtome in the form of ribbon. The ribbons were cut into small pieces and mounted on clean slides with the help of Haupt's adhesive and 3% formalin and stained with safranin and fast green (Sass1951). The slides were taken out from xylene. The mounting medium was applied on the surface of slide before evaporation of xylene and cover slip was lowered gradually. Finished slides were left at room temperature at least for 24 hours and then kept in an incubator at 40^0C.The slides were examined under light microscope and necessary photographs were taken.

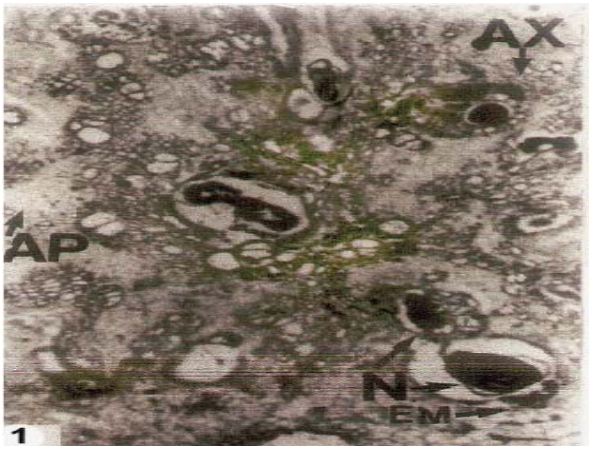

Fig 1: Showing heavy infestation of Meloidogyne incognita (N). The mature females have egg masses (EM). Abnormal xylem (AX) and abnormal phloem (AP) are in abundance.(25X)

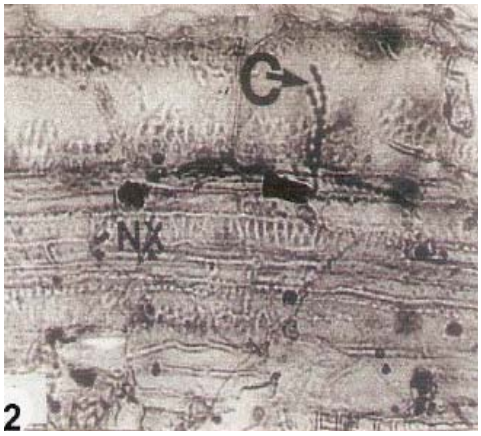

Fig 2: Showing normal xylem (NX) strands with conidiophores and conidial chain C in the lumen of vessel elements.

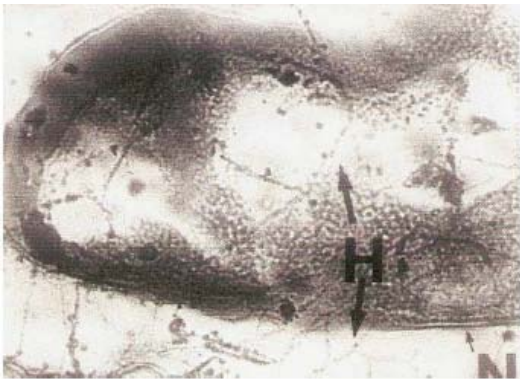

Fig 3: Showing abundant growth of Peacilomyces lilacinus hyphae (H) in and around the females of M. incognita (N).(32 X).

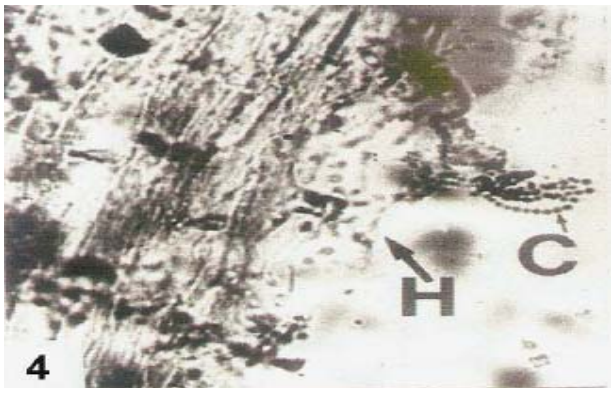

Fig 4: Showing hyphae (H) and conidial chains (C) at the root surface. (12.5 X)

3. RESULTS

Histologically the primary root of Momordica charantia consists of uniseriate epidermis, multilayered parenchymatous cortex and stele. The stele in primary root may be diarch, triarch but generally tetrarch, displaying a typical dicotyledonous pattern. Xylem and phloem are radially arranged alternating with one another. There is small pith consisting of parenchyma cells at the centre of four xylem arches. Meloidogyne incognita inoculated M.charantia plants receiving no disease controlling treatment produced large sized galls. The mature females feeding on giant cells, abnormal xylem and abnormal phloem was observed (Fig 1).All the mature females were found associated with egg masses. Histological examination of the galled roots which were given Paecilomyces lilacinus treatment, one week before the nematode inoculation, revealed that the fungus entered the root tissue and grew successfully. The hyphae and conidiophores, bearing chains of conidia were seen in normal vessel elements of the xylem (Fig 2).The giant cells, though smaller, resembled with those of T2 plants .In the vicinity of the giant cells abnormal xylem and phloem were present in Paecilomyces lilacinus treated plants. The abnormality in vascular elements was less. The fungal hyphae destroyed eggs and egg masses and also entered into the body of the females. Around the nematode body fungal growth was abundant (Fig 3).The root surface also exhibited profused growth of fungus (Fig 4). The simultaneous application of root knot nematode and Paecilomyces lilacinus not only destroyed eggs and egg masses, but also entered into the internal tissues of root, either intercellularly or intracellularly as is evident from the transverse sections of vessel elements (Fig 5).The egg masses were destroyed by the fungus and the growth of fungus was profound inside egg masses. There was no change in the size of giant cells and amount of vascular elements as compared to untreated plants. In plants treated with Paecilomyces lilacinus, one week after nematode inoculation, the fungal hyphae was observed inside the giant cells (Fig 6).The fungal growth was profuse around the body of developing nematodes. In the normal tissue the fungus spreads both inter and intracellularly as is evident from figure (Fig.7)

Fig 5: Showing vessel elements (VE) in transverse sections enclosing conidial chains (C).(40 X)

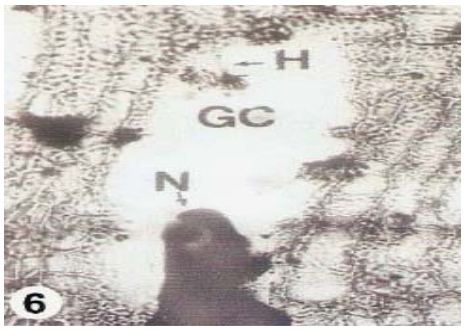

Fig 6: Showing hyphae (H) in giant cell (GC) and nematode (N) adjacent to giant cell.(25 X)

Fig 7: Showing normal xylem (NX) strands with hyphae(H) of Paecilomyces lilacinus traversing through the lumen of vessel elements. (32 X)

DISCUSSION

The fungus Paecilomyces lilacinus shows diverse modes of habits. Basically it is a saprophyte (Domsch et al., 1980) and can easily be grown on artificial culture media. It behaves as an epiphyte and grows on the surface of plant roots (Cabanillas et.al.,1988). It also grows inside the root tissue and behaves as an endophyte and does not cause any damage to the plant. Still at other times it parasitizes eggs and egg masses of Meloidogyne species and destroy them. Because of the lastly stated behaviour, P.lilacinus has been used by several workers, as biocontrol agent against root-knot nematode and other nematodes (Jatala, et al., 1979).

Paecilomyces lilacinus was encountered frequently in and around normal and abnormal xylem. In our opinion, vessels and vessel elements provide sufficient space for its development and also provide an uninterupted passage to grow inside the plant tissues. We consider that P.lilacinus develops inside the root tissues inter and intracellularly. Whether the fungus is beneficial or not to plant but, it is not harmful. In all the sections studied, the fungus was not found damaging the plant tissues even when it was in abundance. Further it did not affect the giant cells in which its occurrence was noted. In all the treatments it was regularly observed that P.lilacinus damage the eggs and egg masses. Various workers have reported egg destroying activity of this fungus (Jatala, et al,. 1979, Jatala, 1985,1986.) It has also been reported that the fungus can destroy neither the juveniles nor the adult females (Jatala ,1986). The eggs however seem to be the most preferred target for obtaining the nourishment by the fungus. Contrary to this (Cardona and Leguizamon,1997) reported 94% infection in Meloidogyne spp. by P. lilacinus strain-9207. KHAN and WILLIAMS (1998) found P.lilacinus entering into the body of the females through natural opening. They did not mention whether the fungus damaged the females or not, although it damaged the eggs inside the egg masses. Small sized giant cells and small amount of abnormal xylem and phloem indicated that the nematode activity and development was influenced by the presence of P.lilacinus. Large giant cells and more quantity of abnormal tissues showed that the nematodes which entered earlier were not affected by the fungus. On the basis of these observations we concluded that the fungus can not check primary infection of nematode when the plants are attacked by the juveniles. However, it can check secondary infection because it destroys eggs as and when these are deposited. As far as time of application of P.lilacinus is concerned, from our studies it can be suggested that incorporation of fungus P.lilacinus one week before and at the time of nematode inoculation is more effective in controlling the root-knot disease, as compared to later intervals of fungus applications.

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Impacts of Sulfidic Materials on the Selected Major Nutrient Uptake by Rice Plants Grown in Sulfur Deficient Soils under Pot Experiment

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ABSTRACT: The impacts of sulfidic materials (SM) and Gypsum (G) application at the rates of 0, 40, 80, 120 and 160 kg S ha^{-1} on the selected major nutrient uptake by rice (*Oryza sativa* L.; var. BR 16: Shahi balam) cultivated in two sulfur deficient soils of Sirajgonj (Kamarkhond series) and Gazipur (Kalma series) were evaluated under pot experiment. The contents of N, P, K, Mg and S in rice shoot at different growth stages of rice were increased by the application of SM and G. But the increments were more striking in case of SM compared to G application. In addition, the applied SM increased the average organic matter and available sulfur contents in the soils by 20 to 43 % and 141 to 229 % increased over control (IOC), respectively, while these increments were 6 to 22 % and 88 to 187 % for gypsum treatments, indicating that the SM has potential and proved to be effective compared with gypsum not only as a source of fertilizer but also to enrich the fertility and productivity status of soils. Moreover, the SM treatment was found to be maintained the high nutrient status in both the soils till the final harvest at maturity of rice, reflecting a good indication for its long term use. [The Journal of American Science. 2009;5(2):9-15]. (ISSN: 1545-1003).

Key words: sulfur deficient soils, sulfidic materials, nutrient uptake of rice, pot.

1. INTRODUCTION

Sulfur is one of nature's super nutrients and one of the oldest elements known to man. It is the thirteenth most abundant element in the earth's crust. Although S is considered a secondary nutrient, it is often called the forth major nutrient ranking just below nitrogen, phosphorous and potassium (http://www.oznetksu.edu/library/CRPSL2/mf 2264.pdf. 21**)**. Sulfur is usually present in relatively small amounts in soils and a majority is in organic forms. Sulfur deficient soils are often low in organic matter, course-textured, well-drained, and subject to leaching. Recently, it has been reported that a large number of finer textured soils have shown sulfur deficiency. Historically, S was found as impurity in mineral fertilizers. However, current chemical fertilizers contain fewer if any impurities. There has also been a decline in the amount of S supplied through atmospheric deposition due to industrial reductions in SO_2 emissions. According to Environment Canada (2003), SO_2 emissions in Atlantic Canada have been reduced by >50 % for the period 1980 to 2000 (from~3.8 million to~1.6 million Mg yr^{-1}). Due to the reduction of anthropogenic SO_2 emission, the use of high purity fertilizers, and continuous cropping with high-yielding varieties, S deficiencies have been reported in Canada and Europe (MacGrath, et al., 1996; Riley, et al., 2002).

Plant nutrition is only one of more than fifty factors which directly affect both crop yield and quality. The availability of required nutrients, together with the degree of interaction between these nutrients and the soil, play a vital role in crop development. A deficiency in any one required nutrient or, a soil condition that limits or prevents a metabolic function from occurring can limit plant growth.
(http://www.ecochem.com/t_soil_nutrients.html-21**)**.

Deficiencies of S have become common in Bangladesh and worldwide. About 7 M ha (about 52 %) of agricultural lands are reported to consists of sulfur deficient soils in the northern region of Bangladesh (SRDI, 1999). The current intensive use of agricultural land for crop production has extended the sulfur deficient areas to about 80 % in the Northern region of Bangladesh (Khan, et al., 2007). Poor crop production as a result of acute sulfur deficiency has frequently been reported by many scientists in different regions of India (Tiwari, et al., 1985) and Bangladesh (Khan, 2000).

The use of sulfidic materials (SM) or layers obtaining from acid sulfate soils (ASSs) as sulfur fertilizer for crop production is very scanty. Khan, et al., (2002) reported that the high organic matter (2-9 %), total sulfur (3-7 %) and micronutrients in ASSs or SM deserve attention to use these soil materials for the reclamation of alkaline, calcareous or sulfur deficient soils and also for the amendment for ASSs themselves by the removal of SM from the soil. Khan, et al., (1994) also reported that the ASSs contained high Mg (1.3 to 2.6 c mol kg^{-1}) and Al (1 to 2 c mol kg^{-1}). But the use of high Al contained ASSs or SM did not notice any harmful effects when applied in the soils having pH > 4.5 (Khan, et al., 2002). The present studied SM in an ASS layer, which occupies

0.7 M ha land area, had low pH (< 3), high sulfate and organic matter (Khan, et al., 2006)

The removal of SM from the ASSs is not only reclaimed the ASSs for a long time but its use in sulfur deficient or non-fertile soils at the rate of about 300 to 1500 kg ha^{-1} may improve the fertility and productivity of the soils. Khan, et al. (2007) reported that the application of SM at the rate of 75 kg S ha^{-1} for sulfur deficient soils had no negative effect on soil pH, nutrient status in the soils and Sunflower production. They suggested that the application of SM was not only effective as sulfur fertilizer but also enriched the organic matter in the soils. Moreover, many studies have been conducted on the mineralization of elements such as N, P, and K from animal manures in various climates and soil conditions (Ebeling, et al., 2003; Egrinya-Eneji, et al., 2003; Eghball, et al., 2002; Schmitt, et al., 2001). However, there are relatively few that focus on nutrients such as Ca and S (Egrinya-Eneji, et al., 2003). Against this background, the present study was considered to evaluate the impacts of SM or ASSs compared with gypsum as sulfur fertilizer in relation to rice production in sulfur deficient soils under pot condition.

2. MATERIALS AND METHODS

2.1 Soil collection and analyses

A large amount of two sulfur deficient soils (surface soil at depth of 0-20 cm) of Kamarkhond series (Sirajgonj soil) and Kalma series (Gazipur soil) were collected from the districts of Sirajgonj and Gazipur, respectively in Bangladesh. The SM (Cheringa acid sulfate soil) used for this study was obtained from the surface soil (depth of 0-20 cm) at Dulahazara in the Cox' Bazar district in Bangladesh. Soils were collected from each replicated pots using Cork borer (2 cm diameter), then air-dried and screened by 1 mm sieve. The soils were oven dried at 105°C before analysis. The particle size distribution of the initial soils was determined by the pipette method (Day, 1965) with 1 M CH_3COONH_4 (pH 5.0) and with 30 % H_2O_2 to remove free salts and organic matter. Soil pH was measured by the soil-water ratio 1:2.5 and for the oven dried soil 0.02M $CaCl_2$ (1:2.5) suspension (Jackson 1973) using a Corning pH meter Model-7. For saturation extract of soils, the electrical conductivity (soil solution has extracted from saturated soil paste through vacuum pump: Richards, 1954), water soluble Na and K (Gallenkamp flame photometry using 589 and 766 nm filters, respectively: Black 1965), Ca^{2+} and Mg^{2+} (Pye UniCam-SP 9 atomic absorption spectrometry: Hesse, 1971) were determined. Organic matter content was determined (Nelson and Somners, 1982) by wet combustion with $K_2Cr_2O_7$. Available N (1.3M KCl extraction, Jackson, 1973), available P (0.002 N H_2SO_4, pH 3 extraction, Olsen, et al., 1954) and available S ($BaCl_2$ turbidity, Sakai, 1978) were determined. Cation exchange capacity was determined by saturation with 1 M CH_3COONH_4 (pH 7.0), ethanol washing, NH_4^+ displacement with acidified 10 % NaCl, and subsequent analyses by steam (Kjeldhal method) distillation (Chapman, 1965). Exchangeable Na^+, K^+, Ca^{2+} and Mg^{2+} were extracted with 1 M CH_3COONH_4 (pH 7.0) and determined by flame photometry (Na^+, K^+) and atomic absorption spectrometry(Ca^{2+}, Mg^{2+}). Total sulfur was obtained by digestion with a mixture of concentrated HCl/HNO_3 (1:3) and determined by turbidity method (Sakai, 1978).

2.3 Pot experiment

A pot experiment was carried out at the premises of the Department of Soil, Water and Environment, University of Dhaka during the period for January to May, 2001 to evaluate the impacts of SM compared with G as a source of sulfur fertilizer in relation to rice production grown in two sulfur deficient soils. Two sets of experiments were set up in a completely randomized design having three replications and three sampling time for each treatment. The experimental treatments on the basis of furrow slice of the soils were: Control, 0 (no application of SM and G); SM_{40}, SM_{80}, SM_{120}, SM_{160} (SM 40, 80, 120, 160 kg S ha^{-1}) and G_{40}, G_{80}, G_{120}, G_{160} (G 40, 80, 120, 160 kg S ha^{-1}).

Six kg of air-dried and screened (5 mm sieve) soil was placed in each earthen pot (size: 36 cm height/28 cm diameter). The soil in each pot was fertilized with N, P and K at the rates of 80, 40 and 60 kg ha^{-1} as urea, triple super phosphate (TSP) and murate of potash (MP), respectively. The full dose of TSP and MP and half of urea were mixed with the soil during pot preparation. The remaining urea was applied in equal splits, one at the active tillering stage of rice and the other at the panicle initiation stage. As per treatments, the soils in the pot were also subjected to the application of SM and G at the rates of 0, 40, 80, 120 and 160 kg S ha^{-1} during pot preparation. Both the SM and G were dried, milled and sieved (1 mm sieve). Thirty days old healthy and uniform seedlings (*Oryza sativa* L., var. BR 16 Shahi balam) were transplanted at the rate of three plants per hill and four hills per pot. The soils in the pots were irrigated by tap water (pH 6.5, EC 0.5 dS m^{-1} and S 0.01 c mol kg^{-1}) whenever necessary to maintain the soil under moist to wet conditions required for the production of rice. Seedlings were collected by the courtesy of Bangladesh Rich Research Institute (BRRI), Gazipur, Bangladesh.

2.4 Plant collection and Analysis

The nutrients content at different stages of growth of rice shoot were determined at 30 (20-35 early tillering stage = ETS), 60 (36-65 maximum tillering stage = MTS) and 110 (harvesting at maturity) days after transplanting (DT). The N contents were analyzed by the H_2SO_4 digestion through the micro-Kjeldhal method (Jackson, 1973) and P contents by spectrometry (Jackson, 1973); K content by Gallenkamp flame photometry (Black, 1965); S contents by turbidometry (Jackson, 1973) and Mg contents by atomic absorption spectrometry (Hesse, 1971) in HNO_3-$HClO_4$ acid (2:1) digest. The level of significance of the different treatments was determined at different stages of growth using Duncan's New Multiple Range Test (DMRT) and least significance different (LSD) techniques (Zaman, et al., 1982).

3. RESULTS AND DISCUSSIONS

3.1 Sulfidic Materials (SM)

The SM was collected from the surface (depth: 0-20 cm) of an acid sulfate soil (Typic Sulfic Halaquept, detailed: Khan, et al., 2006) showed a silty clay loam texture with pH values of 3.3 (0.02 M $CaCl_2$) and 3.8 (field), indicating that the SM had probably accumulated a large amount of pyrite which had produced H_2SO_4 in the laboratory by oxidation. The EC, available and total sulfur and organic matter content in the SM were very high (Table 1). The content of Ca in SM was low compared with the Mg content, which might be due to occasional flooding with sea water rich in Mg. The Na content was also high due to the flooding with high saline water. The SM was in fact a fertile but unproductive soil due to its high acidity, salinity and imbalance of nutrients.

3.2 Conditions of initial and post harvested soils

The Sirajgonj and Gazipur soil had silty loam and silty clay loam textures, initial pH values of 5.8 to 6.2 and 5.2 to 5.8, respectively as determined by the different conditions. These sulfur deficient soils were subjected to the application of SM and G in relation to rice production. The pH values at different conditions of the average soil data of all the treatments at post harvesting were found to be decreased by 0.1 to 0.3 pH units compared with the initial Sirajgonj and Gazipur soil, indicating that the application of acidic SM on these soils had negligible influences on the pH of the soils. On the other hand, the SM strikingly increased the initial low content of organic matter, N, P, K, Ca, Mg, available and total sulfur in both the soils up to 200 % compared with the initial soils (Table 1), which was due to the high nutrient status of the applied SM though there might be a little contribution from the plant roots. The base saturation of the initial Sirajgonj soil was 74 % which was increased to 80 % at the final harvesting of rice, while this increment was 66 to 72 % for Gazipur soil (Table 1). These increases of base saturation were attributed to the high content of basic cations in the applied SM. The EC values of the soils were found to be increased from 1.1 to 1.8 dS m^{-1} for Sirajgonj soil and 1.3 to 2.2 for Gazipur soil, which are attributed to the higher EC values of the SM used. However, these increased levels of EC values might not have remarkable influence on the production of rice.

3.3 Sulfur and organic matters in the soils

By the application of SM and G, the available S contents of the soils were found to be increased but the effects were more pronounced in case of SM and the increments were significantly ($p \leq 0.05$) stronger with the passes of time. Apart from fertilizer rates, the applied SM and G increased the available S contents by 228 and 187 % IOC for Sirajgonj soil; 140 and 88 % for Gazipur soil, respectively at post harvesting of rice at maturity. The SM exerted better response for the increment of sulfur in both the soils (Table 2). This might be due to the contents of other essential nutrients especially N in SM (Table 1), which enhanced sulfur uptake by the rice compared with the G treated pots. On the other hand, S content was found to be increased by the treatments but decreased by the passes of time was attributed to the uptake of rice plant (Table 2).The content of organic matter in both the soils throughout the experimental period was found to be improved a little by the different rates of gypsum fertilization, whereas almost all the doses of SM significantly increased the organic matter status in the both the soils and the increments were more striking with the higher doses of SM (Table 2). The application of SM increased the average organic matter in the soil by 20 to 43 % IOC at post harvesting of rice at maturity, while these increments were 6 to 22 % for G treatments and the increments were more pronounced in Gazipur soil. These increments in organic matter status in the soils were attributed to the high content of organic matter in the applied SM and the little enrichment of organic matter by the G treatments were attributed to the contribution of cultivation processes.

3.4 Nutrition of rice

The contents of N, P, K, Mg and S in rice shoot at different growth stages of rice were increased by the SM and G application. The increments were more striking in case of SM compared to G application (Table 3). The lowest contents of these nutrients were observed for the control treatments in both the soils. The average S contents in plant tissue of all the SM treatments at the final harvesting (110 DT) of rice were increased by 142 % in the Sirajgonj soil and 108 % in the Gazipur soil compared with the control treatments. But these increments of S by the average of all G treatments were 96 % and 45 % for the rice plants grown in Sirajgonj and Gazipur soils, respectively. These findings suggest that the impacts of SM as S-fertilizer were much higher than G and would also be effective for the subsequent crops as indicated by the high contents of nutrient in rice plants at final harvesting (110 DT) stages. The use of SM from ASSs not only recover S deficiency of rice plants but also enhanced the growth of rice and improved the fertility status of the studied soils compared to gypsum. Moreover, the removal of SM from ASSs may lead the reclamation of acute problem of the ASSs. Khan, et al., (2002, 2007) reported that the nutrient uptake by tomato, onion and sunflower were strikingly increased by the application of SM compared to G and $MgSO_4$.

Table1.
Some selected properties of initial soils (depth 0-20 cm, oven dry basis), sulfidic materials and the average soils of all the treatments at post harvesting of rice used during pot experiment

Soil properties	Sirajgonj soil			Gazipur soil			Sulfidic Materials
	Before use	After use	% †IOC	Before use	After use	% IOC	(‡ASSs)
Textural class		Silty loam			Silty clay loam		Silty clay loam
Soil pH (Field)	6.20	6.10	-	5.80	5.60	-	3.80
Soil pH (Soil:Water=1: 2.	6.10	5.90	-	5.50	5.20	-	3.60
Soil pH ($CaCl_2$=1.2.5)	5.80	5.60	-	5.20	4.90	-	3.30
E C (1: 5 dS m^{-1})	1.10	1.80	63.64	1.30	2.20	69.23	18.50
Organic matter (g kg^{-1})	12.20	16.10	31.97	7.10	9.20	29.58	39.10
Extractable N (mM kg^{-1})	0.23	0.30	30.43	0.20	0.25	25.00	3.60
Available P (mM kg^{-1})	0.10	0.12	20.00	0.12	0.14	16.67	0.10
CEC (c mol kg^{-1})	16.85	17.30	2.67	17.10	17.80	4.09	17.20
Base saturation (%)	74.40	80.20	7.80	66.50	72.10	8.42	24.30
Exchangeable cations (c mol kg^{-1})							
Sodium	0.41	0.75	82.93	0.37	0.65	75.68	2.13
Potassium	0.08	0.15	87.50	0.07	0.14	100.00	0.24
Calcium	6.48	6.63	2.31	6.45	6.62	2.64	0.31
Magnesium	3.98	4.52	13.57	3.61	3.99	10.53	0.95
Water soluble ions (c mol kg^{-1})							
Sodium	0.14	0.19	35.71	0.12	0.21	75.00	3.01
Potassium	0.28	0.40	42.86	0.24	0.32	33.33	0.30
Calcium	6.43	6.66	3.58	3.80	3.94	3.68	0.30
Magnesium	2.88	4.22	46.53	2.64	3.60	36.36	3.34
Available sulfur	0.03	0.09	200.00	0.04	0.10	150.00	24.40
Total sulfur	1.40	1.96	40.00	1.56	2.87	83.97	165.60

†IOC = Increased over control, ‡ASS = Acid sulfate soil

Table 2.
Sulfur and organic matter contents of the soils at different growth stages of rice as influenced by the application of sulfidic material (SM: kg S ha^{-1}) and gypsum (G: kg S ha^{-1}) in the sulfur deficient soils.

Treatment	Available sulfur (m M kg^{-1})			Total sulfur (m M kg^{-1})			Organic matter (g kg^{-1})		
denotation	30 DT†	60 DT	110 DT	30 DT	60 DT	110 DT	30 DT	60 DT	110 DT
Sirajgonj soil: Silty loam, pH 6.1, Organic matter=12.2 g kg^{-1}, Total S=14.0 and available-S=0.30 m M kg^{-1}									
Control	0.29e	0.29d	0.27e	13f	12.6d	11.2e	15.7c	14.3c	13.8c
SM_{40}	0.38d	0.46c	0.72c	16.1e	14.2d	12.4e	16.4b	15.7b	14.3b
SM_{80}	0.45c	0.57b	0.85b	28.3c	24.5c	25.6c	17.9b	17.1b	16.6a
SM_{120}	0.51c	0.79a	0.96a	38.4b	33.1b	31.9b	19a	18.1a	17.3a
SM_{160}	0.67a	0.82a	1.02a	43.8a	41.2a	40a	20.5a	19.2a	17.8a
G_{40}	0.34e	0.43c	0.55d	14.7e	13.8d	12.6e	15.8c	14.2c	13.9c
G_{80}	0.42d	0.59b	0.76b	21.2d	20.3c	19.4d	16.4b	14.5c	14.3b
G_{120}	0.48c	0.74a	0.88b	35.1b	34.3b	31.2b	16.6b	15.4b	14.8b
G_{160}	0.60a	0.78a	0.91a	39.7a	37.6a	36a	17.2b	15.6b	15.7b
LSD (5%)	0.06	0.08	0.10	4.10	4.00	3.80	2.00	1.90	1.70
SM-IOC (%)	73.28	127.59	228.70	143.46	124.21	145.31	17.52	22.55	19.57
G-IOC (%)	58.62	118.97	187.04	112.88	110.32	121.43	5.10	4.37	6.34
Gazipur soil: Silty clay loam, pH 5.5, Organic matter=7.1 g kg^{-1}, Total S=15.6 and available-S=0.40 m M kg^{-1}									
Control	0.42d	0.41e	0.41e	16.1c	16e	15.4e	7.3c	6.9c	6.3d
SM_{40}	0.51c	0.69c	0.82c	27.3d	24.5d	22.8d	7.8b	7.5b	7.5c
SM_{80}	0.58b	0.73c	0.87b	35.2c	33.6c	30c	8.4b	8.2a	9.6a
SM_{120}	0.66b	0.82b	0.96b	44.7b	41.8b	37.1b	8.7a	8.2a	9.1a
SM_{160}	0.77a	0.97a	1.3a	49.4a	46.3a	42.5a	9.5a	9.1a	9.8a
G_{40}	0.48c	0.56d	0.63d	19.10	17.30	15e	7.4c	7.1c	6.8c
G_{80}	0.51c	0.59d	0.67d	25.5d	23.10	22.7d	7.5b	7.4b	7.5c
G_{120}	0.62b	0.71c	0.82c	28.6d	26.1d	24.2d	7.8b	7.6b	8b
G_{160}	0.73a	0.85b	0.97b	36.4c	34.3c	31.2c	8.1b	8.1b	8.5b
LSD (5%)	0.07	0.09	0.12	4.20	4.10	4.00	0.90	0.90	0.90
SM-IOC (%)	50.00	95.73	140.85	143.17	128.44	114.94	17.81	19.57	42.86
G-IOC (%)	39.29	65.24	88.41	70.19	57.50	51.14	5.48	9.42	22.22

†DT = days after transplanting, ‡In a column, means followed by a common letter are not significantly different at 5 % level. IOC = Increased over control.

