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RESEARCH ARTICLE

Impact of *Neochetina eichhorniae* Warner. on Biological Control of Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms.) of Singanallur pond, Coimbatore, TamilNadu, India.

Yasotha.D and M.Lekeshmanaswamy*

P G and Research Department of Zoology, Kongunadu Arts and Science College College, Coimbatore-641 029, TamilNadu, India.

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***Address for correspondence**
Dr. M. Lekeshmanaswamy, Associate professor,
PG and Research Department of Zoology,
Kongunadu Arts and Science College,
Coimbatore-641 029, TamilNadu, India.
E.Mail : ml_swamy64@yahoo.co.in. Mobile-07708570555.

ABSTRACT

The use of natural enemies of a weed or pest is to suppress populations of the weed or pest. The programme involves importing, mass rearing, releasing and monitoring the establishment, spread and impact of the host-specific biological control agents, *Neochetina eichhorniae* weevils, on water hyacinth. In this biological control programme growth parameters were evaluated, feeding damage and the impact of *Neochetina eichhorniae* was quantified.

Key words: Natural enemies, biological control, *Neochetina eichhorniae,* water hyacinth

INTRODUCTION

Coimbatore is an important industrial city in the southern part of India, ranking eleventh in terms of population. It is located in the state of Tamilnadu, between 10° 55′ and 11° 10′ N, and 77° 10 and 76° 50′ E at an approximate altitude of 470m. There are more than 30,000 small, medium and large industries including textile mills and foundries in the city employing about 40% of the population. Many textile processing units in Tamilnadu use a number of unclassified chemicals that are likely to be from the Red List Group which is said to be harmful and unhealthy [1].

Coimbatore is located at a distance of 500Kms from the state capital Chennai. The city is traversed in the middle by river Noyyal. It is surrounded by the Nilgiris, a rich tea producing hinterland in the North; Pollachi, the receiving centre for forest produce in the South and the Palakkad of Kerala in the Southwest.

The centre of origin of Water hyacinth is believed to be Amazonia, Brazil, with natural spread throughout Brazil and to other Central and South American countries. It is perennial, herbaceous, aquatic plant of the family Pontederiaceae. The genus *Eichhornia* contains number of other species all of which are aquatic, but only *Eichhornia crassipes* has become a serious weed. The leaves of water hyacinth are compressed of a smooth, glossy, circular to kidney shaped lamina and a swollen spongy petiole. Reported as a weed in 56 countries [10].

Water hyacinth possesses specialized growth habits physiological characteristics and reproductive strategies that allow for rapid growth and expansion in freshwater environments and has spread rapidly throughout the tropics and subtropics [18]. *Eichnoria crassipes* forms large free floating, mono-specific mats that complete with other aquatic species for light nutrient and oxygen [18]. As biomass from mats decomposes, organic input to sediments increases dramatically [6; 11]. *Eichornia crassipes* grows in shallow temporary ponds, wetlands and marshes, sluggish flowing waters and large lakes reservoirs and rivers [4] plants can tolerate extreme of water level fluctuation and seasonal variations in flow velocity and extreme of nutrient availability, pH, temperature and toxic substances [6;18]. It was introduced into many countries during late 19th and 20th centuries, where it spread and degraded aquatic ecosystems. *Eichhornia crassipes* remains the world's most problematic water weed despite wide spreads and various approaches to its control [9]. It has such a high growth rate that according to [15] it can double its area in only five days.

Its control has focused upon biological control measures with the introduction of the *Eichhornia* Weevils (*Neochetina* Spp) in the late 1990s. The exotic water hyacinth weevils *Neochetina eichhorniae* warner are reported to be most effective bio-control agent. In its native range Water hyacinth is attacked by a complex of arthropods. Study of the life history and ecology of some of these has began in Argentina in 1961 [2].The first natural enemies were released as control agents in USA in the early 1970s [17].The two *Neochetina* species are the most widely distributer of the Water hyacinth biological control agents and to date are the most successful.

The role of biological control agent in limiting water hyacinth growth and invasion depends in part on interaction between water and plant nutrients and weevil damage and disturbance factors acting on weed populations. The exotic water hyacinth weevils which is host specific, *Neochetina eichhoriae* warner are reported to be the most effective and widely used bio control agent of water hyacinth and have contributed to the control of this weed in a number of countries [12; 8; 2; 5;14].

Development of biological control agents for weeds has been motivated by the need to reduce the abundance and distribution of a pest plant where chemical and mechanical controls were not cost-effective [7;16].It is necessary to make provision for an important increase and subsequent maintenance in the level of the means and logistics targeted at the prevention and control of this weed.

MATERIALS AND METHODS

The *Neochetina eichhorniae* weevils were imported from College of Horticulture**,** Kerala Agricultural University, Vellanikara, Kerala and reared for mass production.The insect was reared on Water hyacinth plants maintained in cement concrete tanks. Pupae of the insect were collected from culture tanks and when adults emerged, they were sexed eight pairs of freshly emerged adults were used for the study. Individual pairs were released in separate plastic jars (11x8cm) with wire mesh windows on the lids to facilitate aeration. A Water hyacinth leaf retaining 2cm of petiole was introduced into each jar with 1cm of water removed exposed leaves were removed every day and fresh ones were introduced. The collection leaves are then dissected out under a stereo-microscope and the eggs were

transferred for further studies. The egg from the dissected leaves was transferred to a moistened filter paper kept in paired Petri dishes. Incubation period and the hatching percentage were recorded.

Newly hatched larvae were introduced into punctures made with forceps in petioles of water hyacinth plants grown in concrete tanks (diameter). These tanks were covered with nylon net to prevent external infestation. The plants were periodically dissected in order to obtain larvae, which were measured to fix the instars. The larval period was also recorded.

The pupae along with the plants were placed in water filled museum jars (10x10x20cm) and kept undisturbed until the adults emerged and pupal period was recorded.Emerging adults were collected and sexed. Ten pairs of freshly emerged adults were used for the study as described in above paragraph. The exposed leaves were removed every day and fresh ones were introduced. Once a month the tanks are fertilized with 50g of fertilizer containing nitrogen, phosphorus and potassium to enhance plant growth.

RESULT AND DISCUSSION

The weevils are harvested and released in the field. The water hyacinth plants are infested with weevil life stages. Adult weevils are fed on fresh leaves and petioles in plastic jars before transporting them into release sites. Petiole damage by larva, feeding scars by adults and number of adults per water hyacinth plant at release site are recorded. The visible signs are indicators of an established weevil population at the released site. The fresh weight reduction and leaf length reduction occurred at some places but feeding scars of the adult weevil increases in all places. The results recorded are shown in the tables below.

The weevils definitely slowed down plant growth and reduced water hyacinth density even in large growth stage where no complete control could be obtained even at highest inoculation load. Centre and Wright (1991) suggested that plant quality might influence the abundance of *Neochetina* spp. and hence the control of water hyacinth. It was seen that highest quality plants could be controlled faster than the poor quality plants and young plants were controlled earlier than the older ones. They found that adult of *N. eichhorniae* are attracted to young leaves because of presence of some volatile substance that stimulate them to feed especially at previous site of injury.

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TABLE 1: FECUNDITY OF *NEOCHETINA EICHHORNIAE*

SPECIES	DURATION	EGG LAYING
NEOCHETINA EICHHORNIAE	I Week	10(±5)
	II Week	19(±5)
	III Week	27(±3)
	IV Week	33(±2)
	V Week	223(±4)
	VI Week	18(±)
	VII Week	23(±2)
	VIII Week	20(±3)
	IX Week	16(±3)
	X Week	15(±2)
	XI Week	15(±2)
	XII Week	10(±2)
	XIII Week	9(±2)
	XIV Week	5(±2)
	XV Week	3(±2)
	XVI Week	1

TABLE 2: NUMBER OF WEEVIL RELEASED IN LABORATORY CONDITION

SPECIES	NUMBER OF ADULTS RELEASED	NUMBER OF PLANTS	DURATION	FEEDING SCARS/PLANT
NEOCHETINA EICHHORNIAE	2	1	1 week	10
	4	1	1 week	30
	8	2	1 week	25
	10	3	1 week	15

TABLE 3: MASS REARING AND RELEASED IN THE FIELD

NUMBER OF ADULTS RELEASED	1 SQUARE FEET AREA	DURATION	FEEDING SCARS
50	1	1week	300
50	1	3 weeks	750
50	1	1 month	25% damage to whole plant
50	1	2 months	35% damage to whole plant
50	1	4 months	50% damage to whole plant

TABLE 4: MASS REARING AND RELEASED INTO THE FIELD

NUMBER OF WEEVILS RELEASED	1 SQUARE FEET AREA	DURATION	FEEDING SCARS
100	1	1 week	500
100	1	3 weeks	1200
100	1	1 month	25%
100	1	2 months	40%
100	1	4 months	60%

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RESEARCH ARTICLE

A Study on Gross Alpha, Gross Beta and Terrestrial Gamma Radiation in the Coastal Stretch of Bay of Bengal, (South East Coast of India)

G. Satheeshkumar [1], P.Shahul Hameed[2]*, N. Anbusaravanan [1]

[1] PG and Research Dept. of Zoology, Periyar E.V.R. College, Tiruchirappalli--620 023, Tamil Nadu, India.

[2] Environmental Research Centre, J.J. College of Engineering and Technology, Tiruchirappalli--620 009, Tamil Nadu, India.

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***Address for correspondence**
P.Shahul Hameed
Environmental Research Centre,
J.J. College of Engineering and Technology, Tiruchirappalli,
Tamil Nadu- 620 009, India.
E.Mail:drps_zo@yahoo.co.in.

ABSTRACT

The paper reports the radiological profile of a 290 km coastal stretch of Bay of Bengal from Pondycherry to Velanganni by measuring gross alpha, gross beta and terrestrial gamma radiation levels in the beach sand of 10 chosen stations. The gross beta activity is higher than the gross alpha activity for any given station. The gross alpha activity ranged from 2 to 8 Bq g^{-1} with the mean value of 5.2 ± 2.3 Bq g^{-1}. The gross beta activity ranged from 12.2 to 19 Bq g^{-1} with the mean value of 16.6 ± 2.0 Bq g^{-1}. The gamma radiation rate was also non-uniformly distributed ranging from 4 to 81 μR h^{-1} and the mean terrestrial gamma radiation in the study area was 31 ± 25.8 μR h^{-1}. However it was observed that in any given station gamma radiation rate in the beach sand increased with distance from shore (up to 100 m). This condition was attributed to differential distribution of thorium bearing monazite in the beach sand. The study also concluded that in general, Bay of Bengal maintains the low level gamma radiation regime.

Key words: Gross Alpha, Gross Beta and Terrestrial Gamma Radiation, Bay of Bengal.

INTRODUCTION

The gross alpha and gross beta activities are the levels of radioactivity from all alpha and beta emitting radionuclides irrespective of individual contribution in the particular matrix. Measurements of gross alpha and beta activity in various environmental matrices are considered important because it offers quick information on the radioactivity profile of any environment concerned. Such procedures are important preliminary steps in the radioactivity inventory of any ecosystem. Measurements of gross alpha and gross beta activities are carried out to a greater degree in foreign marine environment [6]; [1]; [19]. However such kind of study in Indian environment is limited to the work of [13] in Bombay coast and [21] in Golf of Mannar.

The ^{238}U, ^{232}Th and their daughter products and the singly occurring radionuclide ^{40}K in soil and rocks are the major sources of gamma radiation in the environment. They emit gamma rays of sufficient intensity either directly or from their daughter products and contribute significantly to the gamma absorbed dose of the population [5]. The most important places among the well documented high background radiation areas of the world inhabited by sizable population are Brazil, China, India and Iran [17]; [2]; [23]; [11]. In India, monazite placer deposits have been found along its long coastal line; Ullal (Karnataka), Chavara (Kerala), Manavallakuruchy and Kalpakkam (Tamilnadu) and Chatrapur (Orissa) [8]; [4]; [14]. Since measurements of gross alpha and beta activities are indicative of radioactivity profile of any region, the present investigation is undertaken as a preliminary assessment of radioactivity profile of a coastal stretch of Bay of Bengal.

STUDY AREA

South East coast of India runs along the eastern boundary of Tamil Nadu state, India. It has three distinct marine zones namely, Gulf of Mannar, Palk Strait and Bay of Bengal. For the present study 10 stations were chosen along a 290 Km stretch of Bay of Bengal extending from Pondycherry (S1) (Lat 11^0 59'N; Long 79^0 50'E) to Velanganni (S10) ((Lat 10^0 68'; Long 79^0 84'). Except Pitchavaram Mangrove (S3), all other stations are sandy beaches. The stations are fixed based on accessibility, fishing activity and coastal human population (Fig.1).

MATERIALS AND METHODS

For the measurement of gross alpha and gross beta activity, the coastal sediment was dried in an oven at 105^O-110^OC for 24 h. 10 mg (for alpha) and 50 mg (for beta) of the powered dry sample was uniformly spread over a clean background counted aluminium planchette (3 cm diameter) using a micro sieve and the radioactivity was measured in Radiation Counting System (AT) (Nucleonix Model No: RC605A).

To investigate the levels of terrestrial gamma radiation in the study area, Scintillometric survey was undertaken. The ambient gamma radiation levels were measured with a calibrated SM141 ECIL scintillometer (NaI (Tl) crystal) with a reading range of 0 to1000 μR/h. In each station, gamma radiation was measured at various distances from water mark. Accordingly, scintillometric measurements were taken at 5 m, 10 m, 20 m, 30 m, 50 m and 100 m distance from the water mark along a transect. Multiple measurements were taken at each sampling stations. The mean terrestrial gamma radiation level in the beach area of 100 m wide was assessed and reported.