Table 3.
Effects of sulfidic materials (SM) and Gypsum (G) on the nutrients contents (g kg^{-1}) at different stages of growth of rice shoot on two sulfur deficient soils

Treatment denotation	Nitrogen		Phosphorus		Potassium		Magnesium		Sulfur	
	60 DT*	110 DT	60 DT	110 DT	60 DT	110 DT	60 DT	110 DT	60 DT	110 DT
	(†MTS)	(‡Maturity)	(MTS)	(Maturity)	(MTS)	(Maturity)	(MTS)	(Maturity)	(MTS)	(Maturity)
Sirajgonj soil:										
Control	23.00	10.1c	1.50	1.2e	29.50	15.2b	6.20	3.2c	2.10	1.3f
SM_{60}	24.30	10.7b	1.80	1.4d	30.20	17a	6.50	3.6b	2.60	1.7e
SM_{80}	24.90	11.6b	2.50	2.3b	32.10	17.4a	7.20	3.7b	3.80	3.1c
SM_{120}	25.40	12.8a	2.90	2.5b	32.60	17.5a	7.80	3.9b	4.10	3.6b
SM_{160}	26.30	13.5a	3.20	2.8a	33.00	18.4a	9.00	4.8a	4.80	4.2a
G_{60}	23.40	10.6b	1.60	1.3d	29.70	16.3b	6.40	3.1c	2.50	2.1e
G_{80}	23.90	11.2b	1.80	1.5d	30.10	16.5b	6.90	3.5b	2.80	2.5d
G_{120}	24.50	11.8b	2.60	2.1c	30.50	17.1a	7.60	3.6b	3.30	2.8c
G_{160}	25.20	12.4a	2.90	2.5b	31.20	17.5a	8.20	4.6a	3.60	3c
LSD (5%)		1.30		0.26		1.80		0.50		0.40
SM-IOC (%	9.67	20.30	73.33	87.50	8.39	15.63	22.98	25.00	82.14	142.31
G-IOC (%)	5.43	13.86	48.33	54.17	2.97	10.86	17.34	15.63	45.24	96.15
Gazipur soil:										
Control	22.10	8.2e	1.40	1.1d	25.50	17.5b	6.10	3.2c	2.20	1.6d
SM_{60}	23.20	9.5c	1.80	1.5c	28.30	18.4a	6.50	3.3b	2.80	2.3c
SM_{80}	23.80	11b	2.30	1.9b	28.70	18.5a	7.00	3.7b	3.50	3.1b
SM_{120}	24.30	12.2a	2.60	2.2a	29.60	19.6a	7.60	3.7b	4.10	3.7a
SM_{160}	25.50	12.6a	2.70	2.3a	30.90	20a	8.70	4.2a	4.60	4a
G_{60}	22.50	9.1d	1.50	1.1d	27.80	17.8b	6.30	3.4b	2.40	2d
G_{80}	22.80	9.7c	1.80	1.6c	28.60	17.40	6.60	3.7b	2.50	2.3c
G_{120}	23.40	10.5c	2.00	1.7b	28.90	18.5a	7.10	3.8a	2.90	2.4c
G_{160}	24.10	11.4a	2.20	1.8b	29.20	18.9a	7.80	3.9a	3.40	2.8b
LSD (5%)		1.20		0.22		1.90		0.40		0.38
SM-IOC (%	9.50	38.11	67.86	79.55	15.20	9.29	22.13	16.41	70.45	104.69
G-IOC (%)	4.98	24.09	33.93	40.91	12.25	3.71	13.93	15.63	27.27	45.31

*DT = days after transplantation of rice, †MTS = maximum tillering stage of rice, ‡Maturity = maturity stages of rice, In a column, means followed by a common letter are not significantly different at 5% level.
IOC = Increased over control.

4. CONCLUSION

The contents of N, P, K, Mg and S in rice shoot were increased by the application of sulfidic materials (SM) and gypsum (G). But the increments were more striking in case of SM compared to than that of G. The use of SM and G increased the available S by 228 and 187 % IOC for Sirajgonj soil; 140 and 88 % for Gazipur soil, respectively at post harvesting of rice at maturity, suggesting that the SM compared with G as a source of S-fertilizer was potential and effective for the recovery of S deficiency as well as fertility status of the soils. But further field research is essential to find out the optimum doses of SM for different soils under variable conditions. The high organic matter (39.1 g kg^{-1}), available- S (24.4 c mol kg^{-1}) and total S (165.6 c mol kg^{-1}) and other nutrients specially micro-nutrient of the SM deserve attention to use these soil materials for the reclamation of poor soils like saline, alkaline, calcareous and s deficient soils, etc.

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Research Article

Influence of Subchronic Exposure of Profenofos on Biochemical Markers and Microelements in Testicular Tissue of Rats

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ABSTRACT: To investigate the effect following subchronic exposure to the organophosphorous insecticide of common name profenofos, which extensively used in agriculture, on the key enzymes of fertility and the concentration of microelements in testicular tissues in male albino rats. Methods: Adult male albino rats were orally administered with profenofos at a does of 23.14 mg/kg body weight per day for 60 days, emulsifying in 0.4 ml tap water. The control group received equal volume of tap water. Twenty-four hours after the last treatment the rats were sacrificed using anesthetic ether. Epididymus and testes were collected, cleaned and weight. Then epididymus prepared in buffer saline and spermatozoa were examined with light microscopy for concentration and motility. Testes were fractionated and supernatant of testicular homogenate was obtained by centrifugation, activities of alkaline and acid phosphatases, lactate dehydrogenase and total protein as well as concentration of microelements; Copper, Iron, Zinc and Selenium were measured. Moreover, the testes were histologically examined. Results: The epididymus and testes weights were significantly decreased. Reduction in sperm count was recorded in cauda epididymus in profenofos treated group, associated with decreased motility. Total protein (TP) level exhibited an elevation in testicular tissue in comparison with the control group. There was significant decrease in the activities of alkaline and acid phosphatase (ALP and ACP) and lactate dehydrogenase (LDH). A totally different trend was observed for the level of microelements; Copper (Cu), Zinc (Zn), Iron (Fe) and selenium (Se) where a sharp augmentation in the element levels was noticed in profenofos-treated rats compared with the control group. Treatment-dependent histopathological changes were seen in testes. Conclusion: Profenofos alters testicular functions possible by inhibition the activities of marker enzymes and inducing alteration in microelements levels, thereby disrupting male reproduction. [Journal of American 2009: 5(1), 19-28] (ISSN: 1545-1003)

Key words: Profenofos, Lactate dehydrogenase

1. INTRODUCTION

Organophosphorous insecticides (OPIs) have been considered as genuine alternatives to chlorinated (O'Ch) insecticides due to their broad-spectrum pesticidal properties and relatively shorter persistence after applications (Sharma et al., 2005). OPIs in addition to their intended effects like control of insects or other pests are sometimes found even to effect non-target organisms including human beings (Chantelli-Forti et al., 1993; Chaudhuri et al., 1999). Exposure to low level OPIs is known to produce a variety of biochemical changes, some of which may be responsible for the adverse biological effects reported in humans and experimental animals (Sutatos, 1994). There is growing concern that environmental chemicals both natural and man-made, having estrogenic property may be causing a variety of reproductive disorders in wildlife and human population (Chitra et al., 1999). The testes of humans and other mammals are highly susceptible to damage produced by genetic disorders, environment or occupational exposure to chemical or other means. Specific causes of testicular damage have been catalogued (Jadaramkunti and Kaliwal, 2002). Mainly, much data are available about biochemical analysis of seminal plasma. However, not many studies have been conducted in animals yet (Pesch et al., 2006). Analysis of enzyme activities and concentrations of microelements can estimate integrity and function of testes, in man; analysis of seminal plasma enzymes and microelements has been performed accurately and much is known about the importance of the "right contents" of seminal plasma (Pandy et al., 1983; Chia et al., 2000; Huang et al., 2000 and Stanwell-Smith et al., 1983). It has been reported that, pesticides with such properties have been shown to cause overproduction of reactive oxygen species (ROS) in both intra and extra cellular spaces, resulting in a decline of sperm count and infertility in wildlife and human (Gangadharan et al., 2001).

Trace elements, such as Copper (Cu), Zinc (Zn), and Selenium (Se) have a pivotal role in the spematogensis (Homma-Taked et al., 2003) Ionic environment has a high influence on sperm function (Hamameh and Gatti, 1998), profenofos belongs to the phosphorothioate class of OPIs. It widely used for a variety of agricultural and public health applications, previous studies suggest that profenofos considered as one of the male reproductive toxicant (Moustafa et al., 2007). In spite of the extensive use of profenofos in crop protection and in the household, information related to its effects on health with particular reference to reproductive toxicity are scarcely. Therefore, the objective of this study was to clarify the effect following subchronic exposure to profenofos on testicular functions by measuring the fertility indices (sperm count and motility), the activity of specific enzymes that responsible of spermatogenesis (alkaline and acid phosphatases and lactate dehydrogenase) and total protein level as well as concentrations of the essential microelements; Copper (Cu), Iron (Fe), Zinc (Zn) and Selenium (Se) in testicular tissue of male rats.

2. MATERIALS AND METHODS

The active substance profenofos produced by Syngenta multi national comp. under trade name: Selecron 72% EC was used. Tap water was used for preparing emulsion of profenofos immediately before use and orally administered into animals by osophageal intupation (per OS.). The median lethal dose (LD_{50}) of profenofos (per OS.) was determined according to Weil (1952) and its value was 185.13 mg/kg body weight.

In this investigation, thirty male Wistar albino rats, rattus norvegicus were obtained from the breeding unit of the Egyptian organization for the Biology and vaccine production, Egypt. Male rats initially weighing 150±10g were used. Animals were allowed to be acclimatized to laboratory conditions; of temperature at 25±2^{0}C, humidity (30-70%) and light (12-h dark: 12-h light) and kept on balanced diet and water ad libitum for 2 weeks prior to the experiment. Animals were housed throughout the experiment in polypropylene cages (with each cage housing five animals) containing paddy husk as bedding.

2-3 Experimental Design. Rats were randomly divided into two comparable groups as follows, First group: (n = 10) served as normal control and animals were received the vehicle (tap water). Second group: (n = 20) animals were orally dosed for 60 days with profenofos at 23.14 mg/kg body weight (4 doses/week). Clinical signs were monitored daily and animals were weighed twice weekly throughout the experiment and the dose was adjusted accordingly.

After completion of treatment period (60 days), animals were anaesthetized with ether and sacrified. The testes and epididymus were removed immediately, cleaned of the adhering tissues and weighted. Fertility-related parameters (sperm count and motility) were performed by dissecting out the Cauda epididymus and teasing it in a known volume of normal saline at 37^{0}C. Sperm counting was done using a haemocytometer according to the method of Feustan et al. (1989). The right testes were kept in a deep freezer (-40^{0}C) for biochemical estimations and microelements detection. Left testes were removed and fixed in 10 % formalin for routine histopathology.

Frozen testes were washed with saline solution, then minced and homogenized (10% W/V) in ice-cold saline, using a chilled glass-teflon porter-Elvehjem tissue grinder tube. The homogenate was centrifuged at 10,000 xg for 20 min. at 4 ^{0}C and the resultant supernatant used for determination of protein contents, Tp (Bradford, 1976); alkaline phosphatase, ALP (Babson, 1965) and acid phosphatase ACP (Babson and Read, 1959). Also, a 10% homogenate of testes was prepared in ice-cold 0.1M phosphate buffer, the homogenate was centrifuged at 12,000 xg for 30 min. at 4^{0}C. the supernatant used for determination of lactate dehydrogenase, LDH (Moss and Henderson, 1994).

For the histopathological observations at light microscopic level, fresh testes were immersion fixed in 10% formalin saline. Following an overnight fixation, the specimens were dehydrated in ascending grades of alcohol, cleared in benzene and embedded in paraffin wax. Blocks were made and 5um thick sections were double stained with hematoxylin and eosin and observed under microscope (Banchraft et al., 1996).

The concentrations of the microelements Copper (Cu), Iron (Fe), Zinc (Zn) and Selenium (Se) in testicular tissues were measured according to the procedure which reported in AOAC (2004), by using atomic absorption spectrophotometer (Thermo Jarel Ash-AA-ScanI).

Data analysis and evaluation of statistical significance among different values determined was done using the student's t-test. Statistical differences with a value of $p<0.05$ were considered significant (Snedecor and Cochran, 1980).

3. RESULTS

The variations in the testes and epididymus weights of animals subjected to profenofos treatment are shown in Table (1). There was significant decrease ($p<0.05$) and ($P<0.001$) in weights of the testes and epididymus, respectively, as compared to control group.

Table 1

Effect of oral administration of profenofos on testes and epididymus weights of rats after sub-chronic exposure (60 days)

Parameter	Control group	Profenfos-treated group 23.14 mg/kg body weight
Testes weight (g)	1.52 ± 0.040	1.40 ± 0.004*
Epididymus weight (g)	0.37 ± 0.014	0.02 ± 0.008***

Data represent mean ± SE, n = 5, * P< 0.05, *** P< 0.001 (Student's t-test)

The effect of oral administration of profenofos for 60 days on sperm count and motility in cauda epididymus is shown in Table (2). The spermatozoal density (count) increased significantly ($p<0.05$) in profenofos-treated group in comparison with the control group. Similarly, spermatozoal motility was also found to be significantly decreased ($p<0.001$).

Table 2

Effect of oral administration of profenofos on semen parameters in cauda epididymus of rats after sub-chronic exposure (60 days):

Parameter	Control group	Profenofos-treated group 23.14 mg/kg body weight
Total sperm count (10^7/ml)	100 ± 3.536	80 ± 4.082*
Motility (%)	90 ± 1.58	65 ± 3.227***

Data represent mean ± SE, n = 5, * P<0.05 , *** P<0.001 (student's t-test)

Results of testicular biochemistry have been depicted in Table (3). Alkaline (ALP), acid (ACP) phosphatese and lactate dehydrogenas (LDH) activities were recorded to have decreased ($p<0.001$, $p<0.05$ and $p<0.01$, respectively) in profenofos-treated group as compared to control group. In addition, total protein level was found to

be significantly raised ($p<0.05$) in treated group in comparison with the control group.

Table 3
Effect of oral administration of profenofos on some testicular biochemical parameters in rats after sub-chronic exposure (60 days)

Parameters	Control group	Profenofos-treated group 23.14 mg/kg body weight
alkaline phosphatase (U/mg protein)	0.127 ± 0.002	0.067 ± 0.009***
acid phosphatase (U/mg protein)	0.108 ± 0.002	0.084 ± 0.008*
lactate phosphatase (U/mg protein)	1.60 ± 0.073	1.25 ± 0.042**
total protein (mg/g tissue)	17.28 ± 0.774	20.27 ± 0.348*

Data represent mean ± SE, n = 4, * P<0.05,** P<0.01, *** P<0.001 (student's test)

In addition to the findings listed above, we have observed the presence of microscopic changes in the testes of male albino rats. Histological findings of testes from control and treated groups are presented in figs. 1, 2, respectively. Normal control animals, revealed normal mature seminiferous tubules with complete series of spermatogenesis and high spermatozoal concentration in the lumen (fig.1) Profenofos-intoxicated animals indicated that there were few numbers of sperm cells in the lumen of the seminiferous tubules (fig. 2), in correlation with the control one.

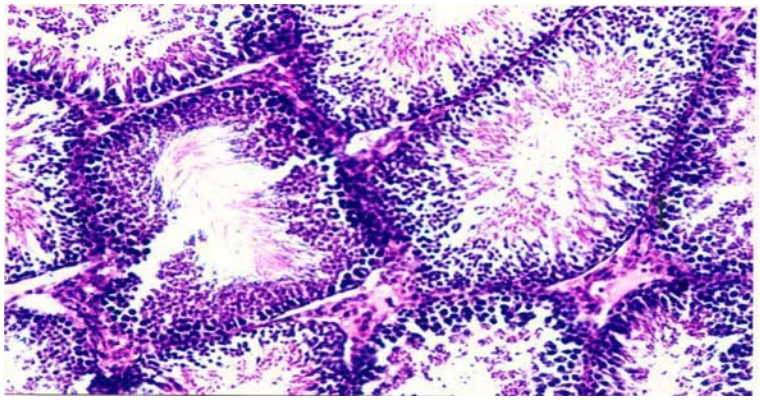

Fig 1: Testes of rat in control has shown the normal histological structure of the seminiferous tubules in nature active condition.

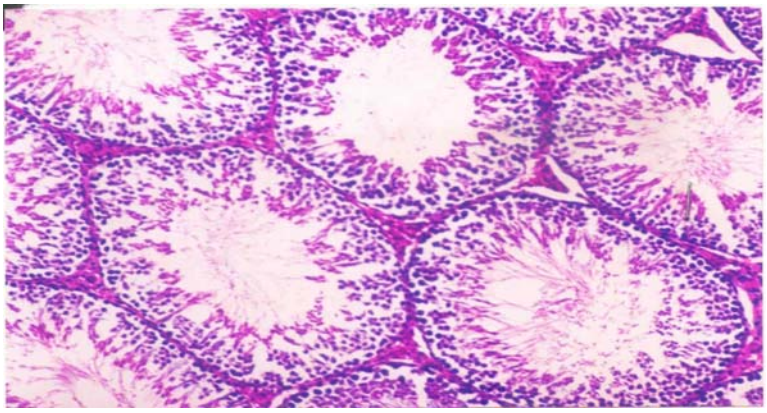

Fig 2: Testes of rat treated by profenofos showing low amount of sperms in the lumen of the semineferous tubules.

Table 4
The Testicular tissue contents of microelements in profenofos-treated rats after sub-chronic exposure (60 days)

Element (ppm)	Control group	Profenofos-treated group 23.14 mg/kg body weight
Copper (mg/kg tissue)	960.24 ± 3.136	1747.22 ± 3.747***
Ferric (mg/kg tissue)	370.36 ± 1.659	700.19 ± 4.827***
Zinc (mg/kg tissue)	9.93 ± 0.143	16.74 ± 0.158***
Selenium (mg/kg tissue)	100.52 ± 0.808	162.37 ± 0.458***

Data presented mean ± SE of five individual values.

The effect of oral administration of profenofos for the 60 days on testicular tissue contents of microelements is depicted in table (4).profenofos treatment produced significant increase ($p<0.001$) in iron (Fe), copper (Cu), zinc (Zn) as well as in selenium (Se) levels.

4. DISCUSSION

Organophosphates (OPIs) are among the most widely used synthetic insect pesticides. The wide spread use of OPIs has stimulated research into the possible extence of effects related with their reproductive toxic activity (Joshi et al., 2007). The present study results demonstrated that 60 day's exposure of male rats to profenofos at the dose 23.14 mg/kg body weight (4 doses/week) resulted in decreased the testes and epididymus weights, male fertility indices (sperm count and motility), and activities of ALP, ACP and LDH but increased levels of total protein and microelements (Cu, Fe, Zn and Se) in testicular tissues. Our results showed that the weights of testes and epididymus were significantly lower in the profenofos-treated rats than in the controls. The decrease in testicular weight in treated rats may be due to reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of leydig cells, a site of steroid biosynthesis (Sujatha et al. ,2001 and Kaur and mangat, 1980). The decrease in testicular weight in profenofos-treated rats may indicate impairment at testicular, pituitary, or hypothalamic level (Chitra et al. ,1991). Similar results were recorded by Ref Joshi et al. (2007), who mentioned that chlorpyrifos (OPIs) at dose levels of 7.5, 12.5 and 17.5 mg/kg b.wt./day, for 30 days, decreased significantly the weight of testes. The epididymus is androgen-dependant organ, relying on testosterone for its growth and function

(Klinefelter and Hess, 1998). On discussing the results with previous reports, it is proposed that profenofos probably imped the activity of testes and epididymus by inhibition of androgen production or its direct action on these organs (Kaur and mangat, 1980), thus, the reduction in the weights of testes and epididymus in our study may be due to lower bioavailability of androgen (Sujatha et al. ,2001). Moreover, the deleterious effects of profenofos on reproductive organ weights might be due to a decrease in the testosterone (T) and thyroid hormone levels after 60 days from the onset of the treatment (Takizawa and Horii, 2002).

The present results confirm the previous reports of (El-kashoury and El-far, 2004) who mentioned that administration of rats with profenofos at 23.14 and 46.30 mg/kg body weight for 28 days and 60 days, respectively, induced significant decrease in thyroid hormone levels, there is ample evidence that thyroid hormone is essential to the normal development of testes in the neonate (Cook et al. ,1994 and Hardy et al. ,1996), as well as an elevation in cholesterol level, a precursor of steroid hormone had occurred. Authors also, mentioned that inhibition of hepatic microsomal 7-hydroxylation of cholesterol by profenofos leads to reduction of cholesterol break down and its accumulation. Sperm count is one of the most sensitive tests for spermatogenesis and it is highly correlated with fertility. Our results revealed that, treatment of rats with profenofos significantly reduced the sperm count and motility. The decreased sperm motility and density (count) after oral administration of profenofos is may be due to androgen insufficiency (Chaudhary and Joshi, 2003) which caused impairment in testicular functions by altering the activities of the enzymes responsible for spermatogenesis (Sinha et al. ,1995 and Reuber, 1981).

Histological structure of the testes confirmed the aforementioned results, where it is revealed degeneration in some of seminiferous tubules associated with low luminal spermatozoal concentration. It is tempting to speculate that the decreased sperm motility in the present study may have been related to our earlier studies on profenofos (El-kashoury and El-far, 2004) which pointed that subclinical hypothyroid state in rats administered with profenofos for 60 days had occurred. Also, men with hypothyroid have been reported to have lower sperm motility than euthyroid controls (Corrales – Hernandez et al. ,1990) and thyroxine (T4) replacement in men with hypothyroidism is reported to improve sperm motility (Kumar et al. 1990). Moreover, it had been reported that chlorpyrifos brought about marked reduction in epididymal and testicular sperm counts in exposed males (Joshi et al., 2007). Also, testicular atrophy and degenerative changes in the seminiferous tubules had been reported in experimental animals administered with various O'Ch and OPIs pesticides (Dutta and Dikshith, 1973). Based on the data obtained in this study, administration of profenofos into male albino rats reduced the activities of acid and alkaline phosphatase and lactate dehydrogenase which reflect suppression in testicular function (Johnson et al. ,1970). Activities of markers enzymes viz ALP, ACP and LDH are considered to be functional indicators of spermatogenesis.

Our results confirm the findings of (Salem et al. ,1989) who investigated the influence of methamidophos (O'ps) on mammals. Results showed that treatment of male rats with methamidophos, at 100 ppm in drinking water for 9 and 45 days, reduced significantly acid and alkaline phosphatase and lactate dehyrogenase in testicular tissue. Also, (Mustafa et al. ,2007) reported that profenofos considered as one of the male reproductive toxicants. ALP is primary of testicular and epididymual origin and, therefore, suitable for differentiation of oligo-and azoospermia (Turner and Sertich, 2001; Turner and McDonell, 2003). Decline in ALP activity indicated that profenofos treatment produced a state of decreased steroidogenesis where the inter and intercellular transport was reduced as the metabolic reactions to channelize the necessary inputs for steroidogenesis slowed down (Latchoumycandane et al. ,1997). Acid phosphatases are enzymes capable of hydrolyzing orthophosphoric acid esters in an acid medium. The testicular acid phosphatase gene is up-regulated by androgens and is down-regulated by estrogens (Yousef et al. ,2001). Activities of phosphatases enzymes have been shown to rise when testicular steroidogenesis is increased (Mathur and Chattopandhyay, 1982).

Also, (Latchoumycandane et al. ,1997) mentioned that a decrease in ACP activity in free state would thus reflect decreased testicular steroidogenesis in rats and this may be correlated with the reduced secretion of gonadotrophins. LDH is associated with the maturation of germinal

epithelial layer of seminiferous tubules and associated with post meiotic spermatogenic cells (Sinha et al., 1997). An inhibition in the activity of LDH in testes of profenofos-treated rats points toward the interference of profenofos with the energy metabolism in testicular tissues (Mollenhauer et al., 1990). The correlation between LDH and motility and living sperm could be a sign that extracellular LDH ensures metabolism of spermatozoa, perhaps even in anaerobic conditions (Pesch et al., 2006).

As regards the testicular protein, results of the present study exhibit an increase in its level in profefos-treated rats. The testicular fluid contains both stimulatory factors as well as inhibitory factors that selectivity alter the protein secretions (Brooks, 1983). Thus, the changes in protein suggested that there is a reduction in the synthetic activity in testes. An elevation in testicular protein in the present study confirms the previous results by (Joshi et al., 2007) who mentioned that the protein content was raised at significant levels in chlorphrifos-treated rats. Gupta et al. (1981) and Singh and Pandey (1989) illustrated that an elevation in the testicular protein may be due to the hepatic detoxification activities caused by endosulfan (O'ch) which results in the inhibitory effect on the activities of enzyme involved in the androgen biotransformation (Dikshith and Dutta, 1972).

Similar results showed the same trend in the protein content caused by several pesticides, at different periods and / or different concentrations, had been also reported (Shivanandappa and Krishnakumari (1981), Bhatnagar and Malviya, 1986; Chitra et al., 1999; Choudhary and Joshi, 2003). In accordance with the findings of the present study, Rao and Chinoy (1983), suggested that the accumulation of protein occurred in testes and epididymus due to androgen deprivation to target organs. This deprivation effect also led to a reduction in testicular and cauda epididymus sperm population, loss of motility in the latter and an increase in number of abnormal spermatozoa, thereby manifesting 100% failure in treated animals. Results of the present investigation showed that administration of profenofos into male rats increased the concentration of trace elements; Cu, Fe, Zn and Se in testicular tissue, which have a pivotal role in spermatogenesis (Homma-Takeda et al., 2007). These findings are not in accordance with those of Salem et al. (1989), who stated that treatment of rats with methamidophos (OPIs), for 45 days, decreased the concentrations of Zn and Se in the testicular tissues. On the other hand, similar results were recorded by Al-Bayati et al. (1988), who mentioned that 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), O'ch, produced atrophy, morphological changes and impaired spermatogenesis in testes of experimental animals. In addition, testicular tissue contents of Fe, Cu, and Zn were significantly increased in the treated rats. Zinc (Zn) markedly increased the ALP and ACP activities and this occurred concomitantly with the appearance of spermatids and mature sperm cells (Guha and Vanha-Perttula, 1983). Selenium is an essential trace nutrient for humans and animals. It is an essential at lower concentrations and toxic at higher concentrations. Se is required for normal testicular development and spermatogenesis in rats (Behne et al., 1996). The selinodeiodinase enzymes (types I, II and III iodothyronine deiodinase) control the metabolism of thyroid hormone, which is essential for the normal development (Defrance et al., 1995) and function (Latchoumycandane et al., 1997) of testes in rats. The above explanation supports our findings where elevated testicular tissue content of Se associated with decrease in testicular weight, sperm count and motility in profenofos-treated rats. In support of these findings, earlier results (El-Kashoury and El-Far, 2004) revealed that treatment of rats with profenofos at the same dose and time interval decreased markedly (T_3) level in plasma in comparison with the control group.

Cupper is necessary for many enzymes like the Cu-Zn-Superoxide dismutase (SOD), which is involved in cell protection against free (Oxygen) radicals. Copper is also needed for the cytochrome C oxidase that is responsible for energy supply and for cellular and humoral immunity (Leonhard-Marek, 2001). As regards Cu concentrations, an administration of rats with profenofos increased testicular tissue contents of Cu by 2-fold, respectively. Elevated Cu concentrations reduced oxidative processes and glucolysis that may cause immotility and reduced viability (Leonhard-Marek, 2001). A proposed mechanism could explain elevated iron concentrations in testicular tissues in profenfos-treated rats, is that iron is known to be essential and mostly bound to transferrin (produced by sertoli cells), haptoglobin (sertoli, leydig and germ cells) and lactoferrin (spermatozoa and vascular gland). These proteins contain catalytic inactive iron which avoids extensive oxidation (Leonhard-Marek, 2001). Results of the present investigation suggested that profenofos may impede the utilization of micro-elements in the testes,

consequently stagnation of Cu, Fe, Zn and Se in the testes occurred. It is concluded that profenofos induced adverse effects on testicular function by altering biomarker enzymes activities as well as disrupting micro-elements levels, thus care should be taken and more studies should be done to increase the validity of those information.

Abbreviation used:

OPIs, organophosphorous insecticides; O'Ch, organochlorine, TP, total protein, ALP, alkaline phosphatase; ACP, acid phosphatase; LDH, lactate dehydrogenase; Cu, Copper; Zn, Zinc; Fe, Iron; Se, Selenium; Ec, Emulsifiable concentrate; T_4, Thyroxine; T_3, Triiodothyronine; T, Testosterone; Ros, Reactive oxygen species.

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Research Article

Effect of Duckweed Meal on The Rate of Mold Infestation In Stored Pelleted Fish Feed

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ABSTRACT: The effect of duckweed (Lemna pauciscostata) meal on the rate of mould infestation in stored pelleted fish feed was carried out. Freshly harvested duckweed was dried and thoroughly ground into powder using a milling machine. Five dry fish feeds were then prepared using duckweed as a replacement for fishmeal at 0%, 10%, 20% and 30% respectively at 40% crude protein, a diet for catfishes. The resultant pelleted feeds were sun dried for 24hrs and stored in airtight polyethylene bags at room temperature. Quantitative mold count using direct colony counts on pour plate technique with 24hr old culture was carried out bi-weekly until profuse growth were recorded within 24hrs in all experimental feeds. Results showed that mold count from experimental feeds decreased with increasing concentration of duckweed. Ethanolic extracts also showed higher inhibitory properties on radial mycelial growth of all the isolates. Isolates identified were Fusarium oxysporium, Penicillium digitatum, Aspergillus niger, A.fumigatus, A..flavus, Rhizopus stolonifer and R..oryzae.
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Key words: Polyethylene, Fusarium oxysporium

1. INTRODUCTION

Feeds are a major cost input into the aquaculture industry and their insufficiency is prominent among the factors responsible for inadequate aquacultural production of fish.

Compounded feeds are prepared with biologically decomposable materials. These materials decompose while in storage due to environmental factors such as temperature and humidity. Change in temperature and humidity affects the moisture content of compounded feed as well as the rate at which chemical changes takes place thereby enhancing invasion and growth of fungi in the feed (Sena and Anderson, 1995; Effiong and Eyo, 1999). Recontamination of feedstuffs by adventitious microorganisms during storage is of primary concern to the feed processor.

Moulds are the principal spoilers of feedstuff in storage (Chow, 1980). Moulds infestation reduces the nutritional value of feeds through loss of dietary lipids and amino acids (Jones, 1987). They also produce mycotoxins, which cause staleness of feed. He also stated that there is no effective way of eliminating fungal growth in stored pelleted feed. Their growth can only be controlled. Research work on the problems of storage of feedstuff \feed has been rather scanty despite the enormous harmful effect it poses on the development of aquaculture in Nigeria (Effiong and Eyo, 2001). Duckweed meal has been reported to resist attacks by mould for more than 5 years (Skillicorn et al., 1993). Duckweed meal is the compounded form of the group of aquatic macrophytes from the family Lemnaceae. The dried powdered and directly pelleted forms of this plant have been observed in storage for 13 years without any signs of fungal growth or physical damage, retaining its nutrient content (Mbagwu, 2001). This study is therefore aimed at determining the effect of duckweed meal on the rate of mould infestation in stored pelleted fish feeds.

2. MATERIALS AND METHODS

Freshly harvested duckweed was thoroughly rinsed with clean water and evenly spread on a mosquito net-size mesh outside to sundry and thereafter dried in a forced air oven at 165 ^{0}c for 48 hours and ground to powder with a milling machine according to Mbagwu and Adeniji (1987).Five dry diets were prepared in which fish meal was replaced with duckweed at 0%, 10%, 20% and 30% levels using the method of Akegbejo Samson (1999) at 40% crude protein, a diet for catfishes. The various feed ingredients were thoroughly

ground into fine meal and mixed together with vitamin premix and salt using hot water.

The resultant mixture was pelleted with Moulinese HV6 model pelleting machine and sun dried for 24 hours. The diets were stored in airtight containers at room temperature for 2 weeks. 1.0g of each feed sample were ground using pestle and mortar, to prepare 10-fold serial dilution. Agar was prepared using sterilized glasswares according to manufacturer's instruction and autoclaved at 121 ^{0}c for 15 minutes. It was allowed to cool to about 37 ^{0}c before 1% streptomycin was added to prevent bacterial contamination (Nwachukwu, 1988). A 48hour old culture of the isolates were subcultured and incubated at room temperature to produce pure cultures from which stock were prepared and stored. A bi-weekly mould count from each experimental diet was carried out quantitatively using direct colony count on pour plate technique (Miles and Misra, 1938) with 24-hour-old culture. Enumeration continued until profuse growth was recorded within 24 hours in all the experimental diets. Mould isolates were characterized during sporulation on the basis of cultural and morphological characteristics as well as microscopic examination (Samson and Reenen-Hoekstra, 1988). Sample of duckweed meal was ground using an Automatic Weed Grinder after it was thoroughly washed and air-dried. 5g of this each was measured and blended with 25ml of sterile distilled water (Oyagade, 1994). After thoroughly blending for 7 minutes, the slurry was filtered through a four –layered muslin cloth. The filtrate was passed through a 0.48 millimicron Millipore filter and transferred into sterile bottle.
In order to compare the efficiency of the extraction process, 95 % alcohol was used as the comparative solvent using the same method.

Radial mycelial growth inhibition tests were carried out on the isolates (Van-Etten, 1973; Oloke et al., 1988). The extracts were separately incorporated into molten PDA at 18ml of media to 2ml of extract. Control plates had either sterile water or ethanol without extract.

Agar- extract mixtures were poured into sterile glass petri dishes and allowed to set (Adedayo, 1994). Mycelial plugs of the test organisms of 5.0mm diameter were cut using sterile cork-borer from the advancing margin of the fungal colonies. These were placed at the center of PDA containing concentrations of 5% sterile distilled water or ethanol. All plates were incubated at 25 0c and radial mycelial growth recorded for 72 hours at 24 h0urs interval

3. RESULTS AND DISCUSSION.

The bi-weekly fungal count (cfu) from the experimental feed at varying concentrations of duckweed showed decrease in fungal growth with increasing concentration of duckweed (Table 1). This observation could be attributed to the antifungal properties of duckweed acting against the growth of fungal species in the feed. Skillicorn et al., (1993) attributed the long storage characteristic of duckweed meal to the presence of high levels of wax. It could be possible that wax presents physical barriers to the growth of molds, which might impair their utilization of nutrients in the feeds. The molds isolates from the experimental feed samples were Fusarium oxysporium, Penicillium digitatum. Aspergillus niger, A. fumigatus, A. flavus, Rhizopus stolonifer and R. oryzae. Chow (1980) reported that the most common molds involved in the spoilage of feedstuffs belong to the *Aspergillus and Penicillium* species among others. The presence of Aspergillus flavus from the feed indicates the possibility of mycotoxins, compounds produced by this species that are toxic to both humans and fish. Feedstuffs known to be contaminated by A. flavus include groundnut cake, maize, sorghum, cottonseed cake, copra and cassava (Chow, 1980). The same author however reported that for aflatoxins to be produced, A. flavus must be present alone in a practically pure culture and that the presence of other molds, yeast or even bacteria seems to interfere with aflatoxin production. These findings have also been reported by Abdulhamid (2008).

The effect of duckweed extracts on the radial mycelial growth of fungal isolates from the experimental feeds is shown in Table 2.Differential efficacy on the test organisms was observed between the aqueous and ethanolic extracts of duckweed meal. Ethanol appeared better as an extractant judging from the wider activity spectrum and the resultant effect on the isolates. This observation perhaps suggests the possibility of the occurrence of bioactive substances that are not only soluble in water but also in organic solvent in the plant material. Majekodunmi et al., (1996), and Martinez et al., (1996) reported that a higher activity of extractable natural products were obtained in ethanol compared with aqueous extracts. Odemena and Essien (1995) also reported that the bacterial activity of alcoholic extracts of the roots of fluted pumpkin, Telfaria occidentalis was better than that of aqueous extracts. Natarajan et al., (2005) reported the antifungal properties of three

medicinal plant extracts against Cercospora arachidicola. They reported that fungal growth was gradually suppressed with increasing extract concentration. Similar findings have been reported by Lucia et al., (2002), Silva et al., (2001) and Costa et al., (2000). These reports are similar to the findings of this study.Olafimihan (2003) working on the antibacterial properties of aqueous and ethanolic extracts of Neem plant reported that the antibacterial activity of the concentrated extract increased with increase in its concentration. This report is similar to the findings from this study with he observation that increasing concentration of duckweed meal in experimental feed resulted in decreasing fungal growth.

The environmental conditions of temperature and relative humidity during the period of the study were high and fell within the ranges that support luxuriant growth of molds in the experimental feed sample. The temperature range varied between 27.2 and 30.6 0c while relative humidity remained constant between 79 and 80%. According to Chow (1980), growth of fungi is only possible at temperature above 250c and relative humidity values at 65%. There any reduction in fungal growth in the experimental feeds could not be attributed to directly affect the rate of fungal infestation of compounded feed in storage.

4. CONCLUSION

The results obtained from this study indicate reduced growth performance in the fungal species isolated from the experimental feed which also signified low infestation rate. Fungal growth decreased generally with increase in concentration of duckweed meal in feed samples.

The result of this experiment have shown that duckweed has the potential of being a beneficial agent for the control of fungal growth in compounded feed in storage.

Table 1
Percentage composition of experimental feed with different inclusion levels of duckweed meal

Ingredients (g)	0%	10%	20%	30%
Duckweed	0	2.6	5.2	7.8
Fish meal	26	33.4	20.8	18.2
Yellow maize	48	48	48	48
Soya Bean meal	15	15	15	15
Groundnut cake	6	6	6	6
Vitamin premix	2	2	2	2
Bone meal	2.5	2.5	2.5	2.5
Salt	0.5	0.5	0.5	0.5
Total	100	100	100	100

Table 2
Bi- weekly fungal counts at varying concentrations of duckweed in experimental feed.

Concentration of Duckweed (%).	Fungal count (cfu\ml)(x 10^7)							
	Time (wk)							
	2	4	6	8	10	12	14	16
0	12	20	31	43	72	Profuse	Profuse	Profuse
10	5	9	21	35	52	108	Profuse	Profuse
20	3	11	18	27	48	90	Profuse	Profuse
30	-	-	9	16	21	54	76	Profuse

Table 3
Effect of duckweed extracts on the radial mycelial growth of fungal isolates

Test Organism	**Mycelial growth (mm)**					
	Aqueous Extract			**Ethanolic Extract**		
	0%	**5%**	**10%**	**0%**	**5%**	**10%**
Fusarium oxysporium	46	21	10	10	-	-
Penicillium digitatum	50	35	24	9	5	-
Aspergillus niger	47	27	18	16	7	2
Aspergillus fumigatus	38	18	12	4	-	-
Aspergillus flavus	50	38	20	16	-	-
Rhizopus oryzae	36	29	16	14	-	-
Rhizopus stolonifer	42	21	13	22	10	4

Table 4
Proximate composition of experimental feed with different inclusion level of duckweed

Feed Sample	% Crude protein	%Ether extract	%Ash content	%Moisture content	%Crude fibre
0%	43.35	14.02	12.30	1.00	6.50
10%	42.56	14.29	12.00	1.00	4.46
20%	41.87	12.83	11.90	2.00	5.13
30%	45.06	11.76	13.29	2.00	4.90

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Research Article

Distribution and Sources of Organochlorine Pesticides (OCPs) in Karst Cave, Guilin, China

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ABSTRACT: Despite the numerous researches done on Organochlorine pesticides (OCPs) in China and in the world, information regarding emissions and concentrations of OCPs in Karst caves is extremely limited. Karst areas have much higher ecological vulnerability and are so easy to be contaminated. This paper presents results of a monitoring program conducted in Dayan cave, Guilin, China that was designed to characterize levels, trends and sources of pesticides in soil (sediment) samples. Thirteen soil samples were collected and OCPs were analysed. Inside the cave a total concentration of OCPs (∑OCPs) detected was 29.659 ng /g with a mean value of 3.295 ng /g and ∑OCPs detected outside the cave was 74.108 ng /g with a mean value of 18.527ng/g. ∑OCPs outside the cave was higher than ∑OCPs outside the cave. The concentration of Chlordane in OCPs was highest among all the OCPs detected with range of 0.12─13.253ng/g and mean value of 3.93 ng /g. The next compound with high level of concentration was Heptachlor which ranged from Non-detected (ND) to 2.465 ng/g with a mean value of 1.4 ng/g. The pollution of OCPs in soil comparing with other countries and other areas in China was light. The analysis of Dichlorodiphenyltrichloroethane (DDT) and Hexachlorocyclohexane (HCH) isomers showed that there was fresh input of Dicofol and Lindane in the study area. By calculating the ratios of Dichlorodiphenyldichloroethane (DDD) to Dichlorodiphenyldichloroethylene (DDE), it was found that the degradation of DDT outside the cave was aerobic and the degradation of DDT inside the cave was anaerobic. [Journal of American Science 2009: 5(1), 35-43] (ISSN: 1545-1003)

Key words: Organochlorine Pesticides, Karst Cave

1. INTRODUCTION

Organochlorine pesticides are a group of persistent organic pollutants (POPs) which are to be eliminated or reduced on their release into the environment in many countries. Because of their persistence in the environment, and biological accumulation through the food web, OCPs can cause environmental damage, and affect human health (Colborn et al, 1996) .Due to their volatility and persistence in the air; OCPs are subjected to long-range atmospheric transport (LRAT). Therefore, OCPs released in the tropical and subtropical environments could be dispersed rapidly through air and water, and tend to be redistributed on a global scale (Tanabe, 1991) .The origin and fate of OCPs in soils with different land use have been extensively studied in many countries. Although the usage of OCPs was phased out for decades, the elevated concentrations were still observed in many agricultural soils (Harris et al., 2000) and the relationship between sites of greatest application and current residue levels was found strong (Shivaramaiah et al., 2002). The release of OCPs from soils continues to be a source of OCPs pollution to the environment (Meijer et al., 2001).

China is a large producer and consumer of Pesticides in the world (Rongbing et al., 2006). Large amount of OCPs were used in past decades to sustain over population in China. HCH and DDT were widely used in China from 1952-1983. Although their use had been discontinued in China since 1983, their persistence has left residual amounts in the soil in many areas (Zhao Ling and Ma Yongjun, 2001). At present the use of DDT is still allowed to control mosquitoes, particularly in the malarial transmission zones in China (Zhang et al., 2005). Accordingly, China still produces a small amount of DDT and China is also allowed to export DDT to other countries for the same

purpose. This paper presents the current status of OCPs residues in Dayan cave (Karst cave).

2. MATERIALS AND METHODS

2.1 Study Area

Region of research was in Guilin located in Guangxi Zhuang Autonomous Region in southeast China.Guangxi province (Southeast of China). The Geographical coordinates are 25° 40' 25" North, 108° 44' 0" East and has an altitude of 150m. It is bounded to the north-east by Hunan province, to the south-east by Hezhou town and it is next to Guangdong province. It has a surface area of 27, 800 square kilometers and a population of 4.76 million.

Dayan is an intermediate upper layer cave of Guilin Maomoatou cave system, located in the middle part of Guangming Mountain at right side of Taohua River in the north-west of Guilin. Guangming Mountain is a large peak cluster in Fenglin Plain, with an area of 0.92km^2, the highest peak altitude of 404.4m and the plain altitude of 151 m.The outcrop is a thick limestone layer of the Devonian system with a high intensity of Karst process. Dayan is a noncommercial karst cave located northeast to Ludiyan cave. The map of Guangxi showing Guilin and plane diagram of Dayan cave are shown in Fig 1 and Fig 2 respectively.

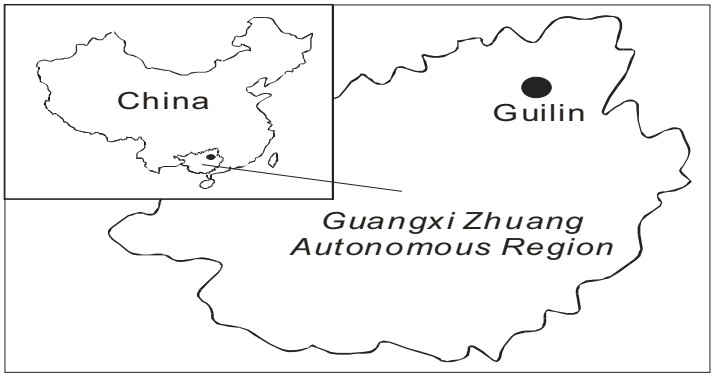

Fig 1: Map of Guangxi province showing Guilin

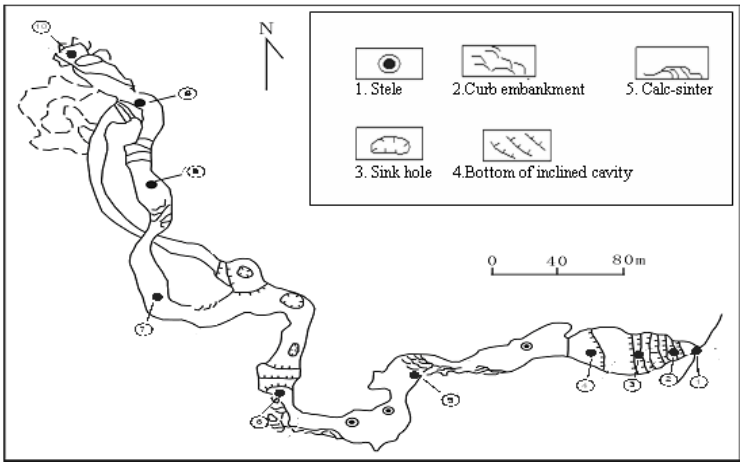

Fig 2: Map of Dayan Cave showing sampling locations
(1 to 10) are sampling locations

2.2 Soil sampling

Ten sampling locations were chosen inside the cave that followed the horizontal section from the east gate as shown in Fig 2. Sampling location 1 was at the east gate (outside the cave) and the serial number was from 1 to10. Three samples (1', 2', and 3') were also taken outside the east gate. Nine samples were obtained inside the cave (2 to10) and 4 samples outside the cave (1, 1', 2', and 3'). Sampling was done with the use of a hand shovel. The weight of each sample collected was 500g. After the collection of samples, they were kept frozen prior to the commencement of the laboratory analysis.

2.3 Analysis

2.3.1 Experimental procedures

Before analyising the samples (before experiment) all glass wares were acid washed and cleansed with distilled water before they were dried in the oven at 200^{o}C for about four hours. Reagents used for the experiment included: dichloromethane (DCM), hexane, acetone, sodium sulfate, alumina gel (100-200 mesh), silica gel (100-200 mesh), mesh hydrochloric acid and vitriol. Filter paper, aluminium foil, absorbent cotton and active copper were also used as materials.

Mixed standard sample of OCPs [2,4,5,6-tetrachloro-m-xylene (TCMX) and decachlorobiphenyl (PCB 209)] were used as surrogate standards and were added to all the samples before the extraction. The whole process of pretreatment was based on US EPA SW-8080A method as reference. 20 g of the sample were weighed with electronic balance and injected with the surrogate (using a syringe) before the sample was Soxhlet-extracted for 48 hours with redistilled Dichloromethane (DCM). Active copper slices were added to the conical flask containing DCM to eliminate the influence of sulphur contained in the sample. After 48 hrs in the soxhlet extractor, the extracted samples were added with Sodium sulphate ($NaSO_4$) to remove unwanted water. After that, the solvents were concentrated to about 5 ml and then passed though a mixture of silica gel and alumina gel (10/3, V/V) for purification and it was rinsed by a mixture of DCM and hexane (2/3,V/V). The solvent was then condensed with high purity Nitrogen. 4 ml of the hexamethyl-benzene and PCNB (5ppb) were added as internal standards to help in quantifying the amount of OCPs present in the samples. Finally samples were stored and kept in the refrigerator until next analysis (Analysis by HP 6890 GC).

2.3.2 Analysis by HP 6890 GC

HP 6890 GC (Gas Chromatography) was equipped with a ^{63}Ni electron capture detector and a 30 m x 0.32 mm i.d (0.25 *lm* film thickness) DB-5 fused silica capillary column. Nitrogen was added as a carrier gas at 1.2ml/min. the oven temperature was kept at 40^{o}C for 5 minutes and increased to 290^{o}C at a rate of 4^{o}C/min. Injector and detector temperatures were maintained at 250 and 300^{o}C respectively. 2 Microliters (µl) of each sample was injected for analysis.

2.3.3 Quality control and Quality assurance (QC/QA)

Quality control and Quality assurance was made by the use of the US EPA method in the process of the experiment. Method blanks (solvents), duplicate samples, and spiked blanks (standards spiked into solvent) were analyzed. In addition, surrogate standards were added to each of the samples to monitor procedural performance and matrix effects. The concentrations of OCPs were corrected for the recovery ratios for the surrogates. The recovery ratios for the surrogates in the samples conform to the reported ranges by US EPA. The recovery rates and standard deviation of OCPs during separation and testing are within the limiting value of the US EPA 610 method. Recovery rates of TCMX and PCB209 are 69±6% and 76±7% respectively.

3. RESULTS AND DISCUSSIONS

3.1 Concentration and distribution of OCPs

A summary of concentrations of OCPs detected in soil samples of Dayan cave is shown in Table1. Inside the cave ∑OCPs detected was 29.659 ng /g with a mean value of 3.295 ng /g and ∑OCPs detected outside the cave was 74.108 ng /g with a mean value of 18.527ng/g. ∑OCPs outside the cave is higher than the total concentration outside the cave (Fig 3).

The levels of OCPs outside the cave compared to the levels inside indicated that despite the relatively closed environmental system of the cave and less human interference inside the cave, it still had OCPs contamination due to air transfer, rain water filtration and other processes, but the degree of contamination was not high.

Table 1
Levels of OCPs in soil samples of Dayan Cave

	OCPs overall level range Min─Max(mean value）	OCPs level range inside the cave Min─Max(mean value) （2 to 10 samples）	OCPs level range outside the cave Min─Max(mean value) （1，1′，2′，3′ samples）
α-HCH	0.014─0.170 (0.087)	0.014─0.126(0.043)	0.095─0.170(0.130)
β-HCH	0.026─0.219 (0.102)	0.026─0.219(0.087)	0.100─0.138(0.117)
γ-HCH	0.015─0.285 (0.092)	0.015-0.180(0.044)	0.075─0.285(0.140)
δ-HCH	0.009─0.072 (0.034)	0.009─0.045(0.024)	0.020─0.072(0.044)
TC	0.021─6.119 (1.841)	0.021─1.674(0.279)	3.226─6.119(3.403)
CC	0.085─7.134 (2.221)	0.101─3.111(0.849)	3.899─7.134(4.198)
Hep	ND─2.465 (1.399)	ND─1.087(0.139)	1.871─2.465(1.632)
Hep-Epo	ND─1.908 (0.911)	ND─1.022(0.379)	1.000─1.908(1.185)
EndoI	ND─0.230 (0.067)	ND─0.040(0.021)	0.103─0.230(0.122)
EndoII	ND─0.161 (0.046)	ND─0.057(0.021)	0.026─0.161(0.080)
Endosulfate	0.030─0.500 (0.175)	0.030─0.180(0.086)	0.200─0.500(0.294)
p,p'-DDE	0.011─0.342 (0.108)	0.011─0.109(0.041)	0.115─0.342(0.174)
p,p'-DDD	ND─0.121 (0.079)	ND─0.077(0.038)	0.011─0.121(0.059)
o,p'-DDT	0.049─0.467 (0.212)	0.049─0.226(0.113)	0.302─0.467(0.310)
p,p'-DDT	ND─0.090 (0.031)	ND─0.039(0.011)	0.046─0.090(0.050)
ΣDDTs[b]	0.094─0.875 (0.371)	0.094─0.384(0.162)	0.532─0.875(0.434)
ΣHCHs[a]	0.100─0.665(0.269)	0.100─0.453(0.197)	0.313─0.665 (0.430)
ΣOCPs[c]	1.159─23.625(10.911)	1.159─11.180(3.295)	13.250─23.625(18.527)

ND=Non- detected
ΣHCHs[a]= α-HCH + *β*-HCH + *δ*-HCH + *γ*-HCH.
ΣDDTs[b]= *p, p'*-DDE + *p, p'*-DDD + *o, p'*-DDT + *p, p'*-DDT.
ΣOCPs[c] =ΣHCHs+ΣDDsT+Σother OCPs.
Σother OCPs = Heptachlor (Hep) + Heptachlor epoxide (Hep-Epo) + TC (Trans-Chlordane) + CC (Cis-Chlordane) +EndoI (*α*- Endosulfan) +EndoII (*β*-Endosulfan) +Endosulfate.

The concentration of Chlordane (TC+CC) in OCPs was highest among all the OCPs detected inside and outside the cave with a total concentration of 39.689ng/g and mean value of 9.92 ng /g inside the cave and a total concentration of 4.52 ng/g outside the cave with a mean value of 1.13 ng /g. This is because South china have been using Chlordane to kill termites, so the high concentration of Chlordane observed may be predominantly due to the use of technical Chlordane as a termiticide in this area in previous years . In China, technical chlordane is still being extensively used against termites in buildings, with an estimated amount of over 200 tons year^{-1} in recent years (Xu et al., 2004).

The next compounds with highest levels of concentration were Heptachlor (Hep) and Heptachlor epoxide (Hep-Epo.) Heptachlor (Hep) was also used and produced in large quantity in China. From 1967 to 1969 the amount of Heptachlor produced was 17 tons, to kill the termites and other insects in the soil. It is shown in Fig 4 that the majors parts of OCPs (HCHs and DDTs) at the cave's innermost sampling locations 9 and 10 did not show the lowest values, but rather slightly greater than the values of sampling locations 7 and 8 at the middle of the cave. This suggests that there may be a fracture pore near the north mouth that allows some air to come in.

Fig 4 shows that the total concentration of DDTs (∑DDTs) in soil samples was higher than the total concentration of HCHs (ΣHCHs). This trend is consistent with the previous observations on the contamination of OCPs in soil in China (Zhou et al., 2001). A most likely explanation for the current low concentration of HCHs in soil is due to the difference in the physicochemical and biochemical properties, wherein HCHs have higher water solubility, vapor pressure and biodegradability, and lower lipophicility and particle affinity compared to the DDTs (Rui et al., 2005). DDTs tend to remain in the particulate phase longer than HCHs. (Nhan et al., 2001).

In comparison with recent research reports, the concentrations of ∑DDTs and ∑HCHs measured in the study area were in the same low range with other pristine areas such as Tibet plateau where the concentration of ∑DDTs ranged from ND to 2.83 ng/g and ∑HCHs ranged from 0.18 to 5.38 ng/g (Fu et al., 2001), and European high altitude mountains that had ∑DDTs and ∑HCHs residual level in the range of 1.7-13 ng/g and 0.08-0.49 ng/g respectively (Grimalt et al., 2004).