RESULTS

Data on the gross alpha and gross beta activity in the beach sand samples collected from 10 coastal stations of Bay of Bengal are presented in Table 1. It is evident from the results that gross beta activity is higher than the gross alpha activity. A minimum level of 2 ± 0.4 Bq g^{-1} of gross alpha activity was recorded in Tirumullaivasal (S4) while Velanganni (S10) as well as Devanampattinam (S2) registered a maximum level of 8 ±1.0 Bq g^{-1}. The mean gross alpha activity of entire stretch was found to be 5.2 ± 2.3 Bq g^{-1}. The gross beta activity in Bay of Bengal coast ranged from 12.2 ± 1.6 Bq g^{-1} (Pitchavaram Mangrove, S3) to 19 ± 2.2 Bq g^{-1} (Poompuhar, S5 and Taragambadi, S6) with the mean gross beta activity of 16.6 ± 2.0 Bq g^{-1} was recorded for the entire stretch.

The terrestrial gamma radiation levels at different distances from water mark ranging from 5 to 100 m in all the 10 sampling stations of Bay of Bengal are presented Fig 2. and the mean terrestrial gamma radiation levels in the sampling stations of Bay of Bengal are presented Table 1. It is evident that the gamma radiation rate is non-uniformly distributed with overall range of 4.0 to 81.0 μR h^{-1} and the mean terrestrial gamma radiation in the study area of Bay of Bengal was found to be 31 ± 25.8 μR h^{-1}. However it was observed that in a given station, gamma radiation rate was found to increase with the distance from shore (water mark). This increasing trend was maintained up to a distance of 50 m to 100 m from the shore. The data also revealed that Karaikal (S7) and Poompuhar (S5) beaches maintained a maximum gamma radiation levels ranging from 60 μR h^{-1} to 80 μR h^{-1}. Stations like other Devanampattinam (S2), Tharangambadi (S6) and Nagappattinam (S9) Maintained an intermediate levels ranging from 30 to 45 μR h^{-1} and stations like Pondycherry (S1), Pitchavaram Mangroves (S3), Tirumullaivasal (S4), Nagore (S8) and Velanganni (S10) maintained low levels of gamma radiation range from 4 μR h^{-1} (Pitchavaram Mangroves, S3) to 24.1 μR h^{-1} Nagore (S8).

DISCUSSION

The present study indicated that the accumulation of beta emitting radionuclides was higher than the alpha emitters. The higher beta activity observed could possibly be due to higher concentration of ^{40}K in the sand as suggested by [9]. Nagaiah [10] reported the gross beta activity of soil samples of Mysore (Srirangapatana) environment to be 652 Bq g^{-1}. Havlik [3] reported the gross beta activity in the Czechoslovakia sediment to be 2516 Bq g^{-1}. The Gulf of Mannar coastal sediment registered gross alpha activity of 86 Bq g^{-1} and gross beta activity 818 Bq g^{-1} as reported by Somasundaram [21]. The gross alpha and gross beta in beach sand observed in the present study are much lower compared to adjacent coast, Gulf of Mannar.

The mean value of gamma radiation levels in Bay of Bengal coast is lower (31.0 ± 25.8 μR h^{-1}) when compared to mean value of coastal environment of Karnataka (74 μR h^{-1}; [12] and also to mean value reported for different regions of India (80.7 μR h^{-1}; [7]. The gamma level of Bay of Bengal is less (range:4.0 to 81 μR h^{-1}) than that of Gulf of Mannar (range 10 to 450 μR h^{-1}; [16] and Kerala coast (100 to 3000 μR h^{-1}; [15] and relatively higher than the adjacent coastal Palk Strait (range 5 to 25 μR h^{-1}; [18]. The mean value for Bay of Bengal (31.0 μR h^{-1}) is less than the world average of 56 μR h^{-1} [22] to the normal background and this clearly establishes that in general, Bay of Bengal stretch under study maintains a low background level of Gamma Radiation.

The differential distribution of thorium bearing monazite in the coastal sand was responsible for the non-uniform distribution of gamma radiation recorded in coastal region of Bay of Bengal. The same pattern of distribution was recorded in the adjacent Palk Strait [18] and Gulf of Mannar coast [16]. It is interesting to compare the results of these measurements with the findings of [20] who have reported the ambient radiation levels over a 300 km stretch along the west coast of India from Mangalore to Karwar in Karnataka. Only in Ullal beach near Mangalore city, gamma level as high as 24 μR h^{-1} were otherwise gamma radiation level was observed to be low (3 - 16 μR h^{-1}) and uniform throughout the environment of coastal Karnataka up to Karwar. Gamma radiation level in the Kalpakkam coast

ranges from 20 to 400 μR h^{-1} [4] and this value is higher as compared to the reported background level of Bay of Bengal. From the present study it is concluded that Bay of Bengal maintains a non-uniform distribution and a low level of Gamma radiation in the entire stations and mangrove ecosystem maintain a lower gamma radiation level (4.0 μR h^{-1}).

The increase in concentration of gamma radiation with distance from water mark can be attributed to the tidal impact. During high tide more monazite laden sand are deposited on the shore while the water recedes back to its normal level. In this process a gradation of gamma radioactivity was formed. It is because the heavier black monazite sand particles get deposited farthest to sea and small sand particles nearest to sea as the tide return back to sea.

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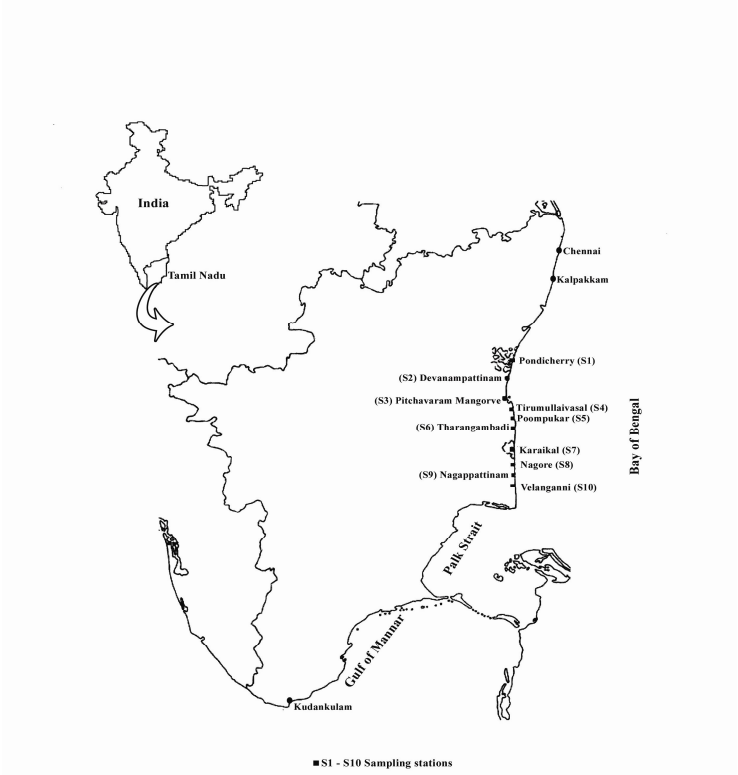