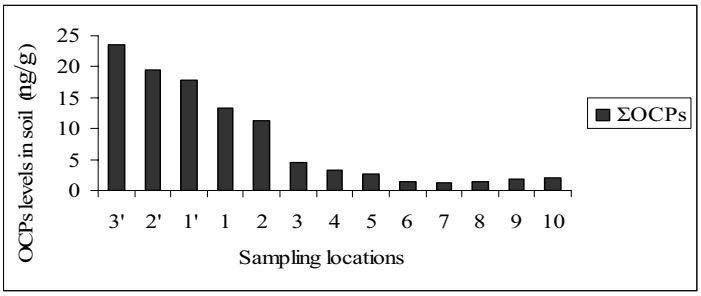

Fig 3: Distribution of ΣOCPs in soil of Dayan cave

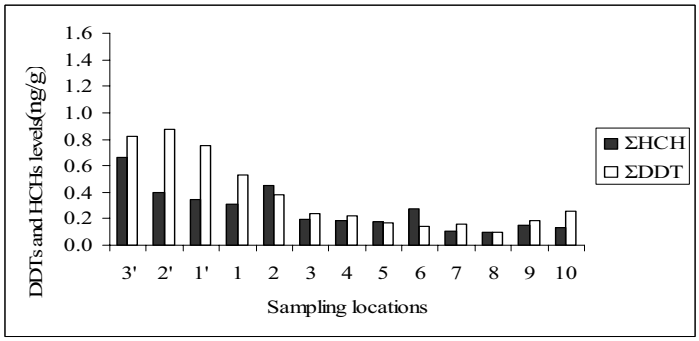

Fig 4: Distribution of ΣHCHs and ΣDDTs in soil of Dayan cave

The average concentration outside the cave and inside the cave of ∑DDTs and ∑HCHs was lower than the average concentration of ∑DDTs and ∑HCHs in Hong Kong soils which was 0.52 ng/g and 6.19ng/g respectively (Zhang et al, 2006), and they were much lower than the average concentrations of ∑DDTs (37.6 ng/g) and ∑HCHs (12.2 ng/g) found in soils of Pearl River Delta Region (Fu et al., 2003). Some other studies reported around China, had higher residual levels of OCPs such as Beijing (Zhu et al, 2005), Tianjin (Tao et al., 2005), Nanjing (An et al., 2005). In Europe, ΣDDTs and ΣHCHs levels were in the range of 4.3-2400 ng/g and 0.36-110 ng/g in Poland soils (Falandysz et al., 2001). In comparison with similar research the levels of OCPs in Guilin were low and the reason is because there are mainly rice farms in the vicinity of Guilin city in which small amounts of OCPs were used with the rotary method of planting rice. The existence of alternating wet and dry conditions was beneficial to the aerobic and anaerobic degradation of OCPs, leading to a reduced amount of soil OCPs.

3.1.1 Distribution and degradation of HCH isomers

It has been widely recognized that HCH is available in two formulations: technical HCH and lindane. Technical HCH contains isomers in the following percentages: α, 55–80%; β, 5–14%; γ, 8–15%; δ, 2–16%; ε, 3–5% (Qiu et al., 2004), and Lindane contains > 90% of γ-HCH. The ratio of α- to γ-HCH has been used to identify the possible HCH source. If the source of HCH comes from fresh input of technical HCH, the ratio of α - to γ - HCH is between 3 and 7 (Yang et al., 2008). However, a lindane source will reduce the ratio to close or <1 (Willet et al., 1998). A higher ratio of α - to γ -HCH than 7 can be explained by long-range transport or re-cycling of technical HCH, because α -HCH has a longer atmospheric lifetime than γ isomer by about 25% (Willet et al., 1998). As shown in Fig 6, the ratios of α-HCH/γ-HCH in all soil sampling locations were lower than 3. Accordingly, the contamination of HCHs in this region probably came from local use of lindane and also indicated Lindane inputs in the past several years. By analyzing the individual HCH isomers (Fig 5), it was found that β-HCH had the highest level of concentration among all the samples and it accounted from 20.03-79.13 %, especially in sample 3 to 7 where it accounted from 23-79% of the total HCHs detected. The β-HCH was higher because of its persistence in soil. The persistence of β-HCH in soils is mainly due to the higher K_{ow} (log K_{ow} =3.78) and lower vapor pressure value ($3.6x10^{-7}$ mmHg, 20^{o}C) (Zhang et al., 2006). These will make β-HCH easier to be absorbed to the soil organic matter and less evaporative loss from soils (Mackay et al., 1997). Furthermore, the spatial arrangement of Chlorine atoms in the molecular structure of β-HCH was supposed to be more resistant to microbial degradation in soils (Middeldorp et al., 1996).

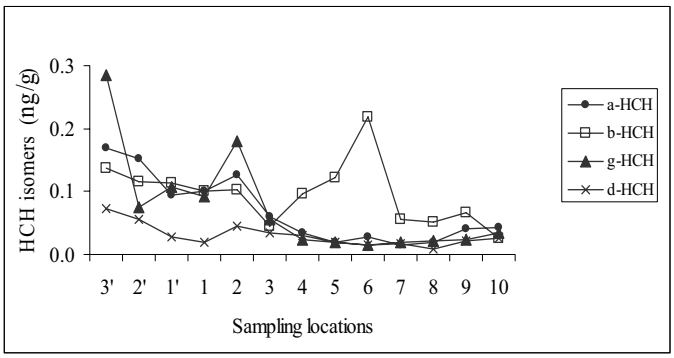

Fig 5: HCH isomers in soil of Dayan cave

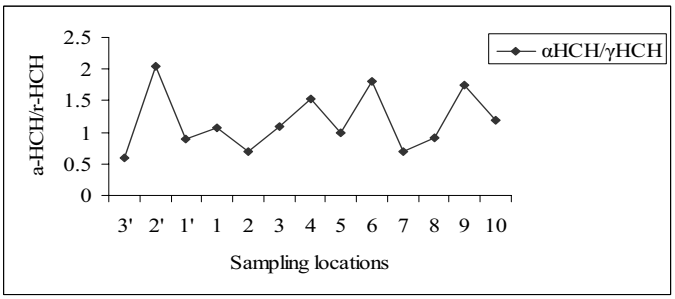

Fig 6: Ratios of α-HCH to γ-HCH in soil of Dayan cave

3.1.2 Distribution and degradation of DDT isomers
Commercial DDT generally contains 75% *p,p'*-DDT, 15% *o,p'*-DDT, 5% *p,p'*-DDE, <0.5% *p,p*'-DDD, <0.5 *o,p'*-DDE and <0.5% of unidentified compounds (WHO, 1979), but in Dicofol, the concentration of *o,p'*-DDT is more than *p,p*'-DDT (Qiu et al., 2005). DDTs isomers have a long persistence in the environment and their levels of concentrations in this study are shown in Fig.7. DDT can be biodegraded under aerobic conditions to DDE and under anaerobic conditions to DDD (Bossi et al., 1992). The ratio of DDD/DDE greater than 1 indicates that the soil was dominated by DDD, the product of anaerobic degradation of DDT, and the ratio lesser than 1 indicates that the soil was dominated by DDE, the product of aerobic degradation of DDT (Zhou et al., 2006). DDE and DDD Changes in the ratio of DDE and DDD to ΣDDTs has been regarded as an indication of either no or decreasing inputs to the environment. The ratio of (DDE+DDD)/ΣDDTs greater than 0.5 can be thought to be subjected to a long term weathering (Dong et al, 2002) and More *o*, *p'*-DDT than *p*, *p'*-DDT in the environment can demonstrate the Dicofol type DDT usage (Qiu et al., 2004).

The ratios of (DDE+DDD)/ ΣDDTs are shown in Fig.9. The ratios were in the range of 0.26-0.61 with most values being less than 0.5 (mean value is 0.4) and in Fig.7 it is shown that the concentration of *o,p'*-DDT was more than the concentration of *p,p'*-DDT as in Dicofol, this suggests that there was fresh input of Dicofol in the study area. Also, most values of DDD/DDE ratios as shown in Fig. 8 were greater than 1 inside the cave and ranged from 0.092 to 7 with an average value of 2.31, and the ratios of DDD/DDE outside the cave ranged from 0.052 to 0.53 with an average value of 0.35.

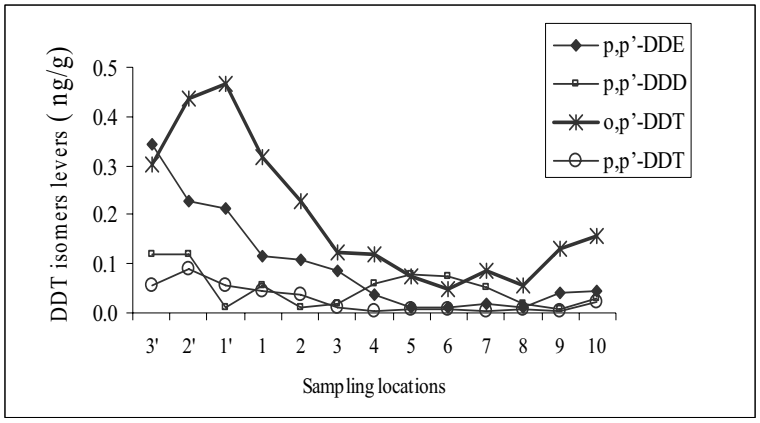

Fig 7: Distribution of DDT isomers in soil of Dayan Cave

The results obtained clearly indicated that DDT in soil inside and outside of the Dayan cave may be derived from Dicofol and DDT was retained under anaerobic conditions inside the cave and under aerobic condition outside the cave.

The use of Dicofol in China is mainly in the southern and eastern provinces, mostly on litchi, longan, citrus crops and cotton (Yang et al., 2008).

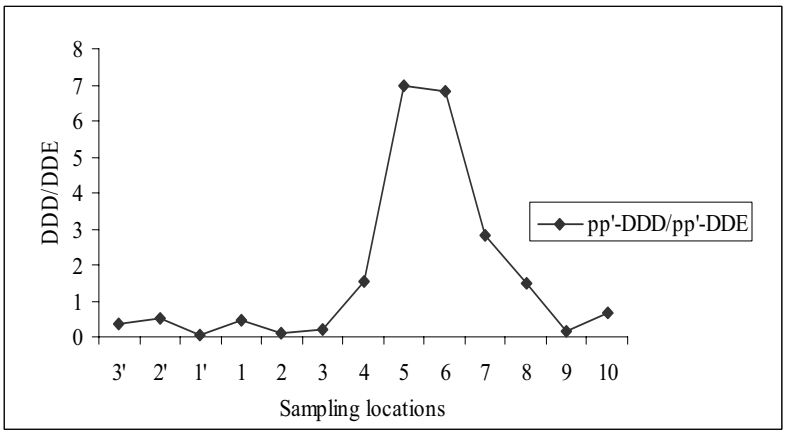

Fig 8: Ratios of DDD/DDE in soil of Dayan Cave

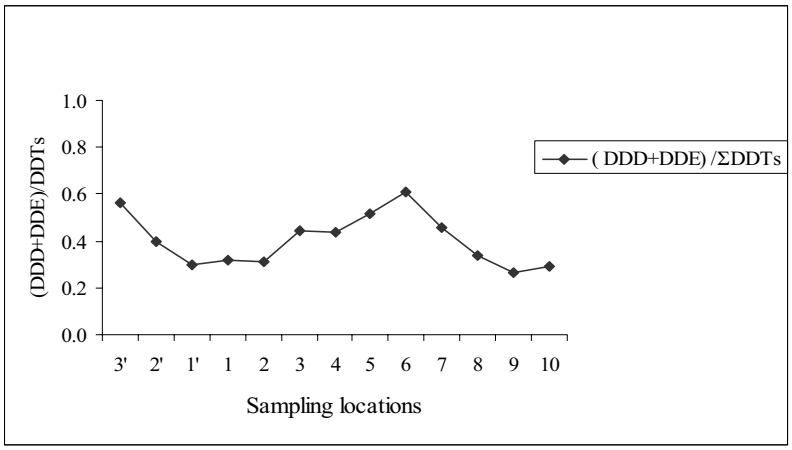

Fig 9: Ratios of (DDD + DDE)/ ΣDDTs in soil of Dayan cave

4. CONCLUSION

The use of HCHs and DDTs in China has been banned for 20 years and this sanction resulted in a tremendous decrease of OCPs concentrations in soils of Dayan cave. The residual levels of OCPs in soils outside Dayan cave were less than corresponding national values and among all the OCPs detected the concentration of chlordane and heptachlor were highest because they have been used in the study area.

ΣDDTs and ΣHCHs in soil inside the cave were low in comparison with worldwide background mountains and polar regions. As conclusion the pollution of OCPs in the soils inside and outside Dayan cave was light.

The analysis of isomers of DDTs and HCHs showed that there is fresh use of Dicofol and Lindane respectively in the study area. DDT degradation outside the cave was aerobic while inside the cave the degradation of DDT was anaerobic.

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Research Article

An Incidence of Substratum Discolouration in a Tropical West African Lagoon.

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ABSTRACT: A greenish discolouration of the lagoon floor at the Bayeku area of the Lagos lagoon was observed in January 2006. We report here an investigation of the area between December, 2005 and Feburary, 2006 as part of a larger study. A total of 19 species from 13 genera were reported. *Oscillatoria tenuis* (95,800 trichomes per ml) was implicated as the causative organism for the substratum discolouration. Increased insolation, especially reaching the lagoon floor, low salinity, absence of flood conditions, suitable sediment type (fine – medium sand) and high nutrient (PO_4 - P > 0.24 mg/L; NO_3 – N > 4.40mg/L) levels possibly encouraged the algal proliferation and subsequent substratum discoloration. It is suggested that improving water quality indices and salinity after January caused the disappearance of the discolouration on the substratum. [Journal of American Science 2009: 5(1), 44-48] (ISSN: 1545-1003)

Key words: algae, water quality indices, substratum.

1. INTRODUCTION

Coastal algal blooms respond to nutrient load from anthropogenic sources (Lee, 1999; Onyema, 2007). South-western Nigeria is endowed with an intricate network of rivers, creeks and lagoons, that serve as conduits transferring highly nutrified waters from hinterland to coastal areas. Bloom conditions have been reported in some of these waters (Nwankwo *et al.*, 2003a; Nwankwo *et al.*, 2008). Blooms of *Microcystis aureginosa*, *M. flos-aquae* and *M. wesenbergii* were reported in the Lagos lagoon (Nwankwo, 1993), Ogun river at Iju (Nwankwo, 1993) causing bluish colouration, anoxia, odour, impacting taste to the water (Nwankwo *et al*., 2003a) and kuramo lagoon (Nwankwo *et al.*, 2008). Blooms of *Trichodesmium thiebautii* have also been reported off the Lagos coast (Nwankwo, 1993) during thermocline conditions and more recently a bloom of *Bellerochea malleus* that caused brownish discolouration off the Light house beach, Lagos (Nwankwo *et al*., 2004) was documented. Blooms of *Anabaena flos-aquae, A. spiroides* (cyanobacteria), *Cerataulina bergoni*, *Chaetoceros convolutus, Coscinodiscus centralis* (diatoms) and *Ceratium furca, C. fusus, C. tripos* and *Noctiluca scntillans* (dinoflagellates) are known to induce harmful effects in waters of south-western Nigeria (Nwankwo, 1993; Nwankwo *et al.*, 2003a, b, Onyema, 2008). There is at present a report of substratum discolouration in the Lagos lagoon system (Onyema and Nwankwo, 2006) implicating *Beggiatoa alba* and *Oscillatoria* spp as causative species.

Between December, 2005 and Feburary, 2006, a greenish discolouration of the substratum at Bayeku was observed and thoroughly investigated. We report here the composition of the organisms before, during the bloom period and after the collapse. Water quality indices before, during and after the substratum discolouration were also estimated and investigated. This report is part of a larger study that was already ongoing at the time of the occurrence.

2. MATERIALS AND METHODS

Description of study area:

The Lagos lagoon opens into the sea via the Lagos habour all through the year. The tidal height is low (<1.5m) and the tidal exchange weak. It is shallow (<2m) and connected to the Epe lagoon to the east. The area investigated was (Fig 1) the Bayeku area of the Lagos lagoon (Latitudes 6^0 32^lN and 6^0 31^lN and Longitudes 3^0 31^lE and 3^0 32^lE). A greenish, slimy covering of suspected algae on the lagoon floor was observed for the very first time in this area. Nutrient rich water is known to flow from eutrophic creeks and creeklets systems in the area. Furthermore, poor sewerage systems are the common state of the rural dwellers of the immediate area. Hence direct dumping of domestic wastes is carried out in the closet water body.

Collection of samples

Water samples for determining water quality characteristics were collected at the site before substratum sample collection. The boat was anchored throughout sample collections. Water samples were collected in 1L plastic bottles with screw cap from 0.5m depth from the water surface. This was labeled and transported to the laboratory for chemical analysis.

Substratum samples (top 5cm) were collected within a 5cm² quadrat carefully placed on the greenish material / lagoon floor. A spatula was gently used underwater to scrape the topmost part. After carefully scooping up the greenish scum, it was gently spooned into a plastic bag while still underwater. Duplicate samples were collected on each occasion. Out of water and in the boat, samples were transferred to 75cl screw capped plastic containers. Samples were fixed with formalin (4% unbuffered) and labeled appropriately on the field before onward transportation to the laboratory. This process was carried out on each sampling occasion.

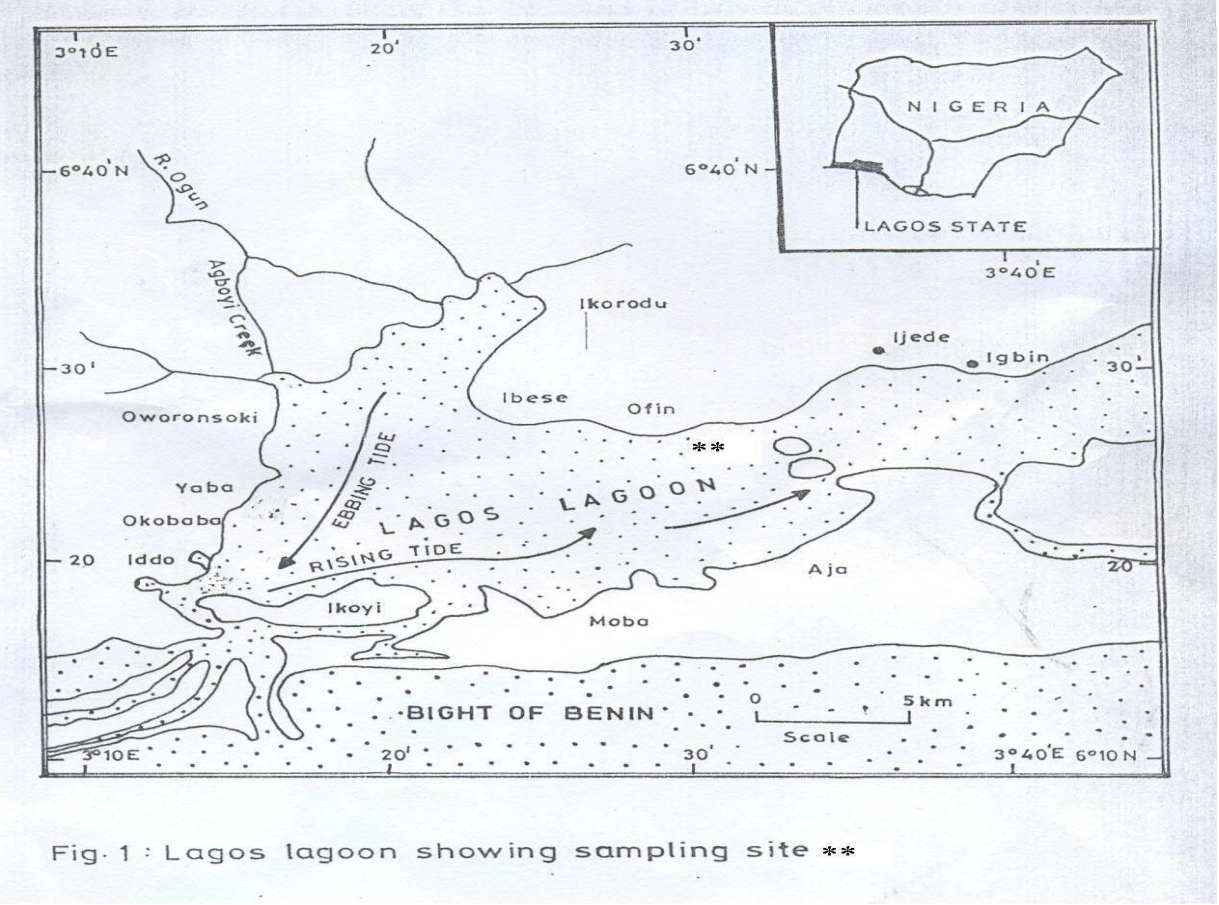

Fig. 1 : Lagos lagoon showing sampling site **

Physico-chemical analysis

Air and surface water temperatures were measured in-situ using a mercury thermometer while water depth was estimated with a calibrated pole. Total dissolved solids was determined by evaporating 100ml aliquot at 105°C and total suspended solids estimated by filtering 100ml of sample through a pre-weighed filter paper, dried to constant weight and reweigh. Conductivity was measured using the HANNA instrument while salinity was determined using the silver-nitrate chromate method. The surface water pH was determined with a Griffin pH meter (Model 80) while dissolved oxygen was measured using a Griffin oxygen meter (Model 40). Biological and chemical oxygen demands were measured using methods described in APHA (1998) for water analysis. Calorimetric methods using a Lovibond Nesslerier were adopted for the direct determination of phosphate-phosphorus and nitrate-nitrogen values while sulphate levels were measured using the gravimetric method. Calcium and magnesium ions were determined using a 400 single channel, low flame photometer. Concentrations of copper, iron and zinc were determined with an atomic absorption spectrophotometer (A.A.S.) Uni cam 99model.

Biological Analyses

In the laboratory, the drop count microscope analysis method described by Onyema (2007) was used to estimate the substratum algal flora. Microscope analysis was carried out on samples within 48hours of collection. Identification materials were used to assist and confirm identification of species (Smith 1950; Hendey, 1958, 1964; Desikachary, 1959; Wimpenny, 1966; Patrick and Reimer, 1966, 1975; Whitford and Schmacher, 1973;

Vanlandingham, 1982; Nwankwo, 1990, 2004a; Bettrons and Castrejon, 1999; Siver, 2003; Rosowski, 2003).

3. RESULTS

Physico-chemical

Air (31 - 32 ^{o}C) and water (30 - 31 ^{o}C) temperatures were high through out the sampling period while the sampling depth was averagely 1.31m. The water remained slightly alkaline throughout the study (7.01 – 7.10). The total dissolved solids (20 - 33 mg/L), salinity (2.30 - 20.60 $^{o}/_{oo}$), chloride content (770.0 – 6930 mg/L), conductivity (2335 – 12,500 µS/cm), acidity (3.0 - 8.8 mg/L), alkalinity (28.5 - 100.3 mg/L), total hardness (562.5 - 4687.0 mg/L), sulphate (6.1 – 60 mg/L) and cation content (Calcium 111- 500, Magnesium 35.6- 859 mg/L) increased as the dry season progressed, while there was a corresponding decrease in total suspended solids (1590 – 8260 mg/L), nitrate (2.5 - 4.8 mg/L), biological (5 - 11mg/L) and chemical oxygen demands (10 – 49 mg/L) and heavy metals levels (Iron 0.14 - 0.35, Zinc 0.003 - 0.006mg/L) (Table 1).

With regard to the algae, just one species each was recorded for December 2005 (*Microcystis aureginosa* Kutzing) and January 2006 (*Oscillatoria tenuis* Agardh), However, 17 species were recorded in February (Table 2). Although, total biomass in terms of cell numbers was high in January (95,800 trichomes per ml) it was for a sole species. This organism (*Oscillatoria tenuis* Agardh) is the implicated microalgae responsible for the greenish discolouration of the lagoon floor at Bayeku. Furthermore, February recorded 3 cyanobacteria, 8 centric diatoms and 6 pennate diatoms species. *Actinophycus splendens* Ralfs and *Biddulphia laevis* Ehrenberg were important diatoms and *Oscillatoria limnosa* Agardh for the cyanobacteria in terms of numbers in February.

Table 1
Monthly variation in water quality characteristics
at Bayeku area of the Lagos lagoon (Dec., 2005 – Feb., 2006).

Physico-chemical parameters	Dec., 2005	Jan., 2006	Feb., 2006
Air temperature (°C)	32	31	31
Water temperature (°C)	30	31	30
Depth (m)	1.42	1.24	1.41
Total Suspended Solids (mg/L)	33	27	20
Total dissolved Solids (mg/L)	1590	5120	8260
Salinity ($^{o}/_{oo}$)	2.30	9.20	20.60
Chloride (mg/L)	770.0	3086.0	6930
Conductivity (µS/cm)	2335	7877	12500
pH	7.05	7.01	7.10
Acidity (mg/L)	3.0	8.8	8.1
Alkalinity (mg/L)	28.5	30.4	100.3
Total Hardness (mg/L)	562.5	360.0	4687.0
Nitrate- Nitrogen (mg/L)	4.4	4.8	2.5
Sulphate (mg/L)	6.1	10.8	60
Phosphate- Phosporus (mg/L)	0.24	0.26	0.04
Silica (SiO_2 mg/L)	1.9	2.6	2.1
Dissolved Oxygen (mg/L)	5.5	4.2	4.3
Biological Oxygen Demand (mg/L)	11	9	5
Chemical Oxygen Demand (mg/L)	49	27	10
Calcium (mg/L)	165	111	500
Magnesium (mg/L)	35.6	50	859
Copper (mg/L)	0.002	0.002	0.002
Iron (mg/L)	0.35	0.22	0.14
Zinc (mg/L)	0.005	0.006	0.003

Table 2

Substratum algal composition (before, during and post bloom) at Bayeku (per ml).

Algal Taxa	Dec., 2005	(Bloom) Jan., 2006	Feb., 2006
Class – Cyanophyceae			
Order I – Chrooccocales			
Microcystis aureginosa Kutzing	170	-	-
Order II – Hormogonales			
Lyngbya limnetica Lemm	-	-	5
Oscillatoria curviceps C.A. Agardh	-	-	10
Oscillatoria limnosa Agardh	-	-	60
Oscillatoria tenuis Agardh	-	95,800	-
Class – Bacillariophyta			
Order I - Centrales			
Actinophycus splendens (Sch adbolt) Ralfs	-	-	205
Biddulphia laevis Ehrenberg	-	-	125
Coscinodiscus centralis Ehrenberg	-	-	10
Coscinodiscus eccentricus Ehrenberg	-	-	10
Coscinodiscus radiatus Ehrenberg	-	-	5
Cyclotella meneghiniana Kutzing	-	-	15
Melosira moniliformis (O.F. Muller) Agardh	-	-	10
Melosira nummuloides Agardh	-	-	35
Order II – Pennales			
Cymbella affinis Kutzing	-	-	15
Navicula mutica Kutzing	-	-	5
Nitzschia palea (Kutzing) Wm Smith	-	-	5
Pleurosigma angulatum (Quekett) Wm Smith	-	-	55
Pleurosigma elongatum Wm Smith	-	-	15
Synedra crystallina Kutzing	-	-	20
Number of species (S)	**1**	**1**	**17**
Species abundance (N)	**170**	**95,800**	**605**

4. DISCUSSION

The water quality status at the site ranged between low and high brackish water conditions. Low brackish condition (S=2.30‰) was experienced in December while high brackish condition (>9.20‰) reflected the dry months. As the rain ceased, turbidity reduced while transparency increased. Furthermore, insolation increased probably reaching the lagoon floor. This coupled with high nutrient levels (PO_3^{2-} > 0.24mg/L, NO_3^- > 4.4mg/L, SO_4^{2-} >6.1mg/L), low brackish condition (<9.2$^0/_{00}$) and low depth (<1.42m), favorable sediment type (fine – medium sand) and absence of flood conditions probably encouraged the proliferation of the epipelic algal population in January. According to Valangdiham (1982), *Oscillatoria tenius,* the causative cyanobacterium, in the substratum discolouration, is a saprobiont which can exist either as plankton or as an attached form. Palmer (1969) reported that *Oscillatoria tenuis* is the second most tolerant *Oscillatoria* species to organically induced stress. It's important to note that both sole species in December and January are known pollution tolerant cyanobacteria forms for the region (Nwankwo, 2004b). Importantly, the highest level of nitrate (4.8 mg/L) recorded for this study was in January at the time of the greenish occurrence. *Oscillatoria* spp are reported in literature to have wide tolerance limits to pH, salts and organically enriched environments (Valangdiham, 1982; Lee, 1999; Nwankwo, 2004b; Onyema, 2008). In Nigeria, Onyema *et al*., (2003) has reported *Oscillatoria tenius* in organically polluted parts of Lagos lagoon. Similarly, Chindah and Pudo (1991) have reported *Oscillatoria tenius* from the Bonny river associated with oil related effluent. According to Valangdiham (1982) *Oscillatoria* species are heavily favoured in organically nutrified waters. The existence of high BOD levels in excess of 9mg/L at this site may be pointer to the probably stressed water quality status. According to Hynes (1960), BOD above 8.0mg/L may indicate severe organic pollution. The disappearance of the bloom in February may be associated with increased salinity (≥20.6$^0/_{00}$) and reduced nutrient load (PO_4 – P 0.04mg/L; NO_3-N = 2.05mg/L). Onyema and Nwankwo (2006) reported a high abundance of epipelic algal forms in the dry months at some organically polluted sites of an estuarine creek in Lagos. This investigation highlights the bane of increasing levels of pollutants from anthropogenic sources in the Lagos lagoon and the role of algal indicators in capturing changes in water quality.

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Research Article

The Influences of Extremely Low Frequency AC Magnetic Fields At 60Hz on Mung Beans Growth

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ABSTRACT: There are many reports about the biological effects of extremely low frequency magnetic fields (ELF MFs),but few of them investigate how different intensity MFs act upon the growth of living organisms. This study aims to assess the influences of the different intensity of ELF MFs on the early growth of living organisms using mung beans as test materials. We used 60Hz 110Vrms AC electric power as the source and made a toroidal magnetic coil by self for this experiment. The ELF MF is induced using a magnetic circuit with a toroidal magnetic coil and a 60W lamp in series, which is driven by 60Hz 110V AC electric power, the maximum intensity of ELF MF is 950mG. To utilize the magnetic field intensity decay when distance increase, to choose the three kinds different magnetic field intensity (such as 875mG,155mG and 1.8mG rms value). We used three groups of mung beans (each group is 50 beans) were exposed to the three kinds different magnetic field intensity separately, and observed the lengths of stems and leaves of mung beans after five days growth. The results indicate that the magnetic field intensity is 875mG and 155mG have an enhancing effect on the early growth of mung beans. [Journal of American Science 2009: 5(1), 49-54] (ISSN: 1545-1003)

Key words: ELF MF; biological effect,AC electric power,mung bean

1. INTRODUCTION

Because popularization of electricity and modernization of life, to place in the electric power line generally and use home electrical appliances frequently on the human inhabitancy space, there are ELF MFs produced also exists around the living space. We used a magnetic meter (TES-1390 ELF Magnetic Field Meter, Bandwidth:50~300Hz,TES Electrical Electronic Crop. made in Taiwan) to measure the root mean square value of ELF MF intensity of home electrical appliances such as hairdryer, desk lamp, razor, etc. We can get magnetic field intensity greater than 100mG (rms value), when to measure home electrical appliances closely (5cm to 10cm away). Because most countries adopt the reference levels which were announced by ICNIRP in 1998 for general public exposure to time-varying electric and magnetic fields as the standard. The formula of reference level for general public is 50/f (f is the frequency ,unit:KHz), the reference level is 833mG when f is 60Hz. For understanding the biological effect of different kinds magnetic field intensity, we made a toroidal magnetic coil by self, the coil produced the maximum ELF MF intensity is 950mG. To utilize the magnetic field intensity decay when distance increase, to choose the three kinds different magnetic field intensity (such as 875mG,155mG and 1.8mG rms value). We exposed

test materials (mung beans) in the three kinds different magnetic field intensity, and observed different magnetic field intensities act upon the early growth of test materials.

2. MATERIALS AND METHODS

2.1 Plant material

Mung beans were used as the test subject in this study. We selected 150 mung beans of almost the same weight (0.09 g) and similar appearance, so that the sample error can be greatly reduced, and divided into three groups of 50 mung beans. Two groups of them are grown in a magnetic field (exposed group 1 under higher magnetic intensity and exposed group 2 under lower magnetic field intensity), and the other group is placed in an ambient weak magnetic field (control group). We used a rectangular culture plate (dimension is 47×27× 3.5cm) which was spread the fine sand of depth 3cm to grow three groups of mung beans together. The environmental parameters of three groups that were maintained in the test room were almost the same, and the light was supplied by white fluorescent lamps .The close environmental parameters of three groups can be achieved so that the growth difference between them only comes from the magnetic field variable. The environmental parameters such as temperature is 28±2℃, humidity is 60±6％ ,illumination is 1120±50LUX(day) and 563LUX(night).

2.2 Exposure System

The purpose of this study is mainly to assess the influence on the early growth of mung beans exposed to the different magnetic field intensities. The equipment needed in this experiment included a 60-Watts incandescent lamp, a toroidal magnetic coil, an oscilloscope/ frequency analyzer, etc. In order to produce the environment of higher magnetic intensity, we made a toroidal magnetic coil with air gap by self is shown in Figure 1.

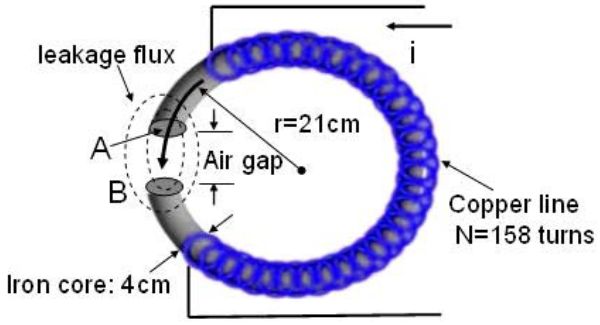


Fig 1: The toroidal magnetic coil with air gap

We entwined Iron wire (cross-section diameter =2mm) to become a toroidal iron core with diameter of 21cm and a 9 cm air gap (cross-section diameter=4cm). The core was wound 158 turns with copper wire (cross-section diameter =2mm) to become a toroidal magnetic coil. The magnetic flux density (B) circulating in the coil and air gap can be theoretically expressed in the following equations:

$$B = \frac{Ni}{RA}, \qquad R = \frac{l_c}{\mu A} + \frac{l_g}{\mu_0 A}$$

where R is total magnetic reluctance of the core and air gap, μ and μ_0 are the magnetic permeability of the core and air respectively (μ ~5000 μ_0), A is the cross-section area of the toroidal iron core, N is the

number of turns of coil, i is the current flowing through the coil, lc and lg are the core circumference and air gap distance, respectively. The exposure system is shown in Figure 2. We used 60Hz 110Vrms AC electric power as the source and a 60-Watts incandescent lamp as the load, and covered on lamp with an iron bucket to hide the light of lamp, to avoid other interference for mung beans growth.

We measured the highest magnetic field intensity of the air gap of coil is 950mG. The air gap of coil was to be placed the mung beans of exposed group one. In order to get more experimental data for statistics, we used 50 mung beans of each group which were put on culture plate will take larger area. Because the magnetic field intensity decay when distance increase, we measured the magnetic field intensity of the relative position of each group on culture plate is shown in Figure 2. We got more accurate data were the magnetic field intensity of exposed group one is 875±75mG, exposed group two is 155±55mG, control group is 1.8±0.8mG.

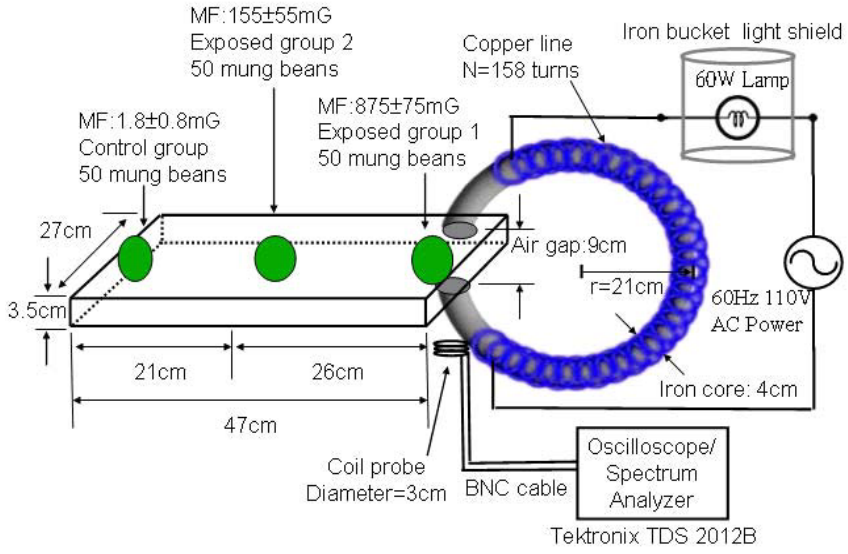


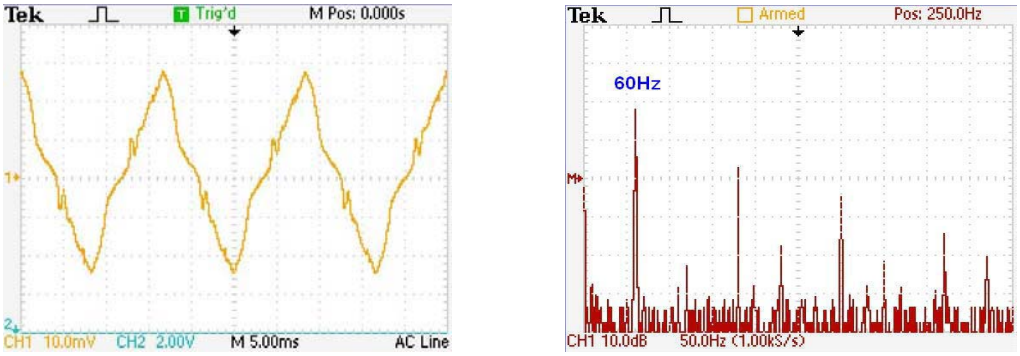
Fig 2: The exposure system of this experiment

The MF source came from the toroidal magnetic coil that was driven by the 60Hz 110V AC electric power. To measure the waveform and spectrum of the ELF MF, we used a little probe coil of diameter 3cm (Misakian, 1993) to induce an electromagnetic force close to the coil . The probe was connected to an oscilloscope/frequency analyzer (Tektronix TDS2012B, Bandwidth:100MHz) to obtain the components of 60Hz 110V AC electric power magnetic field in time and frequency domain are shown in Figure 3 a and b. We found the waveform of 60Hz 110V AC electric power is distortion and the frequency spectrum with harmonics.

2.3 Methods

We prepared three cylindrical containers with diameters of 5cm and poured into 50ml distilled water, then put three groups of mung beans in the cylindrical container, respectively. We moved three cylindrical containers in the positions of rectangular culture plate be shown in Figure 2. After the three groups of mung beans have been imbibing water for 8 hours, so dehydrated beans were simply rehydrated to allow enzyme reactivation, they

were taken out. The three cylindrical containers were removed and three groups of beans were put back in their original positions of culture plate to continue growing, and then were sprayed into appropriate distilled water by a sprinkler every 12 hours. Because three groups of mung beans grew on culture plate together, so the environmental parameters of three groups were almost the same. After mung beans have been growing for 5 days are shown in Figure 4, three groups of mung bean sprouts were taken out, in general mung bean sprout have two leaves, and the stem length and leaves length of each mung bean sprout was measured.



(a) (b)

Fig 3: (a) The waveform of 60Hz 100V AC electric power magnetic field is distorted sine wave. (b) The frequency spectrum of 60Hz 100V AC electric power have harmonics, the measured bandwidth of analyzer is 500Hz.

3. RESULT

We observed the growth of two exposed groups was faster than the growth of control group during 5 days. The average stem lengths and average leaf lengths of each group mung bean sprouts were recorded are shown in Figure 5. We analyzed experimental data by statistical method are shown in Table 1. The average stem lengths of mung bean sprouts exposed to 875±75mG and 155±55mG ELF MF were great than those of control mung bean sprouts ($P<0.01$,one-tailed paired sample t-test). The average leaf lengths of mung bean sprouts exposed to 875±75mG and 155±55mG ELF MF were great than those of control mung bean sprouts ($P<0.01$, one-tailed paired sample t-test), too. We can find an enhancing effect on the growth of mung bean is exposed under 875±75mG and 155±55mG ELF MF. Otherwise, there is no significant different between the exposed group one and exposed group two mung beans ($P>>0.05$, one-tailed paired sample t-test).

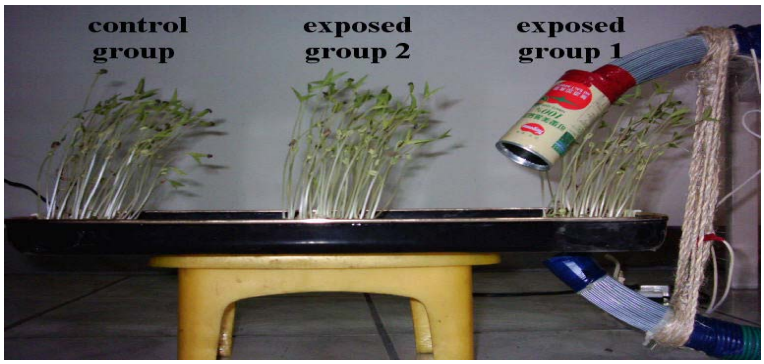


Fig 4: The early growth of exposed 1,exposed 2 and control group mung beans after five days

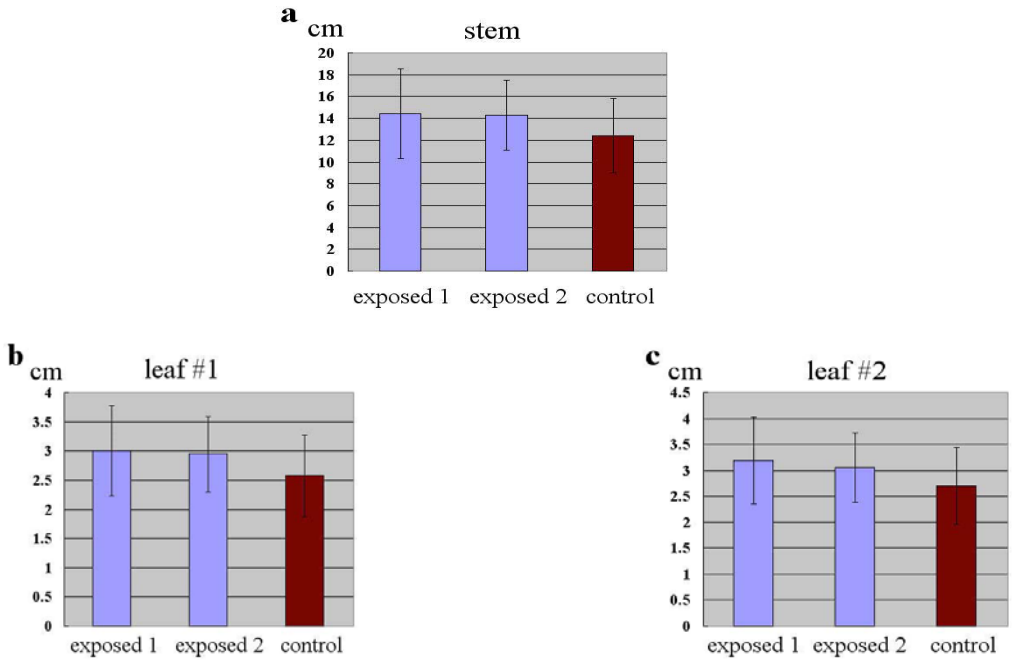


Fig 5 a: The average stem lengths of each group mung beans. b: The average lengths of first leaf of each group mung beans. c: The average lengths of second leaf of each group mung beans.

Table 1

The statistical analysis of three groups mung beans growth

statistics	analyze exposed 1 and control group	analyze exposed 2 and control group	analyze exposed 1 and exposed2 group
P value for stem	0.0054	0.0043	0.4213
P value for leaf#1	0.0025	0.0039	0.3709
P value for leaf#2	0.0017	0.0088	0.2156

4. DISCUSSION

According to the reference levels which were announced by ICNIRP in 1998 is 833mG (f=60Hz) for general public exposure to time-varying electric and magnetic fields, to prevent the influence that may cause to the nervous function of human. However, the experiment results show that the magnetic field intensity is 875±75mG and 155±55mG have an enhancing effect on the growth of mung beans (Smith,1993;Davies,1993; Soja,2003 ;Huang,2007). So, the growth of plant would be modified when plant exposed ELF MF intensity above 100 mG for a long time. The enhancing influence is abnormal phenomenon for growth of plant, because the motion of Ca^{++} ion on the cells of plant is changed (Lednev,1991;Smith,1993). Therefore, we worry about body health would be influenced when human exposed ELF MF intensity above 100 mG for a long

time. We can get magnetic field intensity greater than 100mG (rms value), when to measure home electrical appliances closely (5cm to 10cm away). To use home electrical appliances closely then we would expose higher magnetic field intensity, maybe influence the health of human body. So should avoid exposing ELF MF intensity above 100 mG for a long time in order to reduce the biological effect of extremely low frequency magnetic fields. For electrical appliances and high-voltage line can induce higher magnetic field, we should keep the appropriate distance to protect the health of human body.

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Letter

The Inflation Dynamics of the ASEAN-4: A Case Study of the Phillips Curve Relationship

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ABSTRACT: The conventional Phillips curve argues that there is a trade-off or negative relationship between unemployment and inflation. The aim of this study is to investigate the validity of the Phillips curve for the ASEAN-4 countries: Philippines, Thailand, Indonesia, and Malaysia from 1980 to 2005. Besides unemployment, the relationship of interest rate, exchange rate, and supply shocks to inflation, were also investigated. Using various econometric techniques like Ordinary Least Squares and Instrumental Variables, it was found out that for the ASEAN-4, there seems to be no stable one-to-one trade-off between unemployment and inflation. Variables that could help control inflation were also different for the four countries. For Thailand, the inflation lag, unemployment and oil dummy were significant. As for Indonesia, the interest rate, 1997 East Asian Financial Crisis dummy, and oil dummy were significant in affecting inflation. The OLS regression gave the best linear unbiased estimate for both countries. For the Philippines, serial correlation was detected. Thus, Prais-Winsten method was employed. It was then shown that the unemployment lag, interest rate, and exchange rate lag were significant at the 10% level of significance. [Journal of American Science 2009: 5(1), 55-57](ISSN: 1545-1003)

Key words: Phillips curve; inflation; unemployment; ASEAN

1. INTRODUCTION

The empirical studies on the Phillips curve analyzing the relationship of unemployment rate to the inflation rate are the results of the search for a tool for forecasting inflation and implementing monetary policy. The conventional Phillips curve argues that there is a trade-off or negative relationship between unemployment and inflation (Dornbusch, *et al.* 2005). Economists soon modified the Phillips curve theory to focus on inflation in relation to unemployment. The aim of this paper is to investigate the validity of the Phillips curve for the ASEAN-4 countries: Philippines, Thailand, Indonesia, and Malaysia from 1980 to 2005. Some variables that could affect inflation are also analyzed. Thus, this paper will explore some tools that could aid in the inflation targeting strategies of the ASEAN-4 economies.

2. EMPIRICAL MODEL

I used annual Consumer Price Index, exchange rate (domestic currency per dollar), and money market interest rate data sets supplied by the United Nations Statistical Database (UNSD). For each country, the inflation rate was computed as the percentage change in the Consumer Price Index. That is, inflation rate= $(CPI_t - CPI_{t-1})/ CPI_{t-1} * 100$. All CPI and inflation rates data would have 2000 as the base year (CPI= 100). In addition, since the UNSD only have survey data for unemployment, we acquired more reliable unemployment rates from the National Economic Development Authority of the Philippines website. All of the annual data sets covered the period from 1977 to 2005.

For the empirical model, I modified the equations by Gordon (1997), Dua (2006), Stiglitz (1997), Staiger, Stock, and Watson (1997), and Smith (2000). In the formulation of a simple augmented Phillips curve, I also utilized Wan's (2001) linear model involving lagged inflation and cyclical unemployment as explanatory variables.

For Equation 1, I use the augmented version of Stiglitz's model to capture inflationary expectations by including the lagged inflation rate as a measure of the expected inflation rate. In addition, I include an unemployment lag to determine if such would provide a better fit. I also have additional explanatory variables: interest rate, lagged exchange rate, 1997 East Asian financial crisis binary dummy, and oil shock dummy variable for oil price fluctuations.

$$\pi_\tau = \beta_{0\tau} + \beta_1 unemp_t + \beta_2 \pi_{\tau-1} + \beta_3 unemp_{t-1} + \beta_4 intrate_t + \beta_5 xr_{t-1} + \delta_0 97 + \delta_1 oil + \upsilon_\tau \quad (1)$$

The following are the hypotheses for the signs of the explanatory variables:

- Unemployment, unemp, and unemployment lag, $unemp_{t-1}$, as stated by the Phillips curve, is negatively related to inflation. That is, if the demand for labor increases due to an expansionary monetary expansion, the unemployment rate would fall causing wages/ prices to rise. Thus, creating a trade-off between inflation and unemployment.
- The inflation lag, $\pi_{\tau-1}$, the assumed expected inflation, is positively related to inflation. I assume this using the adaptive expectations theory.
- The interest rate, intrate or mmintrate, is positively correlated to inflation. Increasing interest rates results to higher costs for businesses, which causes prices to rise.
- Due to policy lags, the current exchange rate may be endogenous. Thus, I assume that the exchange rate lag is exogenous and use it in the model. The exchange rate I use is in the form: domestic currency per dollar. I use xr_1 to account for trade prices. I hypothesize that an increase in xr_1, a depreciation of the local currency, would increase inflation because of a higher import prices.
- Binary dummies, 97 and oil, were added to account for price shocks brought by the 1997 financial crisis and oil crises. Such control variables are expected to have a positive sign because they serve as supply shocks. To account for East Asian financial crisis, the years 1997 and 1998 have their 97 dummy equal to one. Meanwhile, the oil dummy for 1980, 1990, and 2005 is equal to unity since oil price fluctuations occurred during those years.

For Equation 2, I use first differencing. This model will only be used if the equation experiences unit root problems. Such unit root behavior was tested using the Phillips-Perron test.

$$\Delta\pi_\tau = \alpha_0 + \Delta\beta_1 unemp_t + \Delta\beta_2 \pi_{\tau-1} + \Delta\beta_3 unemp_{t-1} + \Delta\beta_4 intrate_t + \Delta\beta_5 xr_{t-1} + \delta_0 97 + \delta_0 oil + \upsilon_\tau \quad (2)$$

To have more efficient estimates, I tested Equations 1 or 2 for heteroskedasticity and serial correlation. If either problem exists, corrections are employed to ensure consistent estimates. As will be discussed later, I also used Instrumental Variable method for Malaysia. More specifically, since unit root behavior occurs in the inflation variable, I used an instrument, the inflation lag of Singapore, for the inflation lag of Malaysia.

3. Discussion of Regression Results

Using t-test, with an H_o: $B_j=0$, and a two-sided alternative of H_1: $B_j \neq 0$, the results for Equation 1 can be summarized as follows:

Table 1. Fully-corrected regression results for Equation 1
Dependent variable: Inflation
Significance level: 10%

Explanatory Variable	Indonesia	Malaysia	Thailand	Philippines
Infla_1	-.0488733 (.1096472)	.3483649 (.2066877)	.3773198 (.1637375)	-.0074889 (.1133733)
Unemp	0.2920096 (.7119024)	-.4904054 (.5649236)	-.9443959 (.4813811)	1.164838 (1.157631)
Unemp_1	.0188285 (.8245843)	.3516186 (.5329042)	-.389395 (.5979983)	-2.008291 (.7032352)
Mmintrate	1.200941 (.1402781)	-.060483 (.1525026)	-.3699473 (.3366451)	2.594078 (.2925591)
Xr_1	.0001955 (.0008092)	-1.265882 (1.38491)	-.1813196 (.1655893)	.8965375 (.3458095)
D97	-14.92133 (5.921631)	1.656048 (1.073852)	4.670027 (2.812547)	-3.558471 (3.915062)
Doil	4.907661 (2.690442)	1.596374 (1.078417)	5.304789 (1.658721)	4.543862 (4.269187)
Constant	.5815264 (.9750957)	.07467 (.830654)	-.9713239 (.566889)	-.2621564 (.9492679)
R^2	0.8855	0.4730	0.6918	0.8244
Adj R^2	0.8409	0.2680	0.5720	0.7561
n	26	26	26	26

For Thailand and Indonesia, the OLS regression gave the best linear unbiased estimate (BLUE). For both countries, the error terms have constant variance and have no autoregressive conditional heteroskedasticity (ARCH). There were also no random walk and serial correlation problems. For Thailand, using the adjusted R^2 value, 57.20% of the inflation variation was explained by the model. This is an improvement compared to Equation 1's adjusted R^2 value of 23.44%. Moreover, the inflation lag, current unemployment rate, and oil dummy were significant at the 10% level. The significance of the inflation lag is consistent to the findings of Dua (2006). This may signal that inflation is inertial (Smith, 2000). For Thailand, past inflation influences people's expectations on future inflation. Meanwhile, it was also estimated that a one percentage increase in unemployment, decreases inflation by .94439 percentage points. Such value is very near to one. Thus, I could say that the Phillips curve is present in Thailand using 1980-2005 data. Unemployment and inflation have a trade-off. This finding is similar to that found by Bhanthumnavin (2002). Oil price shocks also influenced Thai inflation values immensely. It was implied that, controlling for all other variables, when there is an oil price shock, predicted inflation is about 5.30 points higher than for a year without an oil price shock (i.e. inflation= doil + constant). That is, when there are oil price fluctuations, inflation increases by about 5.30 + (-.917)= 4.329 percentage points. From these, I could see that as the price of energy rises, the inflation rate will increase as production becomes more expensive. For Indonesia, the significant variables at the 10% significance level were the interest rate, oil and 1997 financial crisis dummies. The dummy for the 1997 East Asian financial crisis, surprisingly, depresses predicted inflation by about 14.92 percentage points. More specifically, ceteris paribus, during the 1997 East Asian financial crisis, inflation decreases by approximately .581- 14.92= |-14.339| percentage points. Such result, most probably, was due to lower productivity growth and aggregate demand in the economy. This finding was similar to that of Vong (2001) in his study of Macau's Phillips curve. Meanwhile, the oil dummy indicated that, in the presence of oil price shocks, ceteris paribus, predicted inflation for Indonesia, is 4.9076 percentage points higher than usual. That is, when there are oil price shocks, inflation increases by .581 + 4.9076= 5.4886 percentage points. In addition to these, it was seen that unemployment and its lag were not statistically significant. The unemployment variables also had positive signs which could indicate that there maybe no trade-off between inflation and unemployment. Being a developing country, it seems to be that Indonesia suffers from both persistent high inflation and high unemployment rates.

For the Philippines, using OLS, it was found out that the unemployment lag, interest rate, and exchange rate lag were significant at the 10% level. For a one percentage point increase in the unemployment lag, inflation decreases by 2.0367 percentage points. Such supports the trade-off between unemployment and inflation as indicated by the Phillips curve. That is, if the demand for labor increased due to an expansionary monetary policy, the unemployment rate would fall. Then, wages and consumer prices will tend to rise. Moreover, the significance of the unemployment lag could indicate that fiscal policies relating to inflation might not have an immediate effect. There could be policy lags. Meanwhile, a percentage point increase in interest rates increases inflation by 2.613 percentage points. In addition, when the exchange rate lag increases by one percentage point, inflation increases by 0.8893 percentage points. This supports our hypothesis that depreciation in the domestic currency makes local goods more competitive. Such increases aggregate supply and results to an increase in the price level. However, even though the Philippines' OLS model gave significant results, it is not BLUE. Using Durbin's alternative test for autocorrelation, with a p-value of 0.0167, at the 10% significance level, there was evidence that the Philippines' Equation 1 regression suffers from serial correlation. Generally, when corrected for serial correlation, I have seen that the standard errors decreased. Although they are characterized by lower coefficients, unemployment lag, interest rate, and exchange rate remain significant. For a one percentage point increase in the unemployment lag, inflation decreases by 2.0082 percentage points. On the other hand, a percentage point increase in interest rates increases inflation by 2.594 percentage points. In addition, a one point percentage increase in the exchange rate lag increases inflation by 0.8965 percentage points.