Fig. 1 The Map Showing the Study Area

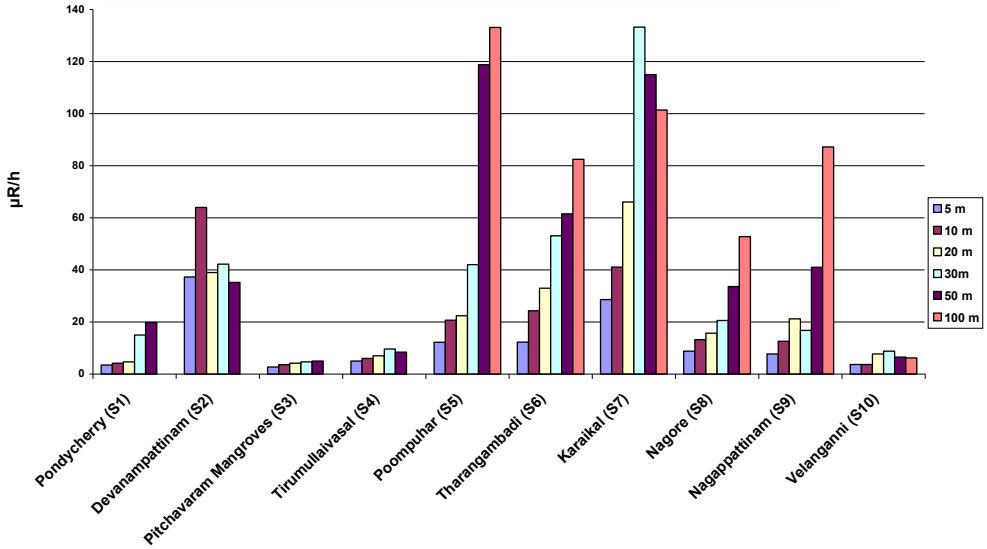

Fig 2. Terrestrial Gamma Radiation Level at Different Distances from Shore(Water Mark) of Sampling Stations in Bay of Bengal.

Table 1. Measurement of Gross Alpha, Gross Beta and Terrestrial Gamma Radiation in Coastal Sand Samples in Bay of Bengal.

Sta. Code	Name of the Station	Gross Alpha	Gross Beta	Terrestrial Gamma
		Bq/g	Bq/g	μR/h
S1.	Pondycherry	3.0 ± 0.5	17.0 ± 2.2	9.4 ± 7.4
S2.	Devanampattinam	8.0 ± 1.0	18.0 ± 3.1	43.5 ± 11.7
S3.	Pitchavaram Mangroves	6.0 ± 1.2	12.2 ± 1.6	4.0 ± 1.0
S4.	Tirumullaivasal	2.0 ± 0.4	16.0 ± 2.6	7.2 ± 1.8
S5	Poompuhar	7.0 ± 2.0	19.0 ± 1.8	58.2 ± 53.5
S6.	Tharangambadi	4.1± 0.6	19.0 ± 2.2	44.4 ± 26.0
S7.	Karaikal	6.0 ± 1.2	15.0 ± 1.8	81 ± 42.0
S8.	Nagore	6.0 ± 0.8	16.0 ± 1.6	24.1 ± 16.4
S9.	Nagappattinam	4.0 ± 0.6	17.0 ± 2.1	31.1 ± 29.7
S10.	Velanganni	8.0 ± 1.0	17.0 ± 1.6	6.1 ± 2.0
	Over All Range	**2.0 – 8.0**	**12.2 – 19.0**	**4.0 – 81.0**
	Over All Mean ± SD	**5.2 ± 2.3**	**16.6 ± 2.0**	**31.0 ± 25.8**

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RESEARCH ARTICLE

Studies on Methicillin- Resistant *Staphylococcus aureus* (MRSA) Prevalence Rate in Misurata, Libya.

Muftah M Zarmouh[1]*, A. K. Sajith Khan[2], R. Nazeerullah[3]

[1]& [3]Dept. of Microbiology, Faculty of Medicine, Misurata University, Libya.

[2]Dept. of Microbiology, Gulf Medical University, Ajman, Dubai.

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***Address for correspondence**

Muftah M Zarmouh
Associate Professor & Head,
Dept. of Microbiology, Faculty of Medicine,
Misurata University, Misurata, Libya.
E.Mail: nazee2001@gmail.com

ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains were identified by a gold standard method Multiplex PCR that targets detection of *mec*A genes from Methicillin- Resistant *S. aureus* (MRSA) strains in the hospital patients. A total of 164 *Staphylococcal* clinical isolates were studied that included 32 isolates of MRSA detected by using oxacillin (1µg) disc diffusion test. mec A genes were detected from the isolated *Staphylococcal* strains by multiplex PCR. Antimicrobial susceptibility study was done for all the 32 MRSA isolates. Of the total 164 clinical samples studied, 112 samples were *Staphylococcus aureus* and 52 samples were Coagulase –negative *Staphylococcus* (CoNS). Out of the 112 *S. aureus* isolates, 32 (28.5%) were MRSA and 80 (71.4%) were Methicillin sensitive *S. aureus* (MSSA) isolates detected by oxacillin disc diffusion method. Of the 52 CoNS, 14(26.9%) isolates were Methicillin resistant coagulase – negative *Staphylococcus* (MR-CoNS) and 38 (73.0%) isolates were MS-CoNS. 4 isolates (5%) were determined as Methicillin susceptible by the phenotypic method, were able to detect the presence of mecA genes by PCR. 7 strains (18.42%) of MS-CoNS yielded *mec A* gene by PCR which were recognized as Oxacillin susceptible by disc diffusion method. All the MRSA isolates were 100% susceptible to Vancomycin and Chloramphenicol. Clindamycin and Cefotaxime showed 82% and 56% susceptibility. Ampicillin was 100% resistant to the MRSA isolates. The prevalence rate in our hospital was 19.51%. The present study, concludes that PCR- based assays and Oxacillin disc diffusion method with Mueller Hinton Agar medium are more accurate for the detection of MRSA and MSSA, but for detecting Methicillin resistance in coagulase negative strains, molecular methods are the most reliable method

Key words: Methicillin resistant *Staphylococcus aureus*, Methicillin sensitive *Staphylococcus aureus*, coagulase-negative *Staphylococcus aureus*, *mecA* gene.

INTRODUCTION

The incidence of *Staphylococcus aureus* infections especially hospital acquired infections are increasing and are found to be resistant to various antibiotics [1]. Methicillin – resistant *Staphylococcus aureus* (MRSA) is an important nosocomial pathogen. During late 1970s and 1980s emergence of Methicillin-resistant *Staphylococcus aureus* infections reported world over and their introduction and spread in hospitals increased the overall incidence of nosocomial infections in the institutions and is a matter of great concern [2]. Therefore rapid and accurate detection of Methicillin resistant strains of *Staphylococci* and differentiating them from susceptible strains by clinical microbiology laboratories have a great importance in the therapy of infectious diseases of *Staphylococci* [3].

Methicillin resistance in *Staphylococci* is due to the presence of the *mecA* gene, which encodes the low-affinity penicillin-binding 2a. Presence of this gene defines the *Staphylococcus* as Methicillin resistant while absence of the gene from a *Staphylococcal* strain indicates Methicillin susceptibility [4]. Methicillin resistance can be difficult to detect because *mecA* –positive strains can differ in their level of expression of Methicillin resistance because few cells show heterogenous type of resistance, one in 10^4 or 10^6 [5].

In addition, Methicillin resistance is influenced by culture conditions such as temperature, medium pH and NaCl content in the medium. These factors complicate the detection process, especially strains with low-resistance level [6]. Phenotypic methods are usually preferred in many laboratories for the detection of MRSA, but it is time consuming and some difficulties in detection of some strains. Hence, the tests based on detection of genotype are more accurate than phenotypic methods [5, 7]. The *mecA* gene is highly conserved among *Staphylococcal* species, therefore detection of this gene by polymerase chain reaction is considered as the gold standard for Methicillin resistance. Early detection of MRSA and formulation of effective antibiotic policy in hospitals are of paramount importance from the hospital epidemiological point [8].

In the present study, we used the Multiplex PCR for the detection of *mecA* genes among the MRSA, MSSA and Coagulase-negative *Staphylococcus* isolates and determined the antimicrobial susceptibility profile of these isolates and the prevalence rate. The purpose of the study was to set up a rapid and accurate detection procedure for Methicillin resistance among *Staphylococcal* isolates obtained in our hospital through the amplification of specific gene determinants by Multiplex PCR in order to efficiently support therapy and eradication of the pathogen.

MATERIALS AND METHODS

Clinical isolates

In the present study, a total of 164 Staphylococcal clinical isolates collected between the periods 2008 to 2009. All the strains were clinical isolates (Pre-operative and post-operative samples) obtained from the surgery department of Misurata Central Hospital, Misurata, Libya. The samples include pyogenic materials, aspirates and wound secretions. A reference strain of *Staphylococcus aureus* (NCTC 6571) was included in the study. The specimens were collected with sterile aseptic condition and as much as possible before instituting the antibacterial therapy. The pyogenic materials were collected by swabbing the wound site with two sterile swabs. The aspirates from abscesses were collected with sterile disposable syringe. The specimens were dispatched immediately to the Microbiology department, Faculty of Medicine, Misurata university and processed immediately or if delayed then refrigerated for not more than 30 minutes. Identification of Staphylococcal isolates was done by morphology, Gram stain, Standard biochemical characteristics and *Staphylococcus aureus* was identified by using the Standard tube coagulase test.

Disc diffusion tests

The disc diffusion method for the detection of MRSA was performed by following the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) [9]. Disc diffusion tests were performed using 1 µg/ml Oxacillin discs. Few colonies of *Staphylococcus* were inoculated into peptone water and incubated for 4 hours. The test organisms were then streaked by using a swab onto Mueller-Hinton agar (MHA) with 4% NaCl supplementation [10]. Oxacillin 1µg disc were placed onto each plate and the zone of inhibition was determined after 24 hours of incubation at 35°C. Organisms with a zone equal to or greater than 12mm were interpreted as susceptible while those with an inhibition zone equal to or lesser than 10 mm were considered as resistant to Oxacillin. Inhibition zones of 11-12 mm were interpreted as intermediate [11].

Antimicrobial susceptibility pattern of MRSA isolates

After confirmation of MRSA, antimicrobial susceptibility test was performed for all the 14 MRSA isolates by Kirby–Bauer's disc diffusion method using *Staphylococcus aureus* NCTC 6571 as the control strain [11,12]. The following antimicrobials with their disc contents in µg/ml disc were used –Ampicillin(10µg), Amikacin(30µg), Clindamycin(2µg), Chloramphenicol (30µg), Cotrimaxazole (25µg) Tetracycline (30µg), Gentamicin (10µg), Ofloxacin (5µg), Ciprofloxacin (5µg), Cefotaxime (30µg), and Vancomycin (30µg). The antibiotic discs were obtained from commercial source (oxford).

Genomic DNA isolation

Total genomic DNA was extracted by standard method Smoker and Barnum [13]. All the isolated *Staphylococcus aureus* strains were grown in appropriately labeled 10ml Brain Heart infusion broth at 35°C for 24 hours. Cell pellet was collected from 5ml of the culture broth by centrifugation at 6000rpm. The pellet was suspended in 500µl of lysis solution (50mM Tris-HCl pH 8.0, 5mM EDTA pH 8.0 and 50mM NaCl). Lysozyme was added to obtain a final concentration of 1mg/ml and the solution was incubated at 55°C for 30minutes. 10µl of proteinase K (1mg/ml) was added in the incubated solution and 20µl of 10% SDS was also added and incubated again at 55°C for 10 minutes or until the solution cleared. The solution was chilled on ice, and extracted with an equal volume of Phenol: Chloroform: Isoamylalcohol (25: 24: 1), thoroughly mixed this solution and supernatant was collected. Equal volume of 4M ammonium acetate (pH - 5.5) was added with this supernatant and double the volume of isopropanol was added with that mixer. Total genomic DNA was precipitated by centrifugation at 12,000 rpm for 10 minutes at - 4°C. The DNA pellet was allowed to air dry and the dried pellet was dissolved in TE buffer (10mM Tris - HCl pH - 8.0 and 0.1mM EDTA pH-8.0) and stored the pellet at -20°C. Like this the remaining *Staphylococcus aureus* strain's genomic DNA was extracted and stored.