For Malaysia, the OLS model explains 43.15% of the variation in inflation. Only unemployment was significant at the 10% level. A one percentage point increase in unemployment decreases inflation by 1.543 percentage point. Such finding is still consistent with the OLS estimates of Tang and Lean (2007): that there exists a trade-off between unemployment and inflation in Malaysia. However, while this might support the Phillips curve hypothesis, we should be careful with the interpretation of results. This is because, when tested for unit root behavior using the Phillips-Perron test, with a p-value of 0.1298, it was found out that the past values of inflation were correlated. In addition, the inflation lag might be endogenous. It might be correlated with the

error term. To solve for this problem, I use the inflation lag of Singapore as an instrumental variable for Malaysia's inflation lag. I use Singapore data since I thought that its price levels might be highly correlated with that of Malaysia. Such may be a result of their geographical proximity and trading relations. The simple correlation of Malaysia's inflation lag with Singapore's inflation lag was 0.7162. In addition, when Malaysian inflation lag was regressed with all other exogenous variables and the Singaporean inflation lag, it was found out that Singapore's inflation lag, with a p-value of 0.079, was significant. This supports one of the assumptions for an instrument. The covariance of our instrument, Singapore's inflation lag, and our x_j, Malaysia's inflation lag, is not zero. Meanwhile, I assume that Cov(Singapore_inflation_1, u)= 0. When I used Singapore_inflation_1 as an instrument for infla_1 in our Malaysian OLS model, the inflation lag and unemployment were significant at the 10% level. The inflation lag fulfilled our expected sign. However, again, we could not be sure as to the reliability of these results. Using the Phillip-Perron test, there was an evidence of a highly persistent time series. The past values of inflation are still correlated. Thus, I use Equation 2, the first-differenced model, for our analysis. The regression with Equation 2 showed that there seems to be no significant variables which could affect inflation. Such results might be consistent but not efficient. This is because of the presence of large standard errors caused by either heteroskedasticity or serial correlation. When tested for both stationary and autoregressive conditional heteroskedasticity, the first-differenced model was characterized by homoskedasticity. However, when tested for serial correlation of order 1, AR(1), and higher order correlation using the Breusch-Godfrey LM test for autocorrelation, it was evident that the Equation 2 for Malaysia suffers from serial correlation. With these, we have seen that although differencing could eliminate most of the serial correlation, it has not done so for our model. Most probably, our model suffers from higher order serial correlation. To correct for serial correlation, I use Prais-Winsten estimation. When corrected for serial correlation, the first-differenced equation, Equation 2, had lower standard errors. This shows that the existence of serial correlation produced large standard errors. For the fully-corrected model, it was only the inflation lag, with a p-value of 0.109, which is nearly significant at the 10% level. From the regression results, it can be seen that as the instrumented inflation lag increases by one percentage point, inflation increases by .3483 percentage points. The nearly significant value might have been the result higher order autocorrelation. The model might not have been fully-corrected because I also used Prais-Winsten method—a method which only employs feasible GLS estimation of AR(1). In addition, we should also take note that the FGLS is not unbiased and therefore, is not BLUE. Moreover, although it may be asymptotically more efficient than the OLS estimator in the presence of serial correlation, we cannot fully assume weak dependence because of a small sample size of 26. Another possible reason for our findings is that Singapore_inflation_1 might not be a completely exogenous instrument for Malaysia's inflation lag. Our IV, Singapore's inflation lag might be correlated with the error term. This could happen because Malaysia and Singapore are closely-linked economies. For example, there is a possibility that the exchange rate between the currencies of the two countries is correlated to our IV. Thus, Singapore's inflation lag might not be the best IV for Malaysia's inflation lag. With this, it is recommended, that in future studies, the exchange rate lag, the interest rate lag, and others be tested as possible instrumental variables.

I will now focus our discussion on the theorized Phillips curve relationship: trade-off between unemployment and inflation. I will analyze the signs of the unemployment rate for the four countries without emphasizing their significance at the 10% level. Using the fully-corrected models, it was found out that for Thailand and Malaysia, there exist a trade-off between unemployment and inflation. The negative coefficients for unemployment are the evidences for this. The trade-off is approximately one-to-one for the two countries. Such relationship supports the Keynesian view on the Phillips curve. That is, at least for the short-run, unemployment and inflation have a negative relationship. In contrast, the unemployment coefficients for Indonesia and the Philippines were positive. The findings for the Philippines are consistent with Dua's findings (2007). The positive relationship between unemployment and inflation is supported by Rational Expectations Theory. There may be no trade-off between unemployment and inflation because markets respond quickly to changes in prices and wages.

4. Conclusions and Recommendations

For the ASEAN-4, significant or not, there seems to be no stable one-to-one trade-off between unemployment and inflation. I also found out that the variables which could help control inflation were different for the four countries. Meanwhile, to have more conclusive results and achieve normality, I suggest obtaining a bigger sample size, e.g. usage of quarterly data. In conducting tests, such would give us higher degrees of freedom. In addition, for serial correlation problems, error terms such CPI minus unit labor cost can be used (Smith, 2000). I also suggest a lag for interest rates. There maybe a possibility that the previous year's monetary policy regarding interest rates might have a significant effect on the inflation rate. In addition, to better explain inflation dynamics, stock prices, energy/ petroleum prices, and other functional forms (e.g. quadratic or logarithmic form) can be utilized in future studies. Furthermore, panel data analysis could be utilized. Lastly, cointegration tests may be employed to explore the feedback dynamics of employment- inflation relationship.

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Research Article

Estimating Soil Loss Using Universal Soil Loss Equation (USLE) for Soil Conservation planning at Medego Watershed, Northern Ethiopia

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ABSTRACT: Water erosion is a major part of land degradation that affects the physical and chemical properties of soils and resulting in on-site nutrient loss and off-site sedimentation of water resources in arid and semi-arid areas of Ethiopia. The heavy reliance of some 85 percent of Ethiopia's growing population on an exploitative kind of subsistence agriculture is a major reason behind the current state of land and soil degradation. Tackling on-site effects of soil erosion requires understanding of the rates of soil loss as well as identification of the major controlling factors that enhance or retard these processes. Therefore, the objective of this study was to predict the amount of soil loss in different landforms and land uses using USLE which is modified and adapted to Ethiopian conditions, at Medego watershed, northern Ethiopia. This study was conducted after massive SWC practices have been implemented in the past 15-year in the study watershed. Primary data and secondary data were collected related to the factors that influence soil loss estimated by USLE and for area description. The land surfaces in the watershed is mainly a reflection of past erosion processes as indicated by many researchers. In this study, the lowest soil loss is estimated on flat plains (< 2% slope) about 1.59 tons ha^{-1} y^{-1}, which is less than the minimum tolerable soil loss (2 tons ha^{-1} y^{-1}) for the country. However, the highest soil loss is from steep slopes (30-50%) which is 35.43 tons ha^{-1} y^{-1}, about twice the maximum tolerable soil loss (18 tons ha^{-1} y^{-1}). The average soil loss rate at watershed level is 9.63 tons ha^{-1} y^{-1} about half of the maximum tolerable soil loss. The implication is the contribution of the implemented SWC measures in decreasing the rate of soil erosion is encourageable as compared to the results related to high soil loss estimated in the past studies i.e., before massive SWC implementation. However, the present value indicates still a need for wise SWC planning that decreases the amount of soil loss in the watershed at least below the maximum tolerable soil loss rate of the country. Therefore, to maximize the available resources in targeting the effect of water erosion on soil loss, those landforms and land uses having large rate of erosion should be given first priority during the introduction of intensive and well designed SWC interventions at Medego watershed, northern Ethiopia. [Journal of American Science 2009: 5(1), 58-69] (ISSN: 1545-1003)

Key words: Medego watershed, northern Ethiopia, soil loss, tolerable soil loss.
Abbreviations: SWC- soil and water conservation, USLE-Universal Soil Loss Equation.

1. INTRODUCTION

Growing degradation and loss of soil means that the expanding population in many parts of the world is pushing this resource to its frontier. In its absence, the biospheric environment of humans would collapse with devastating effects on humanity. Judson (1965) was one of the first geologists to assess the world soil erosion. He estimated that the amount of river-borne soil carried into the oceans had increased from 9.9 billion tons a year before the introduction of agriculture, grazing and related activities, to the present rate of 26.5 billion tons a year. Hydrologists estimated that one-fourth of the soil lost through erosion in a watershed actually makes it to the ocean as sediment (FAO/UNEP, 1978). The remaining three-fourths are deposited on foothill slopes, in reservoirs, in river plains and other low-lying areas or in the river-bed itself, which often causes channel shifts. In an overview of global erosion and sedimentation, Pimental (1995) stated that more than 50% of the world's pastureland and about 80% of agricultural land suffer from significant erosion.

The causes of land degradation are complex and have diverse nature and dimensions, depending on peculiarities of different countries,

influenced as it is by a combination of natural and socio-economic-cultural factors. In Ethiopia, the heavy reliance of some 85 percent of Ethiopia's growing population on an exploitative kind of subsistence agriculture is a major reason behind the current state of land degradation. Moreover, land degradation is a long-term process in which the effect and steady expansion is hardly noticed until it manifests itself with disastrous drought and famine. Most studies indicate that sheet and rill erosion by water and burning of dung and crop residue are the major components of land degradation that affects on-site land productivity (Hurni, 1993; Zeleke et al., 2001).

Water erosion is a major part of land degradation that affects the physical and chemical properties of soils and resulting in on-site nutrient loss and off-site sedimentation of water resources in arid and semi-arid areas like Ethiopia (Boardman, 1998; Lal, 1999; Bartsch et al., 2002; Emrah et al., 2007). The off-site effects of erosion such as reservoir sedimentation and water resources pollution are usually more costly and severe than the on-site effects on land resources (Phillips, 1989). Therefore, proper management of on-site effect of soil erosion could reduce the risks and negative impacts of down stream water resources due to water erosion. Tackling the on-site effects of soil erosion requires an understanding of the rates of erosion processes as well as identification of the major controlling factors that enhance or retard these processes. The knowledge of "what are the factors and where" may help to distinguish the potential causes and the associated reasons behind the respective causes even though this may not be enough to design site-specific management, as the factors playing a major role in erosion may be widely distributed within watersheds (Ferro et al., 1998; Mirco et al., 2003).

Soil erosion by water and its associated effects are recognized to be severe threats to the national economy of Ethiopia (Hurni, 1993; Sutcliffe, 1993, Tamene, 2005). Since more than 85% of the country's population depends on agriculture for living, physical soil and nutrient losses lead to food insecurity. Hurni (1990, 1993) estimates that soil loss due to erosion in Ethiopia amounts to 1493 million tons per year, of which about 42 tons ha^{-1} y^{-1} is estimated to have come from cultivated fields. This is far greater than the tolerable soil loss as well as the annual rate of soil formation in the country. According to an estimate by FAO (1986), some 50% of the highlands of Ethiopia are already 'significantly eroded,' and erosion causes a decline in land productivity at the rate of 2.2% per year. The study also predicted that by the year 2010, erosion could reduce per capita incomes of the highland population by 30%. Hence, soil and water conservation measures have been implemented to alleviate both problems of erosion and drought, which are symptoms of two different extremes of rainfall conditions since the 1980s in the country. However, so far, little or no sufficient documented information has been available on the contribution of the different SWC measures implemented on soil loss reduction since the last 15-years at the study watershed in the semiarid areas of Ethiopia as compared to the tolerable soil loss determined by Hurni (1985) to Ethiopia condition. Such information is vital to take additional measures and soil conservation planning at the watershed and other similar areas in the semiarid areas. Therefore, the purpose of this study was to estimate the amount of soil loss in different landforms and land uses using USLE at Medego watershed, northern Ethiopia.

2. MATERIALS AND METHODS

2.1. Study Area Description

The study was carried out at Medego watershed in the administrative unit of Lalay-maychew district in Tigray region, northern Ethiopia (Figure 1), from August 2007 to July 2008. Its altitude ranges from 2000 to 2720 m above sea level. The study area is bounded by latitudes N14^{o}05.955'and 14^{o}05.937', and longitudes E038^{o}42.352' and 038^{o}42.333'. The total area of the watershed is about 1091.5 ha as delineated using Geographical Positioning System (GPS) during the field study. The study watershed is characterized by different landforms which are ranged from flat plains, undulating plains and rolling land to steep mountains and very steep escarpments. The description of the topography is adopted the slope capability classification made by (Chekun, 2002), and the slope ranges and area coverage of each landform was recorded at field using clinometers and GPS, as it is presented in Table 1. The geological setup of the watershed is originated from volcanic. However, alluvial deposits at flat lands are also found in the watershed. The soil type at the study watershed is quite different along the slope. At steep slopes, coarse earth materials, gravels and boulder are dominated where as at flat

plain, the largest portion of the study area is covered by clay loam soil and the smaller portion laid on clay and sandy soil textures. The main soil types are cambisols on undulating plains and rolling landforms; lithosols on hilly and steep to very steep lands and vertisols are found on the flat plateau plains of the watershed (BoARD, 2007).

The number of households and total population at Medego watershed is 397 and 1537, respectively. The land holding size of most farmers in the study area is less than 1.3 ha. The watershed has uni-modal and erratic rain fall patterns. The rainy season is very short and extends from June to first week of September. The mean annual amount of rain fall ranges from 600 - 700 mm from historical rainfall data. The mean monthly temperature during the growing season ranges from 15 - 20 ^{o} C (BoARD, 2007). According to the BoARD (2007), the farming system of the study watershed is principally crop oriented. Tef cultivation *(Eragrostic tef)* account for the majority of arable lands and followed by wheat *(Triticum vulgare)* crop. Other crops such as faba bean *(Vicia faba)*, field pea *(Pisum sativum)*, lentil *(Lens culinaris)*, chick pea *(Cicer arietinum)*, flax *(Linum usitatissimum),* barley *(Hordeum vulgare)* and maize *(Zea mays)* are also important crops in the farming system. Irrigation is also widely practiced at Medego watershed. In spite of the fact that the high crop diversification in the watershed, it observed that there is still a room to improve the crop productivity. Livestock rearing is also an integral part of the farming system, though the number of livestock in the watershed area is reduced from time to time due to animal feed shortage. According to farmers view, cattle are kept mainly for draught power and milking; goat and sheep are kept for live sale; and equines (donkey, mule, horse, camel) for transportation. The study indicated that 83% of the households in the watershed have some livestock. Of these, 75% are cattle (average of 2 cattle per household), 21% are sheep and goats and the rest is covered by poultry and equines.

It was observed that the vegetation in Medego watershed in general is sparse and has been overexploited for long time and at this time consists of shrubs and bushes of little economic value. The available vegetation species in the study area include seraw (*Acacia etbaica*), chea' (*Acacia abyssinica*), acacha (*Acacia decurrence*) and Awhi (*Cordia africana*) on uncultivated land; and momona (*Acacia albida*), tambock (*Croton machostachys*), keyih bahrizaf (*eucalyptus comoldulensis*) and some 'seraw (*Acacia etbaica*) on cultivated and marginalized areas. Leucena (*Leuceana leucacephala*), sesbania (*Sesbania sesban*) and some other grasses are commonly found in the gully of the watershed. Farmers' used such vegetation for the purpose of farm implements, house construction and furniture, fuel wood, soil and water conservation measures and fencing (Table 2). But most farmers have no or little awareness on the function of these tree species for soil and water conservation as compared to the other uses. Hence, awareness creation to farmers in the watershed and other areas should be done in order to the farmers give attention on planting and managing tree species from different perspectives including soil and water conservation, soil fertility improvement.

2.2. Methodology

Primary and secondary data were collected at Medego watershed related to the assessment of SWC measures on soil loss at Medego watershed. Primary data were gathered by topographic transect walk, measuring of input data, informal discussion and observation. The secondary data include climate, demographic and other related data were collected from Bureau of Agriculture and Rural Development (BoARD) at the administrative unit. These data were used to estimate soil loss after tremendous activities of SWC measures have been implemented at Medego watershed, northern Ethiopia. The rapid rural appraisal technique of the topographic transect walk method was employed for its effectiveness in the assessment of the natural resource base and topography of the watershed. In order to obtain as much information as possible, the transect walk was applied in two direction, east to west and south to north. In both directions, the transect walk started at the top edge of the watershed and went all the way across to the other end of the watershed. During the transect walk, observations and estimates of vegetation type and density, and impact of the existing soil and water conservation measures were observed. These were followed by recording land-use types, soil color, soil depth, soil drainage condition, slope gradient and length. The transect walk also provided an opportunity for informal discussions with farmers working on their plots.

Annual soil loss in the form of runoff from different land forms and land uses of the watershed was estimated using the Universal Soil Loss Equation (USLE) (Wischmeier and Smith,

1978) and modified and adapted to Ethiopian conditions by Hurni (1985) and Gebreselassie (1996) as follows.

$$A = R * K * L * S * C * P$$

Where; A = estimated soil loss (t ha^{-1} yr^{-1}), R = Rainfall Erosivity factor, K = Soil Erodibility factor, L = Slope length factor, S = Slope gradient factor, C = Land cover factor, P = Management practice factor

The R-factor is defined as the product of kinetic energy and the maximum 30 minute intensity and shows the erosivity of rainfall events (Wischmeier and Smith, 1978). However, in this study, to determine the value of the R-factor, the average of annual historic rainfall event (10-years) was collected from meteorological station located at 8-Km distance from the watershed. Then the R-value corresponds to the mean annual rainfall of the watershed was found using the R-correlation established in Hurni (1985) to Ethiopia condition. Therefore, the annual R-factor for the average rainfall (650 mm) at the watershed as extrapolated from Hurni (1985) is 357. The soil erodibility (K), slope length (L), slope gradient (S), C, and P-factors of USLE for the entire watershed based on landforms and land use is presented in Table 3.

The K-factor is defined as the rate of soil loss per unit of R-factor on a unit plot (Renard et al., 1997). To determine the value of the K-factor, a systematic observation on soil color of watershed was carried out, based on the approach described in Hurni (1985). This was done by classifying the watershed into similar land uses and land forms (Table 1). For soils having different color in the same land use and landform, the K-factor was taken as their mean value of these colors as it is described on Hurni (1985). As an example, the K-factor for flat plains in Medego watershed is the mean value of the soil color black (0.15) and brown (0.2), which is about 0.18; and the same approach was used in determining the soil color for the other landforms in the watershed (Table 3). SL is the topographic factor expressed as the expected ratio of soil loss per unit area from a field slope to that from a unit plot under otherwise identical conditions. Slope length and slope gradients factors were recorded using meter tape and clinometers, respectively, in the watershed on different landform and land uses. It is taken the weighted value of the slope gradient and slope length range measured at the field for each landform and land use and so extrapolated based on Hurni (1985) to Ethiopia condition (Table 3 and 4).

The C-factor is defined as the ratio of soil loss from land with specific vegetation to the corresponding soil loss from continuous fallow (Wischmeier and Smith, 1978). Assessment of the type of land use-cover was made separately for each land unit and the corresponding land cover was obtained from Hurni (1985) which was developed to Ethiopia condition. For variations in land cover with specific land unit or landform, the C-factor was obtained using weighted value of the different land cover (Table 3).

The P-factor gives the ratio between the soil loss expected for a certain soil conservation practice to that with up-and down-slope ploughing (Wischmeier and Smith, 1978). Specific cultivation practices affect erosion by modifying the flow pattern and direction of runoff and by reducing the amount of runoff (Renard and Foster, 1983). In areas where there is terracing, runoff speed could be reduced with increased infiltration, ultimately resulting in lower soil loss and sediment delivery. Values for this factor were assigned considering local management practices and based on values suggested in Hurni (1985). Management factors were obtained by assessing the different supporting practices in the study watershed and it was taken the weighted value for similar land forms and land uses types (Table 3). The data related to management practices were collected during the field work. The presence and status of conservation activities were assessed with emphasis on the existing conditions of terraces and protected areas. Most of the areas in the watershed are well-terraced, mainly the upslope parts. However, most of the terraces are broken due to high runoff and/or livestock trampling in many parts of the watershed.

The data were analyzed following the interpolation of the values of USLE in Hurni (1985) and Gebreselassie (1996) to Ethiopia condition. The data was then interpreted qualitatively and using descriptive statistics.

3. RESULTS AND DISCUSSION

3.1. Estimated Soil Loss Using USLE at Medego Watershed, Northern Ethiopia

In spite of the fact that tremendous efforts of SWC have been implemented, their contribution in reducing soil loss due to water erosion demands recent assessment for appropriate future conservation planning. It is understood that heavy rainfall cause severe soil erosion in agricultural fields of the semiarid regions of Ethiopia. Soil erosion in agricultural fields affects not only land productivity but also the water environment in the down stream. Many investigations have been conducted for the development of prediction methods of water-induced soil erosion processes. Among the methods, the empirical Universal Soil Loss Equation (USLE) has been applied broadly for predicting the average annual soil loss from upland fields in Ethiopia (Wischmeier and Smith, 1978; Hurni, 1985) for the reasons described in the discussion part of this paper.

The soil loss estimated using USLE on this study from cultivated land on flat plain land form (< 2% slope) of Medego watershed, northern Ethiopia is the lowest as compared to the other land uses or landforms, which is 1.59 tons ha^{-1} y^{-1} (Table 4). This indicates that soil loss due to rill and inter-rill erosion is almost balanced by deposition within the flat landforms of the watershed. Next to the flat land form, the landforms having lower soil erosion are undulating plains (slope 2-8%) and flat- flood prone areas (< 2% slope) which are 3.13 and 4.87 t ha^{-1} y^{-1}, respectively. The highest soil loss at the study watershed was recorded at the landform -steep mountains (slope 30-50%), which is 35.43 tons ha^{-1} yr^{-1}. The small soil loss rate of the landforms is related to the factors of the USLE in the watershed (Table 4). Therefore, more attention should be given to slope ranges between 30-50% while SWC measures is planning to implement in the watershed.

The general trends of the finding indicate that soil loss increases as the slope steepness increases in the watershed (Table 4). However, at the landform of very steep mountains (> 50% slope), the annual soil loss is estimated as 7.63 tons ha^{-1}, which is even less than the landforms such as rolling land forms (8-15% slope), hill landforms (15-30% slope) and steep mountains (30-50% slope). This is because these slope ranges are susceptible to daily human interferences such cultivation and grazing as compared to very steep slopes (> 50%) and also slopes having more than 50% in the watershed have land cover of 'Bad Lands Hard' and stone cover which can retain the impact of the kinetic energy of raindrops and at the same time decrease runoff amount. Moreover, there are rock-out crops, which are difficult to detach or transport by raindrops and water erosion on the very steep escarpment of Medego watershed. Landforms more than 50% slopes are protected areas in the watershed. The C-factor represents resistance of the ground surface to the transport of water-soil mixture on the very steep mountains of the watershed includes badlands hard, and bushes and shrubs which dissipate the force of the raindrops. The P-factor stands for erosion inhibition effect, and reflects partly awareness and control measures implemented to minimize soil erosion more than the other landforms by the community (Table 3). It is also noted that the lower slope landforms are susceptible to daily human interferences where as the steepest landforms are protected areas. This proves that the USLE is useful for assessing the adequacy of conservation measures and management practices in agricultural watersheds.

The average annual soil loss estimated by USLE from the entire Medego watershed is 9.63 tons ha^{-1}. If we interpret the annual soil loss as a proxy to watershed erosion, it is possible to see that the magnitude of annual soil loss reported in Table 4 is generally higher than the tolerable soil loss of 2 – 18 tons ha^{-1} y^{-1} estimated for Ethiopia by (Hurni, 1985) except flat landforms of the watershed. The soil loss rate in all the landforms are below the maximum tolerable soil loss for Ethiopia condition, which is 18 tons ha^{-1} y^{-1}, except the steep mountains (slope 30-50%) landforms that indicate almost double of the maximum soil tolerance value. In general, the average soil loss in the watershed in about half of the maximum tolerable soil loss and five times the minimum soil loss tolerance value given by Hurni (1985). The implication is that there is a need to integrate a sound management practices so that to decrease the amount of soil loss in Medego watershed, northern Ethiopia below the maximum as well as the minimum soil loss tolerable value for the country.

As compared to the soil loss estimated for Ethiopia as 42 tons ha^{-1} y^{-1} from cultivated fields by Hurni (1990, 1993); 21 tons ha^{-1} y^{-1} (Machado et al. 1995), and 30-80 tons ha^{-1} y^{-1} (Tekeste and Paul, 1989) in Tigray region, northern Ethiopia, the soil loss estimated on this study in 2007/08 is by far the smallest. The results of the present study as compare to past findings indicate that the amount of soil loss from a given unit of land is low. This could be due to the contribution of the

different soil conservation interventions implemented for at least the last decades in the country in general and the study watershed in particular. This related to the fact that SWC intervention increases soil moisture, fertility and decrease slope factor and thereby enhance the availability of vegetation covers. The combined effect of such factors will be decreasing the impact of raindrops, detachment and transporting of soils. This was evidenced by the opinion of the respondents which evaluated as less soil erosion after the soil conservation practices were built at the watershed as compared to before the implementation (data not presented here). Therefore, as noted in the above, the soil loss estimated by different scholars has showed discrepancy for the same environment (semi-arid region of Ethiopia). This implies that there is a need to have site specific (watershed level) information on soil erosion in order to support timely information for decision makers so that to plan the correct soil conservation planning. In doing so, it is categorized the severity of erosion in the study watershed's landforms as follows.

According to Singh and Phadke (2006) classes of soil loss range (*very slight, slight, moderate, severe and very severe*), the mean annual soil loss (9.63 tons ha^{-1}) from Medego watershed, northern Ethiopia is categorized under *slight class* of soil erosion (5 – 9.99 tons ha^{-1} y^{-1}). According to them, the only part of the watershed landforms having *very slight class* of soil loss (0 - 4.99 tons ha^{-1} y^{-1}) are the flat plains, undulating plains and the flat-flood prone areas; and followed by *slight soil loss* (5 – 9.99 tons ha^{-1} y^{-1}) for the very steep escarpment of the watershed; and *moderate soil loss* class (10 – 24.99 tons ha^{-1} y^{-1}) on rolling to hill landforms of the watershed, where as *severe class of soil loss* (25 – 44.99 tons ha^{-1} y^{-1}) was estimated using USLE on slopes 30-50% (Table 4). This doesn't mean that to give less attention to those landforms with very slight to slight soil loss classes in the study watershed but this is to indicate that parts of the watershed landforms that need high priority for SWC implementation using the available existing resource. This is because; it may be worth noting that nature takes 200–400 years to build up 1 cm of top soil (Pimental 1995) but thousands tons of soil are lost in a season from a watershed . He also reported that each millimeters of cultivated soil loss could cost 10 kg of nitrogen and 2 kg of phosphorus per ha. Hence, this study suggests for effective control of soil erosion at specific area which would occur under alternative management strategies and practices in order to minimize the costs related to fertilizer and environmental rehabilitation.

4. DISCUSSION

4.1. Soil Erosion Models and Their Potentials and Challenges

Soil erosion is the most serious causes of land degradation have influenced tremendous pressure on productivity and environmental stability of arid and semiarid areas. Serious impacts led the demand for conservation and management measures to reduce the magnitude of soil loss and the extent of its associated impacts in many parts of the arid and semiarid areas. There are many models in existence estimating soil erosion. The USLE has the advantage of being less data demanding than other models. A wide range of models that differ in their data requirement for model calibration, application, complexity and processes considered are available for use in predicting soil loss (Merritt et al., 2003). Physically based spatially distributed soil erosion models can be used to quantitatively determine the amount of soil loss from watersheds and also to identify critical soil loss source areas (De Roo, 1998; Emrah et al., 2007). The successful application of such models, however, depends on the availability and quality of data for calibration and validation (De Roo, 1998; Stefano et al., 1998; Takken et al., 1999). Such problems are more pronounced in developing regions where data availability is scarce, existing data are not easily accessible and data collected and stored are mostly in different formats. In addition, more complex models do not necessarily perform better for watershed-scale management purposes, mainly because input errors can increase with increasing model complexity (Favis-Mortlock, 1998; Mitas and Mitasova, 1998a; Jetten et al., 2003; Merritt, et al., 2003).

Empirical models are frequently used in preference to complex physically based models as they can be implemented in situations with limited data and parameter inputs, particularly as a first step in identifying sources and rate of soil loss (Merritt et al., 2003). However, such models

cannot be directly applied to environments other than those for which they were developed, and extrapolation of results from larger-scale plot-level to small-scale watershed level application is difficult. It is, therefore, necessary to identify models that are not very much simplified and under-represent the physical basis or not too complicated and very expensive to implement. The best example is USLE, which is identified and fit to apply in the case of the study area of Medego watershed, northern Ethiopia. The USLE is an empirically based model developed in the United States by using data on soil erosion rates. This equation has certain limitations but still is the best available method which is used most widely for estimating soil losses as average annual mass per unit area as a function of the major factors affecting sheet and rill erosion in data scarce areas of developing countries. As all landscape positions are not equally sensitive to erosion, one important approach to tackling the problem of erosion could be identifying where the sources of most of the soil loss are in a watershed (Dickinson and Collins, 1998; Kim et al., 2007). Identification of potential areas of erosion for appropriate management interventions to tackle the major causative factors at their specific locations is, therefore, imperative from an economic, management and sustainability point of view. This study was attempted in indicating the areas or landforms of high soil loss in Medego watershed, northern Ethiopia.

4.2. Soil Loss and the Influencing Factors in Medego Watershed

It is a fact that environmental degradation has been a problem in Tigray region, northern Ethiopia. The land surfaces in the region is mainly a reflection of the past erosion processes. The main causes of soil erosion in the area among others were out lined by different researchers ([Hurni, 1985; Gebresilassie, 1996, Tilahun, 1996; Tamene, 2005) and even witnessed by farmers as over-cultivation, deforestation, over grazing, steep topography, high rainfall intensity, unwise land use and management. This is evident by the huge amount of soil loss, by water erosion and very low productivity of the farm lands. Therefore, to rehabilitate the environment and enrich it to a meaningful level, a concerned effort on SWC program has been carried out by the community coordinated by of bureau of agriculture under the umbrella of the Tigray Regional Government, northern Ethiopia. In the name of SWC program, various types of physical and biological SWC measures have been undertaken in the study watershed. These activities are: watershed treatment as area enclosure, afforestation, trench; reclamation of big gullies using check dams, biological; moisture harvesting techniques on farms and degraded grazing lands like soil, stone and trench bunds; and soil faced stone bund on hillsides.