Oligonucleotide primer for *mec*A gene amplification and PCR conditions

The sequences of Oligonucleotide primers used for PCR are listed in Table-1. The design and synthesis of the *mec*A Oligonucleotide forward and reverse primers such as *mec*A1 and *mec*A2 primers are 5′ATCGATGGTAAAGGTTGGC′3 and 5′AGTTCTGCAGTACCGG ATTTGC3′ for 530 base pairs and 5′TCCAGATTACAACTTCACCAGG3′and 5′CCATTCATATCTTGTACG3′ for 162 base pairs [14]. Both the primers were synthesized by XDT Technology (Germany). The PCR reactions were earned out in a 25µl volume containing 1µl (50 pmol) of each primer, 1.5 µl of 1.5mM of dNTPs, 1µl (50µg) of template DNA, 1U (0.5µl) of DyNAzyme™ DNA polymerase (Finzymes, Finland), the buffer supplied (10x Buffer 2.5µl) with the enzyme was used according to the manufacturer's directions and make upto 25µl with double distilled water. The PCR amplification was performed with DNA thermal cycler (Eppendorf Master Cycler Gradient, Germany). The cycling condition is the initial denaturation at 94°C for 4 minutes, denaturation at 94°C for 45 seconds, annealing reaction is 50°C for 45 seconds, extension reaction is 72°C for 60 seconds and final extension reaction is 72°C for 5 minutes. Like this, 30 cycles were

done and after the reaction was completed, 10µl of amplified DNA product was separated on 1.2% low melting agarose (Sigma, USA), stained with ethidium bromide and recorded the separation bands using a CCD camera in UVP gel documentation system (UVP, England). Standard molecular marker λ DNA supplied by DyNAzyme™ II DNA polymerase Kit (Finzymes, Finland) was used as marker DNA in the gel. Like this, the entire genomic DNA extracted from all the subsurface bacterial strains were amplified for *mec*A1 and *mec*A2.

RESULTS

Of the total 164 clinical samples studied, 112 samples were *Staphylococcus aureus* and 52 samples were Coagulase – negative *Staphylococcus* (CoNS). Out of the 112 *S. aureus* isolates, 32 (28.5%) were MRSA and 80 (71.4%) were MSSA isolates detected by Oxacillin disc diffusion method. Of the 52 CoNS, 14(26.9%) isolates were MR-CoNS and 38(73.0%) isolates were MS-CoNS. All the MRSA isolates were 100% susceptible to Vancomycin and Chloramphenicol. Clindamycin and Cefotaxime showed 82% and 56% susceptibility. Ampicillin was 100% resistant to the MRSA isolates (Table-2).

MRSA strains were unequivocally detected within three hours using multiplex PCR with *mecA* gene-specific Oligonucleotides. On the basis of specific amplifications of the *mecA* genes the multiplex PCR procedure allowed the specific identification of the Staphylococcal species and the determination of its susceptibility to β-lactum antibiotics. All the 32 MRSA strains (100%) showed *mecA* gene positive by PCR (Fig.1). We found 4 isolates (5%) that were determined as Methicillin susceptible by the phenotypic method, were able to detect the presence of *mec*A genes by PCR. Also we observed 7 strains (18.42%) of MS-CoNS yielded *mecA* gene by PCR which were recognized as oxacillin susceptible by disc diffusion method. The prevalence rate of MRSA in our hospital was 19.51%.

DISCUSSION

The epidemiology of MRSA has continued to evolve since its first appearance more than three decades ago [15]. Initially, there were sporadic reports of Methicillin resistance amongst nosocomial *S. aureus* but later MRSA became a well known established hospital acquired pathogen with few reports of community acquired isolates [16]. PCR-based assays are considered as the gold standard for the detection of MRSA, since many phenotypic detection methods show problematic results due to the heterogenous resistance displayed by many clinical isolates. Genotypic methods are more accurate in detecting Methicillin resistant *Staphylococci* than conventional susceptibility methods. Many automated detection methods are currently available in market because of their short detection time but their sensitivity for detection of Methicillin resistance is low.

In the present study we evaluated the oxacillin disc diffusion phenotype method for the detection of Methicillin resistance with *mecA* multiplex PCR using new NCCLS criteria. Also we studied the antimicrobial susceptibility pattern of the MRSA isolates and determined the prevalence rate of MRSA in our hospital.

In this study, 32 strains have determined as MRSA by Oxacillin disc diffusion method also showed 100% consistency with PCR results. Also we were able to detect *mecA* genes in 4 MSSA isolates by PCR which were classified as Methicillin susceptible by phenotypic methods. Among the MS-CoNS isolates, 7 strains showed *mecA* genes positive by PCR. This may be due to the fact that heterogenous expression of resistance varies more for MS-CoNS compared to *Staphylococcus aureus* and the subpopulation of resistant cells is smaller for MS-CoNS than for *S. aureus*.

All these cryptic strains should be regarded as potentially Methicillin-resistant isolates bearing the *mecA* gene and should not be classified as Methicillin-susceptible in spite of their susceptibility to β-lactum antibiotics [17]. The cryptically Methicillin resistant strains were most probably derived from typically resistant strains; that is, they were first selected as Methicillin resistant strains by β- lactum antibiotics but later stopped the production of PBP -2′ with

loss of their resistance [18]. The unstable nature of Methicillin resistance has been reported previously and a recent report described *mecA* positive but phenotypically susceptible subclones, as well as *mecA* negative ones, that arose from a Methicillin resistant strain after penicillinase plasmid elimination [5,18]. In this study, all the MRSA isolates were 100% susceptible to Vancomycin and Chloramphenicol. Clindamycin and Cefotaxime showed 82% and 56% susceptibility. Ampicillin was 100% resistant to the MRSA isolates. The prevalence rate of MRSA was found to be 19.52%.

CONCLUSION

In conclusion, both oxacillin disc diffusion method with MHA and PCR- based methods are more accurate in detecting Methicillin resistance among *Staphylococcus aureus* isolates but for CoNS isolates, detection of Methicillin resistance should always depend on molecular methods since conventional phenotypic methods are less reliable. The multiplex PCR employed here were useful both in rapid detection of Methicillin resistance and were able to detect cryptically Methicillin resistant strains which yielded a typically Methicillin resistant subpopulation. The early detection of MRSA by PCR and formulation of effective antibiotic policy in hospitals are of paramount importance from the hospital epidemiological point.

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Table: 1 - *mecA* gene primers

Primer	Oligonucleotide Sequences (5′ – 3′)	Amplican Size (bp)
mec A1 *mec A2*	'TCCAGATTACAACTTCACCAGG CCATTCATATCTTGTAACG'	162
mec A2F *mec A3R*	'ATCGATGGTAAAGGTTGGC AGTTCTGCAGTACCGGATTTGC'	530

Table: 2 - *mecA* gene presence with Oxacillin and coagulase +ve strains in the clinical isolates.

Isolates	Resistant strains	Phenotypic observation		*mecA* gene presence
		Oxacillin resistant strains	Coagulase + ve strains	
MRSA	32	32	32	32
MSSA	80	0	80	4
MS-CoNS	38	0	0	7
MR-CoNS	14	14	0	14

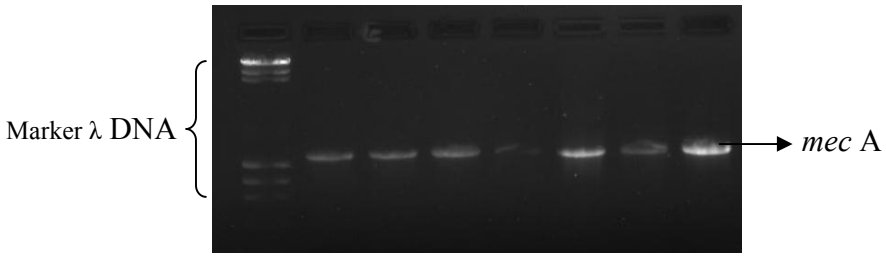

Fig. 1: Amplified *mec*A genome with marker DNA

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REVIEW ARTICLE

Effects of Air Pollution on Health and the Natural Resources

Sirajuddin.M. Horaginamani* and M. Ravichandran

Department of Environmental Management, School of Environmental Sciences, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India.