Soil loss in different landforms of the study watershed is influenced by erosion factors differently. For instance, the soil erodibility (K) factor of the landforms in the watershed is a function of soil texture, drainage condition and soil depth. These sub-factors can influence the soil color, which determined the value of K-factor in USLE, adapted from Hurni (1985). The landforms in the watershed have different in texture, drainage condition, soil depth, soil color, land cover, erosion controlling management practices and slope factors (Table 3). Fine texture soils are dominated on flat land areas where as coarser textural class increases with increasing steepness. The same trend was observed for the soil depth with deeper soil on flat areas and shallow soil on high slope gradient landforms. The drainage condition is extremely high on steeper landforms and poor on flat area of the watershed. Therefore, the principle of Hudson (1992) that describe as fine soil particles resist to detachment by raindrops but they are susceptible to transport easily is soil drainage dependent. This is because if the landform is poor in drainage, so the probability of transporting by waters the fine particles long distance leaving the original area is too low. Transportation and deposition processes are almost balanced in such occasions. Drainage is affected by the slope factor. That is why soil loss estimated on flat landform is below the minimum tolerable soil loss (2 ton ha^{-1} yr^{-1}) determined by Hurni (19985) for Ethiopia condition. This is the lowest soil loss as compared to the other landforms in the watershed (Table 4). Sand dominant soil textures are common on higher slopes of the watershed. Even though they are coarser to transport as compared to clay texture due to high soil drainage condition of steep slopes, they are susceptible to erosion in the watershed.

Of course, the management practices in the watershed also play its own great role in the magnitude of soil loss. Landforms with well land cover indicated less soil loss. Because it dissipates the energy from rain drops and also decreases the volume and velocity of runoff effect. Soil loss estimated from landforms with very step slope (> 50%) in the study watershed is smaller than slopes

in the rage of 15-30% and 30-50%. The reason is cover factors and land managements factor are better in the very steeper slopes of the watershed. This includes less human and livestock interferences, intensive terraces and relatively better vegetation cover of bushes and shrubs. Therefore, the overall implication of this study is that after the implementation of SWC measures the amount of soil loss in a given land unit is decreased in many parts of the landforms by more than 50% in the watershed as compared to the high values indicated in the past studies in northern Ethiopia (e.g., Hurni, 1985; 1990; 1993; Tekeste and Paul, 1989; Gebreselasie, 1996)). However, the present soil loss amount has also a significant influence on the overall productivity of the study watershed unless the correct measures on the targeted landforms are undertaken. This is because as compared to the soil formation in the region which is not more than 2 ton ha^{-1} yr^{-1} (Hurni, 1985); the present soil loss estimated in Medego watershed, northern Ethiopia is not neglected or it is very big. Therefore, base on the landforms identified in this study, soil conservation planning should be undertaken to address the problem of erosion in areas having large soil loss as areas of prioritization in the future.

5. CONCLUSION

The entire watershed area experienced intensive rainfall which coupled with steep gradient slopes, cause highly erosive runoff as in many other arid and semi-arid areas of Ethiopia. It is this high runoff and soil detachment that is responsible for the high rate of soil erosion at Medego watershed, northern Ethiopia that range from 1.59 – 35.43 tons ha^{-1} y^{-1}. There is a need to regulate this soil loss by all possible means so as to decrease the existing amount of soil loss and enhancing watershed rehabilitation and productivity. Suggested watershed rehabilitation as long and short-term measures should be included the following: As long-term measures re-vegetation of denuded hill slopes with trees and perennial grasses such as vetiver strips and belts; introduction of an agro-forestry program that is compatible with crop, livestock; and forestry development; where as short-term soil and water conservation measures are given due attention to: cut-off drains which need to construct that intercept runoff; constructing and maintenance stone and soil bund and trenches on proper slopes and soils and integrating with vegetation intensively. This has to include interventions such as inter-bund management, bund stabilization, buffer zone establishment and re-bank re-vegetation; and gully control by both vegetative and structural measures should be being intensively implemented.

As a result of the implementation of SWC, the hydrological behavior of the watersheds is improved such as base flow in streams and springs increased, sediment load to reservoirs reduced, crop yield improvement due to soil moisture enhancement, vegetation cover improvement and increased availability of forage for livestock were observed. These are some of the indicators of the effectiveness of the implemented soil and water conservation practices in the study watershed. However, maintenance of the existing SWC and introducing additional appropriate land management practices and rules should be given attention by concerned bodies in order to decrease and totally stop the rate of soil loss and then to increase the total biomass production in the watershed, even though the biggest rate (35.43 tons ha^{-1} yr^{-1}) of soil loss is coming from landforms having slopes 30-50% and the lowest soil loss is from slope less than 2%. Therefore, to maximize the available resources in targeting the effect of water erosion on soil loss, those landforms and land uses having large rate of erosion should be given first priority during the introduction of intensive and well designed SWC interventions at Medego watershed, northern Ethiopia.

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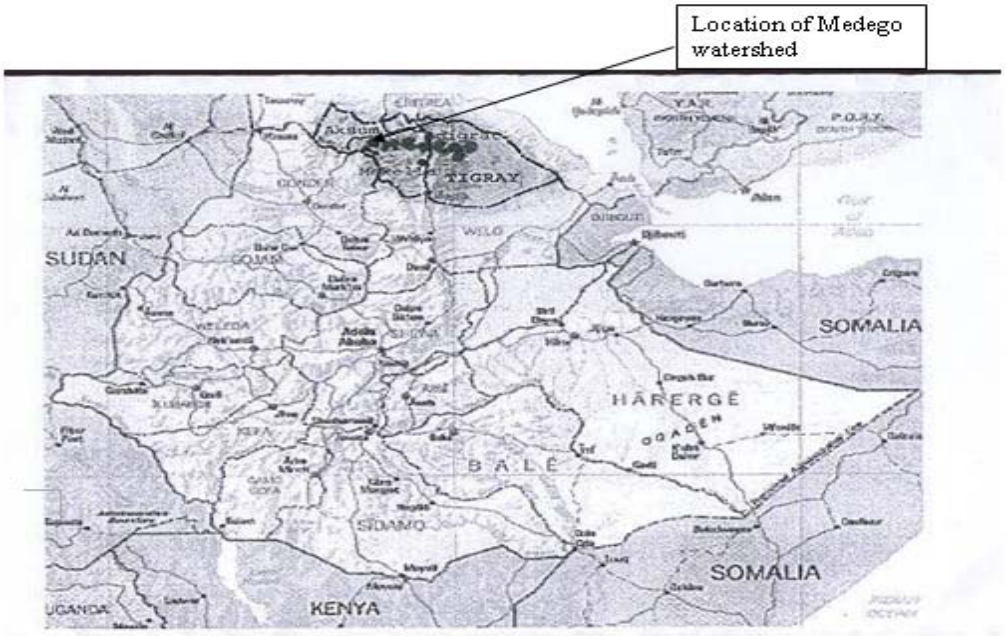

Fig 1: Map of Ethiopia with Tigray region and Medego watershed outlined.

Table 1

Land forms and their area coverage in Medego watershed, Lalay-Maychew district, northern Ethiopia.

land forms/ features	Slope range (%)	Area coverage (ha)
Flat plains	< 2	200
Undulating plains	2-8	300
Rolling land	8-15	50
Hill to rolling	15-30	290
Steep Mountains	30-50	200
Very steep escarpment	>50	50
Flat, flood prone area	< 2	1.5
Total		1091.5

Table 2.

Farmers own ranking of important tree and shrub in the order of importance at Medego watershed, Lalay-Maychew district, northern Ethiopia.

Column number*	1	2	3	4	5	6	7	8	9	10	11	12
Species use for	Soil improve-ment	fodder	Bee forage	fencing	Fire wood	charcoal	Construc-tion	Hand tools	Soil conserve-ation	shading	total	Score/rank
Che'a/*Acacia abyssinica*	1	3	4	4	4	2	2	4	3	3	30	4
Seraw/*Acacia etbaica*	3	2	1	2	5	5	1	4	3	3	29	6
Acacha/*Acacia decurrens*	3	5	4	3	4	3	4	1	3	4	34	1
Eika/*Agave sisalana*	-	1	2	5	-	-	1	-	5	-	14	11
Ere/Aloe berhana	-	1	4	4	-	-	-	-	5	-	14	11
Tebeb/*Becium gradiflorum*	4	-	5	-	2	-	1	-	3	-	15	9
Agam/*Carrissa edulis*	2	1	-	3	3	1	1	1	2	1	15	9
Tahsus/*Dodonea angguistifolia*	3	2	3	3	3	4	3	5	4	3	33	3
Bahrizaf/*Eucalyptus comoldulensis*	3	5	2	1	4	3	5	3	2	2	30	4
Kulkual/*Euphorbia abyssinica*	-	-	3	5	1	-	3	-	4	4	20	7
Awlie/*Olea europea*	2	1	2	1	5	5	5	5	3	5	34	1
Beles/*Opuntia ficus indica*	-	5	2	4	-	-	-	-	5	-	16	8

*- = no/zero value; *(1-5) = 1 with the lowest value and 5 with the highest value for column 1 up to 10 but it is the reverse of this in column 12 which is the top rank starts with 1 and the last/lowest rank ends with 11. Column 11 is sum of columns 1 up to 10.*

Table 3
Selected soil characteristics, average slope length (L) and gradient (S), land cover (C) and management (P) practices collected for different landforms at Medego watershed, northern Ethiopia

Land form	Soil color	Ave. L (m)	Ave. S (%)	C-factor*	P-factor*	Soil depth	texture	drainage
Flat plains	Black and brown	25	2%	Ethiopian teff (0.25)	Ploughing on contour (0.90)*	deep	Clay loam	poor
Undulating plains	Black and red	22	5	Cereal, Ethiopian teff and degraded grass (0.15)	Ploughing on contour and strip cropping (0.73)	Moderately deep	Sandy loam	moderately
Rolling land	Brown and red	18	12	Badlands soft, pulses and degraded grass (0.2)	Ploughing on contour, stone cover 40% and terraces (0.73)	shallow	Sandy silt	High
Hill to rolling	Brown and yellow	25	15	Scattered forest, degraded grass, badlands soft and bush (0.13)	Stone cover 40%, protected areas and terraces (0.63)	Very shallow	sandy	Extremely high
Steep mountains	Red and yellow	15	40	Scattered forest and badlands soft (0.22)	Stone cover 80%, protected areas and terraces (0.53)	Extremely shallow	sandy	Extremely high
Very steep mountains	yellow	10	60	Badlands hard and bushes/shrub (0.04)	Stone cover 80% and terraces (0.53)	Extremely shallow and rock out crops	Coarse sand	Extremely high
Flat, flood prone area	black	20	2	Dense forest and Ethiopian teff (0.13)	Dense intercropping (0.7)	Very deep	clay	Extremely poor

* Numbers in parenthesis are the mean values of more than two C or P practices based on interpolation from Hurni (1985) and Gebresilassie (1996).

Table 4.
KLSCP factors values adapted based on Hurni (1985); Gebreselasie (1996) to land forms and land use at Medego watershed, northern Ethiopia. R factor is 357 for all landforms in the watershed.

Landform	land use	K - factor	L - factor	S- factor	C- factor	P- factor	tons $ha^{-1}yr^{1}$
Flat plains	cultivated	0.18	1.1	0.1	0.25	0.90	1.59
Undulating plains	cultivated; some grazing	0.20	1.0	0.4	0.15	0.73	3.13
Rolling land	cultivated; some grazing	0.23	0.9	1.0	0.20	0.73	10.88
Hill to rolling	scattered natural forest, reforestation, grazing and area closure, some marginal area	0.25	1.1	1.6	0.13	0.63	12.86
Steep mountains	grazing area, some closed area, marginal area	0.28	0.8	3.8	0.22	0.53	35.43
Very steep mountains	Protected area with bushes/shrubs	0.30	0.7	4.8	0.04	0.53	7.63
Flat, flood prone area	Rain fed and irrigated land, dense eucalyptus trees and check dams and biological measures in gullies	0.15	1.0	0.1	0.13	0.70	4.87
Mean soil loss							9.63

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Research Article

Conservation through *in vitro* method: A case of plant regeneration through somatic embryogenesis in *Quercus semecarpifolia* Sm.

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An efficient and reproducible protocol for *in vitro* propagation via somatic embryogenesis (direct as well as indirect) induced on cotyledon halves (with embryo) taken from seeds of *Quercus semecarpifolia* (Sm.) has been developed. Direct as well as indirect somatic embryogenesis was induced from the cotyledons on Woody plant (WP) medium supplemented with 6-Benzyladenine (BA) + Indole-3-butyric acid (IBA), and, BA + 2,4-Dichlorophenoxyacetic acid (2,4-D), respectively. Somatic embryos thus obtained were multiplied profusely on Schenk and Hildebrandt (SH) + Murashige and Skoog (MS) basal as well as BA supplemented media. Germination and conversion of somatic embryos into plantlets was achieved on SH+MS medium supplemented with BA (0.44- 8.88 μM). Rooting of *in vitro* produced shoots was achieved on WP (1/2 macro + full concentration of rest of the constituents) medium supplemented with IBA (14.76 μM). The plants were hardened *ex-vitro* and transferred to earthen pots containing garden soil. [Journal of American Science 2009: 5(1), 70-76] (ISSN: 1545-1003)

Key words: *Quercus semecarpifolia*, brown oak, somatic embryogenesis, micropropagation.

1. INTRODUCTION

The genus *Quercus* has a wide distribution range; mostly trees, either deciduous or evergreen and is of enormous ecological and economical value. One of the species of *Quercus*, i.e., *Quercus semecarpifolia* Sm. (family-Fagaceae); common name-brown or kharsu oak; is the main forest forming evergreen tree species around 2400 m amsl in parts of Indian Himalaya (Singh and Singh, 1987). In view of the general importance of this species and problems associated with its regeneration (Tamta et al. 2008), in the present study attempt has been made for the first time to develop an efficient *in vitro* micropropagation method through somatic embryogenesis.

Micropropagation through somatic embryogenesis offers considerable advantages over other methods of clonal propagation; this route has a high proliferation potential. It has been considered as a very promising method of oak micropropagation (Chalupa, 1995, Wilhelm 2000, Purohit et al.2002), and was found to be highly reproducible in this study on *Q. semecarpifolia*. Efficient protocols on SE induction and plant regeneration have recently become available for many plant species, including *Arabidopsis thaliana*, a model plant in genetics and embryogenesis (Gaj, 2004).

2. MATERIALS AND METHODS

Plant material and surface sterilization

Seeds of *Quercus semecarpifolia* Sm. were collected from well grown adult tree in the natural forests at Kilbury, Nainital (2100-2400 m amsl; 29° 24′ 30″ N- 29° 27′ N lat. and 79° 25′ E- 79° 29′ 40″ E long.), Uttarakhand, India. Following surface disinfection (Tamta et al. 2008), the seed coat was removed and seeds were divided into two halves; one half containing only one cotyledon while the other half contained the other cotyledon along with the embryo. These seed halves were used as explants for inoculation.

Media and culture establishment

Three basal media, namely MS (Murashige and Skoog, 1962), WP (Lloyd and McCown, 1980) and SH+MS, i.e., a combination of macronutrients

of SH (Schenk and Hilderbrandt, 1972) and the remaining constituents of MS, were used. The basal media were supplemented with various concentrations of auxins, cytokinins and gibberellins. The sucrose concentration was 3.0% (w/v) and the media were solidified with 0.8% agar (w/v). The experiments were done using glass petridishes (10 cm dia, 25 ml medium per petirdish) or conical flasks (250 ml volume, 100 ml medium per flask). Incubation of cultures was carried out at 25 ±1 °C in a 16 h light and 8 h dark cycle, with 42.0 µmol $m^{-2}s^{-1}$ and 60.0 µmol $m^{-2}s^{-1}$ irradiance inside and outside the culture flasks, respectively by cool fluorescent tubes (Philips TI 40 W/54).

Production of somatic embryos

Seed halves turned green when inoculated on WP basal medium. After seven days, cotyledons with or without the zygotic embryo, were transferred on to WP or MS medium supplemented with either BA (0.44 µM) alone or in combination with 2,4-D (4.53 µM) or IBA (4.92 µM) or GA_3 (2.89 µM). Direct as well as indirect somatic embryogenesis with the intervening callus phase was induced within 13 weeks and 18 (10 weeks for callus establishment and proliferation + 8 weeks for induction of somatic embryos) weeks of culture, respectively. In both the cases, the presence of zygotic embryo seems to have some role in the production of somatic embryos. The callus raised from cotyledons without the zygotic embryos did not survive on further subculture and degenerated. For germination of somatic embryos, formed both from the direct as well as indirect pathways, SH+MS medium supplemented with BA (0.44-8.88 µM) was used. The somatic embryos germinated to form well developed shoots, leaves and tap root system.

Adventitious rooting of microshoots

The survival rate of plantlets thus obtained; after transfer to *ex vitro* conditions was very poor (data not shown). Therefore, the main tap root was excised and the shoots were transferred to the rooting medium, i.e., WP (1/2 macro + full concentration of rest of the constituents) or SH + MS (macro of SH + rest of the constituents of MS) media supplemented with different auxins (Table 4), containing sucrose (3.0%; w/v) and phytagel (0.25%; w/v). Well developed adventitious roots were found to form within 4 weeks.

Transfer of plantlets to soil

After 5 weeks, the shoots with well developed roots were taken out from the culture flasks, the roots gently washed with water to remove traces of phytagel and the plantlets were then transferred to small plastic cups (8.0 cm ht; 7.0 cm dia) containing garden soil and the cups were covered with a transparent polythene sheet. Plants were kept inside a polyhouse for acclimatization for 1 month. After that the plants were transferred to the earthen pots (18 cm high; 20 cm dia) containing the same soil.

Statistical analyses

Experiments were conducted using a randomized block design to determine the effect of treatments and were repeated as described in individual experiments. For all the experiments explants were used in triplicates.

3. RESULTS AND DISCUSSION

Direct somatic embryogenesis

Globular structures were found to develop directly on the periphery of cotyledons with attached zygotic embryo, after 13 weeks on WP medium supplemented with BA and IBA (Table 1). These structures were loosely attached to the surface of cotyledons (Fig. 1A). On subculture these globular structures were converted into bipolar somatic embryos (Fig. 1B). This has been reported in some other species of *Quercus* (Chalupa, 1995; Gingas and Lineberger, 1989). Bipolar somatic embryos were also observed in *Q. robur* (Cuenca et al., 1999) and in *Q. suber* (Puigderrajols et al., 1996), which were reported to be translucent or opaque-white in appearance. These somatic embryos were multiplied by secondary embryogenesis (Fig. 1C), and the frequency of secondary embryo formation was found to increase when subcultured on SH+MS medium, without any growth regulators. In *Q. suber* also secondary embryogenic lines were mainted on medium lacking PGRs (Fernandez-Guijarro et al., 1995). Proliferation of secondary embryos was most prolific from the root pole of the somatic embryos. Secondary embryos were produced mostly from the root pole end of the primary embryos as also observed by El Maataouti et al. (1990) and Gingas (1991). Cotyledons without the embryonic axes failed to give rise to direct embryos.

Indirect somatic embryogenesis

Callus was induced from the surface of cotyledons inoculated on both MS or WP media supplemented with BA and 2,4-D or IBA (Table 1). The creamy yellow callus developed on MS medium was slow to proliferate and degenerated on further subcultures. On the other hand friable callus was formed on WP medium after 10 weeks on cotyledonary halves with embryo (Fig. 1D); subsequently this callus was subcultured on MS basal medium (half or full strength) supplemented with CH (0.02%, w/v) and activated charcoal (1.0%, w/v) (Table 2). The friable callus developed on WP medium supplemented with BA (0.44 μM) and 2,4-D (4.53 μM) (Table 1) was found to turn embryogenic after 8 weeks (two months) of subculture (Table 2; Fig. 1E) when transferred to the above medium, i.e., MS basal (half or full strength) medium supplemented with CH (0.02%; w/v) + AC (0.1%; w/v). Somatic embryos could be multiplied through secondary embryogenesis on SH + MS medium supplemented with BA (0.44-8.88 μM) (Table 3). BA, a potent cytokinin, alone or in combination with auxins, particularly IBA or 2,4-D, has been known to induce somatic embryogenesis from the zygotic embryos (Chalupa, 1995; Gingas and Lineberger, 1989; Sasamoto and Hosoi, 1992; Kim et al. 2006). Somatic embryos of all stages (globular, heart and torpedo shaped) could be observed on the same medium.

The rate of multiplication of somatic embryos through secondary embryogenesis varied from 1.66 to 3.14 secondary embryos per somatic embryo, over a period of 5-6 weeks, depending upon the PGR supplements (Table 3). It is often reported in case of *Quercus* that calli turn embryogenic when transferred to the basal medium (Gingas and Lineberger, 1989; Guijarro et al., 1995; Kim et al., 1994).

Germination of somatic embryos

Somatic embryos (produced from the direct as well as indirect pathways) were transferred to BA (0.44-8.88 μM) supplemented SH+MS medium for germination. Some of the somatic embryos germinated and produced root and shoot in a well coordinated manner (Fig. 1F). In a number of somatic embryos only the root primordia elongated (Fig. 1G); its frequency varied from 4.0-27.0 per cent depending upon the concentration of BA in the medium. The overall conversion frequency of somatic embryos was only around 10 per cent. BA at 2.22 μM was found to be optimum for germination and conversion of somatic embryos into plantlets (Table 3). The frequency of conversion of somatic embryos into full plants in oaks is usually quite low (Chalupa, 1995); this is a matter of future investigations. Fig. 1H shows the germination of somatic embryo.

Adventitious rooting of microshoots excised from germinating somatic embryos

Out of various media tried (MS, WP, SH+MS) supplemented with various auxins (IAA, NAA, IBA) in different concentrations (4.92 μM - 28.55 μM), WP medium supplemented with IBA (14.76 μM) was found to be most effective (100.0%) in inducing rooting without any callus formation at the basal end (Table 4). The root initials were observed within 10 days and well developed roots were formed in four weeks (Fig. 1I). The average number of roots was 12.46 with maximum length of 6.97 cm (Fig. 1J). WP medium supplemented with NAA or IAA also induced rooting (16.6% and 50.0%, respectively). However, the average number of roots was 3.0 and 3.02 and the length of the longest roots were 0.2 and 2.2 cm, respectively. When IBA was added to SH+MS medium, this combination also induced rooting (100.0%) but the formation of callus was invariably seen at the base of the explant, and the average number of roots (4.3) and length of the longest root (0.21 cm) were also considerably less. The addition of NAA to SH+MS medium totally failed in inducing rooting, whereas IAA induced rooting in 40.0% shoots with the average number roots being 4.0. However, the roots did not elongate and the length of the longest root never exceeded beyond 0.2 cm. Secondary roots were found to develop only on WP medium supplemented with IBA with profuse adventitious rooting. Addition of IBA to the rooting medium gave better results in comparison to another auxin, NAA, in *Q. suber* (Manzanera and Pardos, 1990) also.

Hardening: Well rooted plants were taken out of the culture vessels and the adhering phytagel was carefully removed; the delicate roots were then gently and thoroughly washed before transferring to plastic cups containing garden soil (Fig. 1K). The survival of these plants was only 20.0 per cent. After one month, these plants were transferred to earthen pots containing same soil and maintained inside the polyhouse until new leaves were found to emerge (Fig. 1L). In conclusion, the present study describes, for the first time, the effective multiplication protocol for *in vitro* propagation of *Q. semecarpifolia*.

Table 1
Effect of treatments on seed halves of *Q. semecarpifolia* in different media

S. No.	Treatments	MS medium	WP medium
1	Control	-	-
2	BA (0.44 μM)	-	-
3	BA+2,4-D (0.44 μM+4.53 μM)	Callus	Callus*
4	BA+IBA (0.44 μM+4.92 μM)	Callus	Direct SE
5	BA+ GA_{3} ((0.44 μM+2.89 μM)	-	-

*embryogenic callus, - nil, SE: somatic embryogenesis, data recorded after 10 weeks of culture for callus formation and after 13 weeks for direct somatic embryo formation

Table 2
Callus proliferation and somatic embryogenesis in *Q. semecarpifolia*

Medium constituents	Callus Proliferation	Embryogenesis	No. of embryos/ petri dish
MS	++	***	125
MS+CH (0.02%)	+++	**	96
MS+CH (0.02%) +AC (0.1%)	+	-	NA
1/2 MS + CH (0.02%)	+	-	NA
1/2MS+CH (0.02%) + AC (0.1%)	++	***	110

The callus was initiated on WP medium supplemented with BA and 2,4-D;
MS: Murashige and Skoog medium; CH: Casein hydrolysate, AC: activated charcoal, all concentrations are w/v basis; + poor, ++ medium, +++ prolific; * poor, ** moderate, *** abundant, - nil, NA: not applicable; data recorded after 8 weeks (2 months) of culture; 6 petridishes were used per treatment with 4 callus pieces per petridish; the experiment was repeated twice with similar results

Table 3
Response of somatic embryos of *Q. semecarpifolia* on SH+MS medium supplemented with various concentrations of BA

BA (μM)	No. of somatic embryos transferred	Germination of somatic embryos (%)	Secondary embryogenesis*	Frequency of root formation (%)
0.44	30	0	1.66	26.60
0.88	97	4.50	2.28	18.40
1.78	44	5.20	3.14	18.18
2.22	49	6.90	3.00	14.00
4.44	68	2.90	1.85	4.40
8.87	90	0.89	1.76	4.10

* No. of total somatic embryos after six weeks/no. of somatic embryos initially inoculated per flask; each treatment consisted of 12 flasks, data was recorded 6 weeks after transfer of somatic embryos to the medium. The experiment was repeated twice with similar results.