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***Address for correspondence**
Sirajuddin.M. Horaginamani
Department of Environmental Management,
School of Environmental Sciences,
Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India.
E.Mail : ecosirajuddin@yahoo.com.

ABSTRACT

Like photochemical pollutants, sulfur oxides contribute to the incidence of respiratory diseases. Acid rain, a form of precipitation that contains high levels of sulfuric or nitric acids, can contaminate drinking water and vegetation, damage aquatic life, and erode buildings. When a weather condition known as temperature inversions prevents dispersal of smog, inhabitants of the area, especially children and the elderly and chronically ill, is warned to stay indoors and avoid physical stress. The dramatic and debilitating effects of severe air pollution episodes in cities throughout the world—such as the London smog of 1952 that resulted in 4,000 deaths—have alerted governments to the necessity for crisis procedures. Even everyday levels of air pollution may insidiously affect health and behavior. Indoor air pollution is a problem in developed countries, where efficient insulation keeps pollutants inside the structure. In less developed nations, the lack of running water and indoor sanitation can encourage respiratory infections. Carbon monoxide, for example, by driving oxygen out of the bloodstream, causes apathy, fatigue, headache, disorientation, and decreased muscular coordination and visual acuity.

Key words: Air pollution, health, natural resources, temperature inversions, sanitation, apathy.

INTRODUCTION

Since the onset of the industrial revolution, there has been a steady change in the composition of the atmosphere mainly due to the combustion of fossil fuels used for the generation of energy and transportation. Air pollution is a major environmental health problem affecting the developing and the developed countries alike. The effects of air pollution on health are very complex as there are many different sources and their individual effects vary from one to the other. It is not only the ambient air quality in the cities but also the indoor air quality in the rural and the urban areas that are causing concern. In fact in the developing world the highest air pollution exposures occur in the indoor environment. Air pollutants that are inhaled have serious impact on human health affecting the lungs and the respiratory system; they are also taken up by the blood and pumped all round the body. These pollutants are also deposited on soil, plants, and in the water, further contributing to human exposure. As you read on you can learn about health impacts of specific air pollutants.

Effects of air pollution on health

The magnitude of the London fog of 1952, which affected such a large number of people, was the first incident that made people aware of the damage done to the atmosphere due to industrialization. The SPM levels increased manifold and resulted in over 4000 deaths.

Indoor air pollution can be particularly hazardous to health as it is released in close proximity to people. It is stated that a pollutant released indoors is many times more likely to reach the lung than that released outdoors. In the developing countries a fairly large portion of the population is dependent on biomass for their energy requirements. These include wood, charcoal, agricultural residue, and animal waste. Open fires used for cooking and heating are commonly found in the household both in the rural and the urban areas. The stove is often at floor level, adding to the risk of accident and the hygiene factor. In addition, they are often not fitted with a chimney to remove the pollutants. In such households the children and women are most likely to be affected, as they are the group that spends more time indoors. The main pollutant in this environment is the SPM. In fact, death due to indoor air pollution, mainly particulate matters, in the rural areas of India is one of the highest in the world. Many of the deaths are due to acute respiratory infections in children; others are due to cardiovascular diseases, lung cancer, and chronic respiratory diseases in adults. If emissions are high and ventilation is poor, household use of coal and biomass can severely affect the indoor air quality.

Pollutant emissions per meal are also very high compared to those of other fuels. Household use of fossil fuel is also fairly common in the developing countries, particularly coal—both bituminous and lignite. These are particularly damaging as they burn inefficiently and emit considerable quantities of air pollutants. If emissions are high and ventilation poor, then the exposure levels to the gases emitted are far higher. The most harmful of the gases and agents that are emitted are particulate matter, carbon dioxide, polycyclic organic matter, and formaldehyde. The indoor concentrations of these are far higher than the acceptable levels and are cause for concern in rural areas.

The human health effects of poor air quality are far reaching, but principally affect the body's respiratory system and the cardiovascular system. Individual reactions to air pollutants depend on the type of pollutant a person is exposed to, the degree of exposure, the individual's health status and genetics. People who exercise outdoors, for example, on hot, smoggy days increase their exposure to pollutants in the air.The health effects caused by air pollutants may range from subtle biochemical and physiological changes to difficulty breathing, wheezing, coughing and aggravation of existing respiratory and cardiac conditions. These effects can result in increased medication use, increased doctor or emergency room visits, more hospital admissions and even premature death.

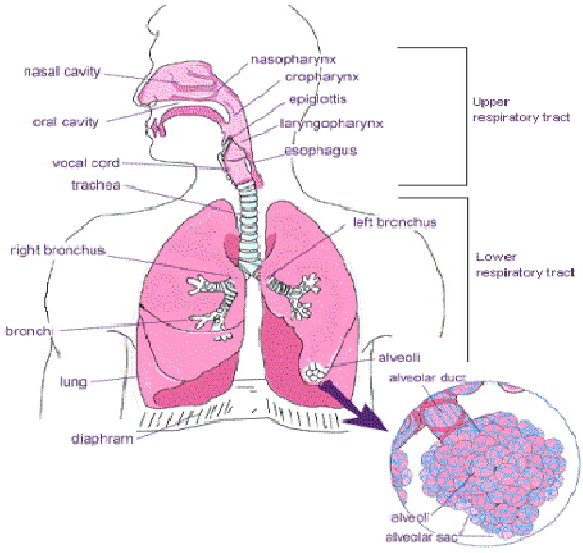
Human Respiratory System

The health of our lungs and entire respiratory system is affected by the quality of the air we breathe. In addition to oxygen, this air contains other substances such as pollutants, which can be harmful. Exposure to chemicals by inhalation can negatively affect our lungs and other organs in the body. The respiratory system is particularly sensitive to air pollutants because much of it is made up of exposed membrane. Lungs are anatomically structured to bring large quantities of air (on average, 400 million litres in a lifetime) into intimate contact with the blood system, to facilitate the delivery of oxygen.

Lung tissue cells can be injured directly by air pollutants such as ozone, metals and free radicals. Ozone can damage the alveoli -- the individual air sacs in the lung where oxygen and carbon dioxide are exchanged. More specifically, airway tissues which are rich in bioactivation enzymes can transform organic pollutants into reactive metabolites and cause secondary lung injury. Lung tissue has an abundant blood supply that can carry toxic substances and their metabolites to distant organs. In response to toxic insult, lung cells also release a variety of potent chemical mediators that may critically affect the function of other organs such as those of the cardiovascular system. This response may also cause lung inflammation and impair lung function.