Table 4
Effect of auxins and media on *in vitro* rooting of SE derived microshoots of *Q. semecarpifolia*

Medium	PGRs (conc.in μM)	Shoot ht (cm) ±SD	% callusing	% rooting	No. of roots/ shoot ±SD	Length of longest root (cm)±SD	Sec. roots
WP	IBA (14.76)	2.20 ±1.04	0.00	100.00	12.46 ±4.87	6.97 ±1.47	+
	NAA(16.11)	1.33 ±0.68	100.00	16.60	3.00 ±1.22	0.20 ±0.03	-
	IAA (17.13)	2.17 ±0.69	0.00	50.00	3.02 ±4.24	2.20± 0.57	-
SH+MS	IBA (14.76)	2.56 ±0.42	100.00	100.00	4.30 ±2.07	0.21 ±0.13	-
	NAA (16.11)	1.93 ±0.89	48.00	0.00	NA	NA	NA
	IAA (17.13)	2.00 ±1.31	0.00	40.00	4.00 ±2.3	0.20 ±0.11	-

WP: 1/2 macro + full concentrations of rest of the constituents; SH+MS : macro (SH) + rest of the constituents of MS; SE: somatic embryo, +: occurred; -: did not occur; NA: not applicable; SD: standard deviation, data recorded 5 weeks after transfer to rooting medium, treatments were carried out in triplicate and each flask contain 9 microshoots

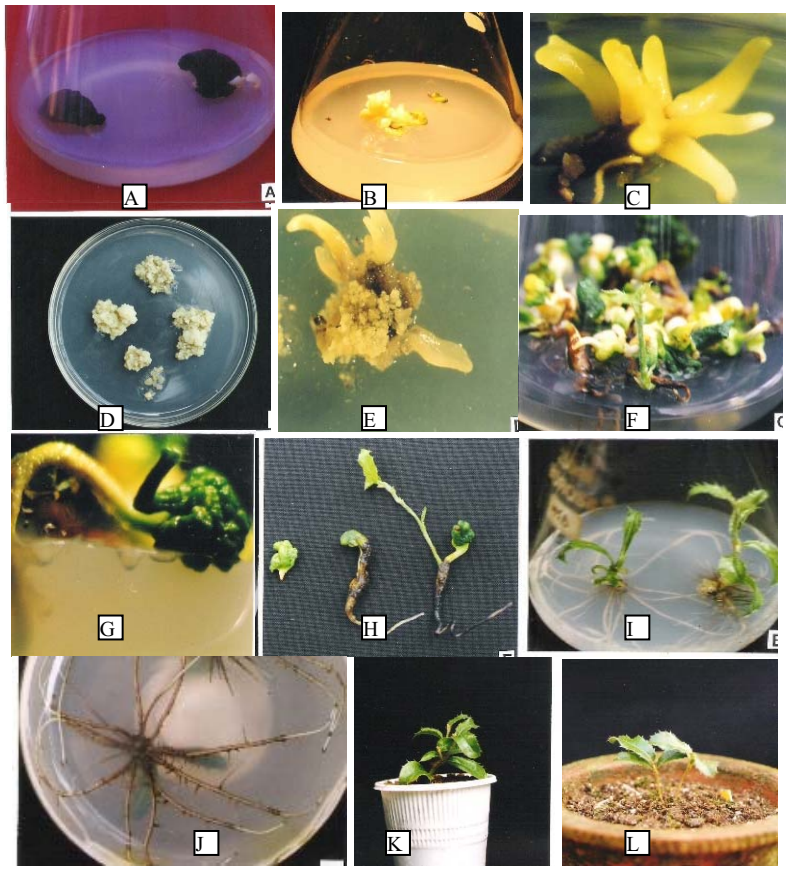

Fig. 1. *In vitro* propagation of *Q. semecarpifolia*
(A) Globular structures loosely attached to the surface of the cotyledon.
(B) Bipolar somatic embryos
(C) Secondary embryogenesis
(D) Friable embryogenic callus on WP medium
(E) Indirect somatic embryogenesis
(F) Germination of somatic embryo
(G) Elongation of root primordial from the somatic embryo
(H) Different stages of somatic embryo germination
(I) Well rooted plantlets after 4 weeks of culture on WP medium supplemented with IBA
(J) Rooting from basal view
(K) Well rooted plant 1 month after transfer to plastic cup containing garden soil
(L) Two –months-old in vitro propagated plant in earthen pot

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Research Article

Synthesis, characterization and Electroluminescence of BPh_2(2-(benzimidazol-2-yl) pyridinato) compound

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ABSTRACT: A novel luminescent boron compound, BPh_2(2-(benzimidazol-2-yl) pyridinato) (**B-BIP**), have been synthesized by reactions of triphenylboron with appropriate ligands, 2-(2-pyridyl)benzimidazole (**BIP**). For the three-layer OLED with the structure ITO/NPB/ **B-BIP** /Alq_3/Mg-Ag, an emission band covering the whole visible region from 400 to 650 nm with the maximum brightness of 50 cd/m^2 was observed, indicating a perfect white light OLED (CIE = 0.32, 0.37). [Journal of American Science 2009: 5(1), 77-82](ISSN: 1545-1003)

Keywords: white light; electroluminescence; imidazole; boron;

1. INTRODUCTION

The chemistry of organoboron compounds have attracted much more attention recently because they are of interest for practical applications [1-3]. Since an organic light emitting diode (OLED) was reported by Tang and Vanslyke [4], LEDs based on organic materials have generated considerable interest and enabled the development of low-cost, full-color, flat-panel displays [5-8]. The best-known EL metal complex used in OLED is Alq_3 which is not only a good emitter but also a highly efficient electron-transporting material, where q is the 8-hydroxyquinolinato ligand [9-12]. Via the modification of the ligands of metal complexes, the emission spectra of devices and other properties, such as thermo stability and carrier mobility, can be tuned. The imidazoles have been known as good chelating ligands [13] and the attachment of the pyridyl group at 2-position of imidazole would allow the new ligand to form stable compounds with the other atoms. In the present work, the syntheses, structures, and electroluminescent properties of two new boron compounds BPh_2(2-(benzimidazol-2-yl) pyridinato)(**B-BIP**) is reported.

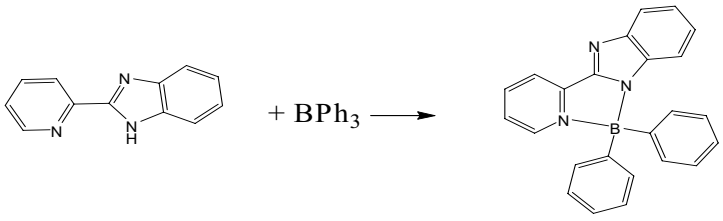
2. EXPERIMENTAL METHOD

The synthesis of the title compound was accomplished by following processes, as shown in Scheme 1. The triphenylboron (1.45g, 6.0 mmole) was slowly added to 100 ml of THF solution containing 2-(2-pyridyl)benzimidazole (2.63g, 13.5 mmole) at 0°C under N_2. After the resulting mixture was stirred at room

temperature for 6 hours, 5 ml isopropyl alcohol was added to quench the reaction. The solvents were removed under vacuum condition at 5×10^{-3} Torr, and the residual solid was sublimed to purify the final product. Light green of **B-BIP** was obtained in 90% yield. The organic light emitting device, Fig. 1, using **B-BIP** as the emitting and electron-transporting layer were fabricated on the transparent conductive indium-tin oxide (ITO) glass substrate. The organic layers and the cathode were sequentially deposited by conventional vacuum vapor deposition in the same chamber without breaking the vacuum under 3×10^{-6} Torr. The cathode composed of magnesium silver alloy (Mg:Ag = 10:1) were deposited onto the top layer of organic materials by co-evaporation of Mg and Ag from different source. Before the deposition, all of the organic materials were purified by the train sublimation method. In the present wok, the

N,N'-bis-(1-naphthyl)-N,N'-diphenyl-1,1'-biphenyl-4,4'-diamine (NPB) was used as the hole-transport material (HTM), and tris (8-quinnolinolato) aluminum (Alq_3) was employed as the electron-transporting material (ETM). The EL spectrum and the Commission Internationale de l'Eclairage (CIE) co-ordinates were measured by Pro-650 Spectroscanner (step size is 1.0 nm and bandpass is 4nm), the current-voltage (I-V) characteristic was measured by Keithley 2400 Source meter.

Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer thermogravimeter (Pyris 1) under a dry nitrogen gas flow at the heating rate of 20°C/min. Glass transition temperature (T_g) and melting point (T_m) of materials were determined by differential scanning calorimetry of the Perkin-Elmer differential scanning calorimeter (DSC-7).



Scheme 1. Synthesis process for the title compound

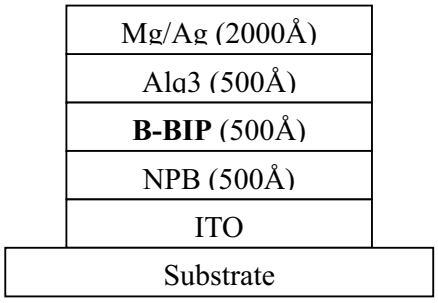


Fig 1: Device structure of organic light emitting device (OLED) fabricated in this work

3. RESULTS AND DISCUSSION

A new boron compound

BPh_2(2-(benzimidazol-2-yl) pyridinato) (**B-BIP**) was prepared by reacting triphenylboron with appropriate imidazole in dry THF (Scheme 1). Both of the compounds are air-stable in the solid state and in solution. The Thermogravimetric analyses (TGA) scans under nitrogen for **B-BIP** powder showed weight loss of 10% at 301 ^oC, respectively, which reveal that **B-BIP** is quite stable in the atmosphere of nitrogen. The DSC results indicate that the compound **B-BIP** possess a very high melting temperatures, 289^oC, respectively, which may serve as an advantage for OLED device fabrication because the materials having high transition temperature could provide the device with greater longevity [14, 15]. The thin films of **B-BIP** used for the analyses of UV-vis and photoluminescence spectra were obtained by depositing **B-BIP** onto quartz substrates under vacuum condition. At room temperature and low concentration (1×10^{-5} M), the absorption spectral features of **B-BIP** in N,N'-dimethylformamide (DMF) consist of two discrete bands (Fig. 2). The strong absorptions centered at 280 nm for **B-BIP**, respectively, can be assigned to the π-π* transition. The other intense band centered at 348 nm shows a vibrational separation of 1000 cm^{-1} with the $\nu_{0,0}$ transition at 2.94×10^3 cm^{-1}. This lower energy band possesses a reasonably high absorptivty ($\varepsilon \sim 3\times10^4$ $dm^3mol^{-1}cm^{-1}$) and a red shift with increasing polarity of solvent, which is typical for a π-π* transition [16-18].

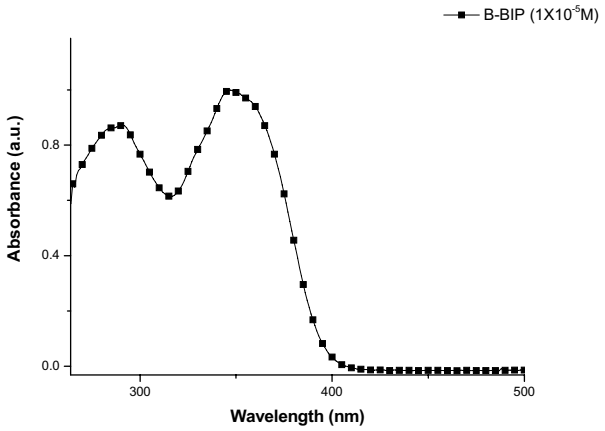


Fig 2: UV-vis spectra of **B-BIP** in N, N'-dimethylformamide

Fig. 3 show the photoluminescence (PL) spectra of the solutions and neat film of **B-BIP** excited with 355 nm laser line. All concentration in DMF, only one emission band was observed with a maximum at 455 nm. Compound **B-BIP** possess the appreciable PL quantum yield, with Φ_f = 0.66 respectively, 10^{-6} M in DMF relative to

3-(2-benzothiazolyl)-7- diethyl-aminocoumarin (C540). To investigate the electroluminescent properties of **B-BIP** typical three-layer device with the configuration of

ITO/NPB/**B-BIP**/Alq3/MgAg was fabricated by using NPB as the hole-transporting layer and **B-BIP** as the emitter and Alq3 is electron-transporting layer. The EL spectrum of organic light emitting device at the bias voltage of 10 V, Fig. 4, shows the broader emission bands ranging from 400 to 650 nm were observed, indicating that the three-layer LED device emitted white light covering the whole visible light region. The band around 455 nm in

EL spectrum can be attributed to emission of **B-BIP**, because its emission position is almost identical with that in PL spectrum of **B-BIP**. The emission band at 535 is Alq3 emission position. The emission is almost fixed in the white region in the CIE coordinate of x = 0.32 y = 0.37. For the small molecular organic materials, to develop the double layer of device with white emission is very important because this kind of material is very seldom prepared so far, and it is very important for the fabrication of display panels. At the same time important role here may play electron-vibration interactions determining the spectral broadening of the emission lines. So the future strategy of the materials design may be in this way also.

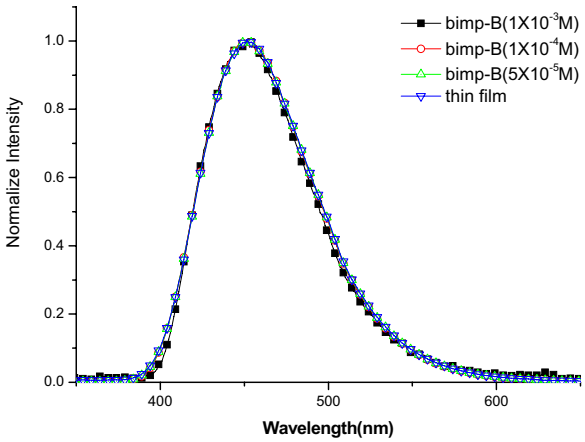


Fig 3: PL spectra of the **B-BIP** in solutions and neat film

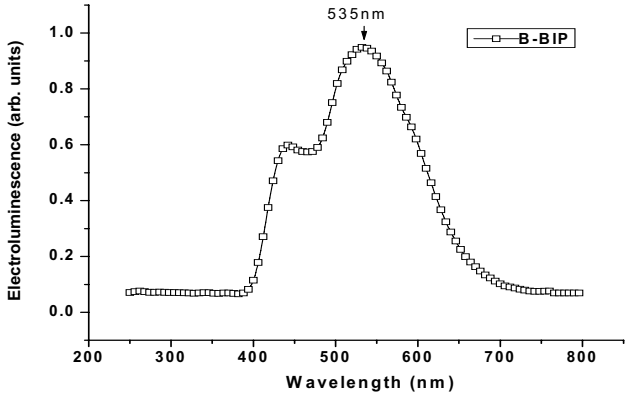


Fig 4: EL spectrum of OLED fabricated in this work.

Figure 5. shows the energy level diagram of the HOMO and LUMO of the different organic materials and the work function of cathode and anode. By using cyclic voltammetry (CV) method obtaining LUMO energy of **B-BIP** is 3.4eV and the optical band gap estimated from the absorption onset, we can determine the HOMO energy at 6.5eV. In Comparison with the energy level of **B-BIP** and NPB, **B-BIP** has much higher hole injection barrier than that of

NPB. As a matter of fact, it is impossible for the hole injection from ITO into **B-BIP** without the assistance of NPB or some HTLs. This diagram pointed out Alq_3 has lower electron injection barrier than that of **B-BIP**. Therefore, the electron injection from the MgAg into **B-BIP** will be enhanced and confines the recombination zone at the interface between NPB and **B-BIP**. Fig.6 shows the current-voltage and luminance-voltage characteristics of this device having a low turn on voltage of about 4.5V for current and luminance. This device shows a brightness of 50 cdm^{-2} at the driving voltage of 12V with current density of 390 mA/cm^2, decaying to 25 cdm^{-2} in 100 hours.

4. CONCLUSION

A new compound of emitter for OLED, BPh_2(2-(benzimidazol-2-yl)pyridinato) (**B-BIP**), has been successfully synthesized and investigated. It has been shown that the novel ligands BIP is capable of chelating to B(III) centers and the resulting compounds possess appreciable photoluminescent efficiency and very high thermal stabilities. This study further indicates that the emission band of the devices could be modified by changing the composition of emitting layer and therefore, OLEDs with different colors could be obtained.

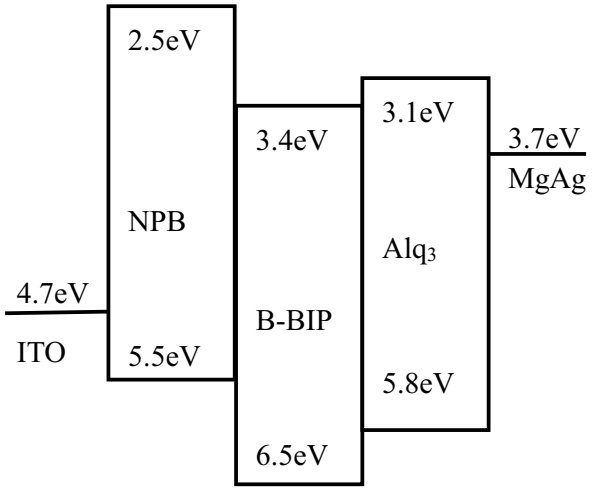


Fig 5: Energy level diagram of OLED materials, ITO, and Mg-Ag alloy

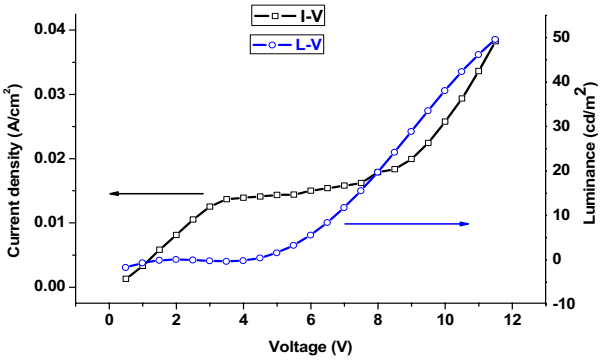


Fig 6: Current-voltage and luminance- voltage characteristics of OLED fabricated in this work

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Research Article

White Organic Electroluminescence Base on a new Aluminum Complex

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ABSTRACT: A bright blue emission material, tris{2-(benzimidazol-2-yl) pyridinato} Aluminum (**AlBIP**) used for white-light of organic light emitting devices has been synthesized. The decomposition temperature was observed at 400 ℃ and no melting transition (T_m) was observed up to 400℃. For three-layer LED devices with the configuration of ITO/NPB/**AlBIP** /Alq3/MgAg, the white light emission covering the whole visible region from 400 to 700 nm with the maximum brightness of 75 cd/m^2 and current density of 330 mA/cm^2 was observed.
[Journal of American Science 2009: 5(1), 83-87] (ISSN: 1545-1003)

Keywords: Electroluminescence; white light; device

1. INTRODUCTION

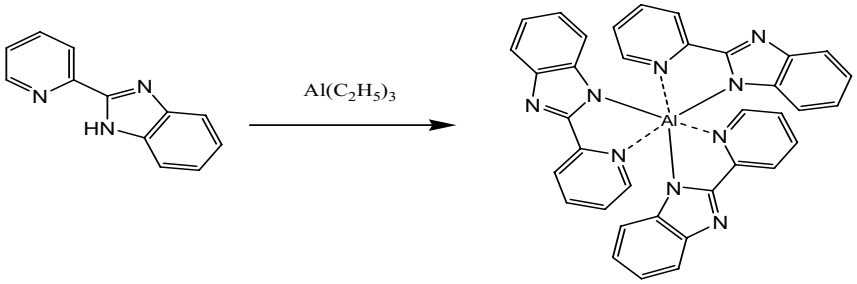
White organic light emitting diodes have attracted much attention, because their potential applications in the backlights of laptop computers and portable panel light sources. In the literatures, several strategies including multi-layer devices have been developed to realize highly efficient white organic electroluminescence [1-5]. Luminescent chelate complexes have been shown to be particularly useful in electroluminescnt (EL) displays because of their relatively high stability and volatility. The most well-known example of such chelate compounds is $A1q_3$, not only a good emitter but also a highly efficient electron-transporting material, where q is the 8-hydroxyquinolinato ligand [6, 7]. Via the modification of the ligand of metal chelate compound, the emission color of a metal chelate compound may be tuned. Other properties, such as thermal stability and carrier mobility, may also be improved upon. In the present work, we report the synthesis and electroluminescent (EL) property of tris{2-(benzimidazol-2-yl) pyridinato} Aluminum (**AlBIP**). The **AlBIP** containing N,N-bidentate ligand instead of N,O-bidentate one such as 8-hydroxyquinoline. Therefore, the thermal stability, an important character for the practical application in the electronic fields, of this metal complex is investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The organic emitting device using **AlBIP** as emitting layer has been fabricated to study the electroluminescent property of this metal complex.

2. EXPERIMENTAL METHOD

The synthesis of the title compound was accomplished by following processes, as shown in Scheme 1. The triethylaluminum solution (25% w/w in hexane 1.86ml, 2.82×10^{-3} mole) was slowly added to 100 ml of THF solution containing 2-(2-pyridyl)benzimidazole (1.75g, 9.0×10^{-3} mole) at 0^{o}C under N_2. After the resulting mixture was stirred at room temperature for 6 hours, 5 ml isopropyl alcohol was added to quench the reaction. The solvents were removed under vacuum condition at 5×10^{-3} Torr, and the residual solid was sublimed to purify the final product. Light green of **AlBIP** was obtained in 85% yield. The formula of this compound has been determined by ^{1}H NMR and

elemental analysis. The organic light emitting device, Fig. 1, using **AlBIP** as the emitting and electron-transporting layer were fabricated on the transparent conductive indium-tin oxide (ITO) glass substrate. The organic layers and the cathode were sequentially deposited by conventional vacuum vapor deposition in the same chamber without breaking the vacuum under 3×10^{-6} Torr. The cathode composed of magnesium silver alloy (Mg:Ag = 10:1) were deposited onto the top layer of organic materials by co-evaporation of Mg and Ag from different source. Before the deposition, all of the organic materials were purified by the train sublimation method. In the present wok, the N,N'-bis-(1-naphthyl)-N,N'-diphenyl-1,1'-biphe nyl-4,4'-diamine (NPB) was used as the hole-transport material (HTM), and tris (8-quinnolinolato) aluminum (Alq_3) was employed as the electron-transporting material (ETM). The EL spectrum and the Commission Internationale de l'Eclairage (CIE) co-ordinates were measured by Pro-650 Spectroscanner (step size is 1.0 nm and bandpass is 4nm), the current-voltage (I-V) characteristic was measured by Keithley 2400 Source meter.

Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer thermogravimeter (Pyris 1) under a dry nitrogen gas flow at the heating rate of 20°C/min. Glass transition temperature (T_g) and melting point (T_m) of materials were determined by differential scanning calorimetry of the Perkin-Elmer differential scanning calorimeter (DSC-7).



Scheme 1. Synthesis process for the **AlBIP** complex**.**

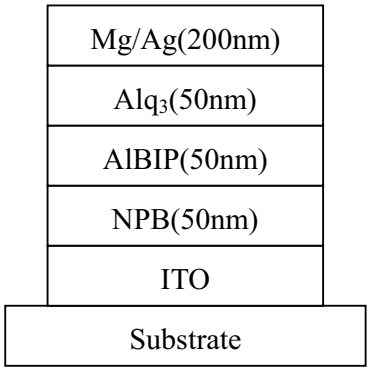


Fig 1: The organic light emitting device (OLED).

3. RESULTS AND DISCUSSION

The TGA of **AlBIP** that possesses a maximum rate of weight loss occurring at 400 ^{o}C and no weight loss was observed at the temperature lower than 350 ^{o}C. Above 600 ^{o}C, there is about 14 wt % of residue composed of aluminum ash. This aluminum complex is reasonably stable upon exposure to air and exhibited a high thermal stability in nitrogen. The melting temperature (Tm) of **AlBIP** was not observed up to 400 ^{o}C with DSC curve. The DSC and TGA results indicate that the **AlBIP** possesses a high thermal stability, which may serve as an advantage for the fabrication of organic light emitting device because the use of the materials with high thermal stability as the active emissive layer or carrier transporting layer may provide the device with greater longevity [11, 12].

The Photoluminescent (PL) spectra of the **AlBIP** solutions and neat film, excited with 350 nm laser line, were illustrated in Figure 2. At low concentration, 1×10^{-5} M in DMF, only one emission band is observed with maximum at 450 nm, corresponding to the relaxation of **AlBIP** from the excited state of a single molecule into ground state. There is red shift emission band that a maximum at 460 nm is observed in the spectrum of the **AlBIP** neat film. To investigate the electroluminescent properties of **AlBIP** typical three-layer device with the configuration of ITO/NPB/**AlBIP**/Alq3/MgAg was fabricated by using NPB as the hole-transporting layer and **AlBIP** as the emitter and Alq3 is electron-transporting layer. The EL spectrum of organic light emitting device at the bias voltage of 10 V, Fig. 3, shows the broader emission bands ranging from 400 to 700 nm were observed, indicating that the three-layer LED device emitted white light covering the whole visible light region. The band around 465 nm in EL spectrum can be attributed to emission of **AlBIP**, because its emission position is almost identical with that in PL spectrum of AlBIP. The emission band at 525 is Alq3 emission position and 565nm can be attributed to the exciplex emission originated from the interface between NPB and **AlBIP** . The emission is almost fixed in the white region in the CIE coordinate of x = 0.32 y = 0.37. For the small molecular organic materials, to develop the double layer of device with white emission is very important because this kind of material is very seldom prepared so far, and it is very important for the fabrication of display panels.

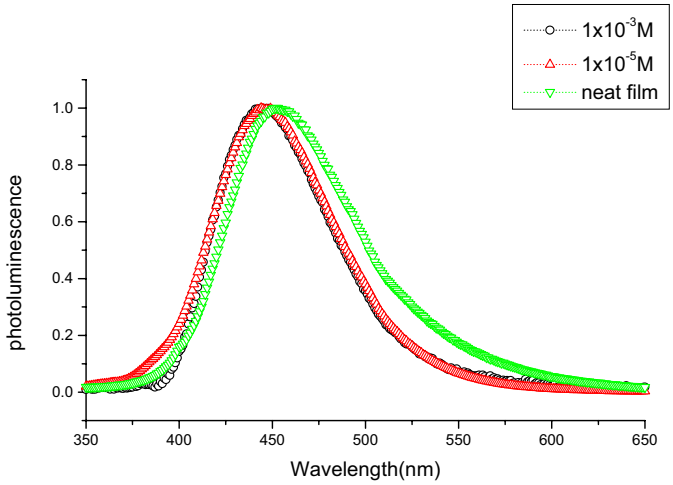


Fig 2: Photoluminescent spectra of the **AlBIP** in solutions and neat film

The change of the spectral wavelength may be achieved also by general conception of search and design of modified materials for wide band emission consists in substitution of the backside groups by electron acceptors like halogens etc. and different kind of donors [13, 14]. At the same time important role here may play electron-vibration interactions determining the spectral broadening of the emission lines. So the future strategy of the materials design may be in this way also.

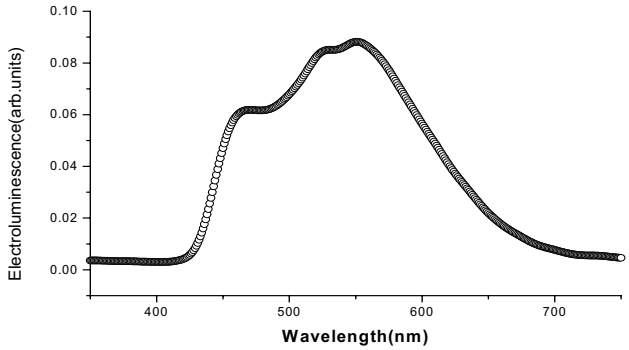


Fig 3: EL spectrum of OLED fabricated in this work.

Figure 4 shows the energy level diagram of the HOMO and LUMO of the different organic materials and the work function of cathode and anode. By using cyclic voltammetry (CV) method obtaining LUMO energy of **AlBIP** is 2.8eV and the optical band gap estimated from the absorption onset, we can determine the HOMO energy at 5.6eV. In Comparison with the energy level of **AlBIP** and NPB, **AlBIP** has much higher hole injection barrier than that of NPB. As a matter of fact, it is impossible for the hole injection from ITO into **AlBIP** without the assistance of NPB or some HTLs. This diagram pointed out Alq_3 has lower electron injection barrier than that of **AlBIP**. Therefore, the electron injection from the MgAg into **AlBIP** will be enhanced and confines the recombination zone at the interface between NPB and **AlBIP**. Fig.5 shows the current-voltage and luminance-voltage characteristics of this device having a low turn on voltage of about 4.0V for current and luminance. This device shows a brightness of 75 cdm^{-2} at the driving voltage of 16V with current density of 330 mA/cm^2, decaying to 30 cdm^{-2} in 120 hours.

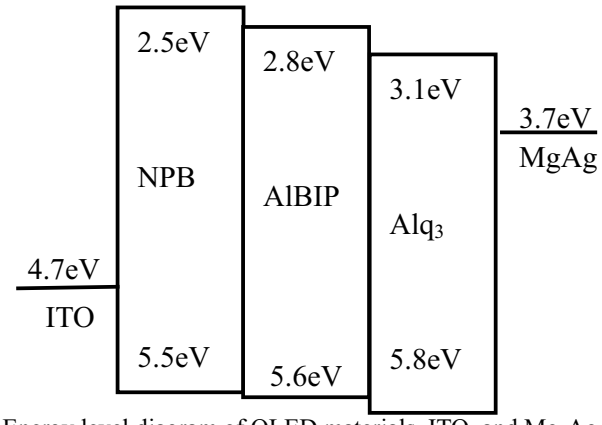


Fig 4: Energy level diagram of OLED materials, ITO, and Mg-Ag alloy

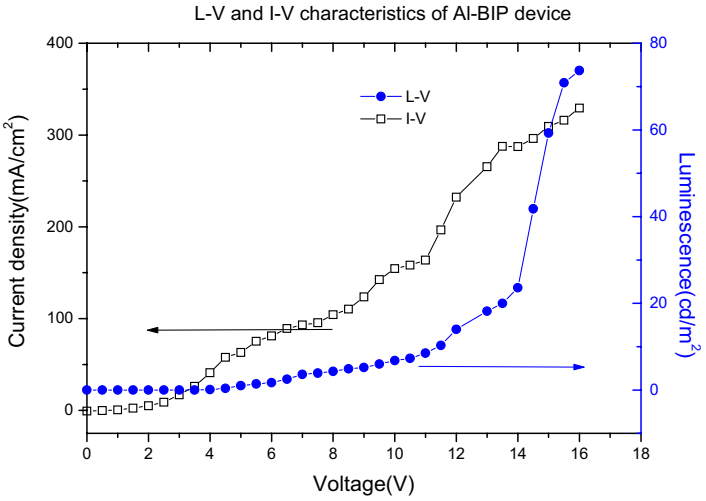


Fig 5: Current-voltage and luminance- voltage characteristics of OLED fabricated in this work.

4. CONCLUSION

A novel bright blue emission material, tris{2-(benzimidazol-2-yl) pyridinato} Aluminum (**AlBIP**), was successfully prepared by the reaction of 2-(2-pyridyl)benzimidazole and trisethylaluminum. Because of its high thermal stability and excellent electrical characteristics, **AlBIP** and its related compound suggest a possible application for the use of white-light of the organic light emitting devices.

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