Structure and Function

The human respiratory system is dominated by our lungs, which bring fresh oxygen (O_2) into our bodies while expelling carbon dioxide (CO_2). The oxygen travels from the lungs through the bloodstream to the cells in all parts of the body. The cells use the oxygen as fuel and give off carbon dioxide as a waste gas. The waste gas is carried by the bloodstream back to the lungs to be exhaled.



The lungs accomplish this vital process - called gas exchange - using an automatic and quickly adjusting control system. This gas exchange process occurs in conjunction with the central nervous system (CNS), the circulatory system, and the musculature of the diaphragm and the chest.

The human respiratory system can be divided into the upper respiratory tract and the lower respiratory tract.
The upper respiratory tract includes the following rigid structures:

Nasal cavities: Filter the air we breathe and provide a sense of smell.

Pharynx: Acts in the respiratory and the digestive system.

Larynx: Link between the pharynx and the trachea. Generates the voice with the presence of vocal folds.

Trachea: The trachea is the bond with the lower respiratory tract. This is a flexible structure allowing the air to go down to the lungs.

In addition to gas exchange, the lungs and the other parts of the respiratory system have important jobs to do relate to breathing. These include:

- Bringing all air to the proper body temperature.
- Moisturizing the inhaled air for necessary humidity.
- Protecting the body from harmful substances by coughing, sneezing, filtering or swallowing them, or by alerting the body through the sense of smell.
- Defending the lungs with cilia (tiny hair-like structure), mucus and macrophages, which act to remove harmful substances deposited in the respiratory system.

The respiratory system is sensitive to air pollution. The cardiovascular system can be affected as well.

Human Cardiovascular System

The cardiovascular system has two major components: the heart and a network of blood vessels. The cardiovascular system supplies the tissues and cells of the body with nutrients, respiratory gases, hormones, and metabolites and removes the waste products of cellular metabolism as well as foreign matter. It is also responsible for maintaining the optimal internal homeostasis of the body and the critical regulation of body temperature and pH.

The inhalation of air pollutants eventually leads to their absorption into the bloodstream and transport to the heart. A wide spectrum of chemical and biological substances may interact directly with the cardiovascular system to cause structural changes, such as degenerative necrosis and inflammatory reactions. Some pollutants may also directly cause functional alterations that affect the rhythmicity and contractility of the heart. If severe enough, functional changes may lead to lethal arrhythmias without major evidence of structural damage to the myocardium.

There also may be indirect actions secondary to changes in other organ systems, especially the central and autonomic nervous systems and selective actions of the endocrine system. Some cytokins released from other inflamed organs may also produce adverse cardiovascular effects, such as reducing the mechanical performance and metabolic efficiency of the heart and blood vessels.

Health impact of specific air pollutants:

Some of these gases can seriously and adversely affect the health of the population and should be given due attention by the concerned authority. The gases mentioned below are mainly outdoor air pollutants but some of them can and do occur indoor depending on the source and the circumstances.

Tobacco smoke: Tobacco smoke generates a wide range of harmful chemicals and is a major cause of ill health, as it is known to cause cancer, not only to the smoker but affecting passive smokers too. It is well-known that smoking affects the passive smoker (the person who is in the vicinity of a smoker and is not himself/herself a smoker) ranging from burning sensation in the eyes or nose, and throat irritation, to cancer, bronchitis, severe asthma, and a decrease in lung function.

Biological pollutants*:* These are mostly allergens that can cause asthma, hay fever, and other allergic diseases.

Volatile organic compounds : Volatile compounds can cause irritation of the eye, nose and throat. In severe cases there may be headaches, nausea, and loss of coordination. In the longer run, some of them are suspected to cause damage to the liver and other parts of the body.

Formaldehyde*:* Exposure causes irritation to the eyes, nose and may cause allergies in some people.

Lead*:* Prolonged exposure can cause damage to the nervous system, digestive problems, and in some cases cause cancer. It is especially hazardous to small children.

Radon.: A radioactive gas that can accumulate inside the house, it originates from the rocks and soil under the house and its level is dominated by the outdoor air and also to some extent the other gases being emitted indoors. Exposure to this gas increases the risk of lung cancer.

Ozone*:* Exposure to this gas makes our eyes itch, burn, and water and it has also been associated with increase in respiratory disorders such as asthma. It lowers our resistance to colds and pneumonia

.

Oxides of nitrogen*:* This gas can make children susceptible to respiratory diseases in the winters.

Carbon monoxide*:* CO (carbon monoxide) combines with haemoglobin to lessen the amount of oxygen that enters our blood through our lungs. The binding with other haeme proteins causes changes in the function of the affected organs such as the brain and the cardiovascular system, and also the developing foetus. It can impair our concentration, slow our reflexes, and make us confused and sleepy.

Sulphur dioxide*:* SO_2 (sulphur dioxide) in the air is caused due to the rise in combustion of fossil fuels. It can oxidize and form sulphuric acid mist. SO_2 in the air leads to diseases of the lung and other lung disorders such as wheezing and shortness of breath. Long-term effects are more difficult to ascertain as SO2 exposure is often combined with that of SPM.

SPM (suspended particulate matter). Suspended matter consists of dust, fumes, mist and smoke. The main chemical component of SPM that is of major concern is lead, others being nickel, arsenic, and those present in diesel exhaust. These particles when breathed in, lodge in our lung tissues and cause lung damage and respiratory problems. The importance of SPM as a major pollutant needs special emphasis as a) it affects more people globally than any other pollutant on a continuing basis; b) there is more monitoring data available on this than any other pollutant; and c) more epidemiological evidence has been collected on the exposure to this than to any other pollutant.

Effects of Air Pollution on Natural Resources along with harming human health, air pollution can cause a variety of effects on natural resources. Some of them are as follows:

Acid rain is precipitation containing harmful amounts of nitric and sulfuric acids. These acids are formed primarily by nitrogen oxides and sulfur oxides released into the atmosphere when fossil fuels are burned. These acids fall to the Earth either as wet precipitation (rain, snow, or fog) or dry precipitation (gas and particulates). Some are carried by the wind, sometimes hundreds of miles. In the environment, acid rain damages trees and causes soils and water bodies to acidify, making the water unsuitable for some fish and other wildlife. It also speeds the decay of buildings, statues, and sculptures that are part of our national heritage. Acid rain has damaged Massachusetts lakes, ponds, rivers, and soils, leading to damaged wildlife and forests.

Eutrophication is a condition in a water body where high concentrations of nutrients (such as nitrogen) stimulate blooms of algae, which in turn can cause fish kills and loss of plant and animal diversity. Although eutrophication is a natural process in the aging of lakes and some estuaries, human activities can greatly accelerate eutrophication by increasing the rate at which nutrients enter aquatic ecosystems. Air emissions of nitrogen oxides from power plants, cars, trucks, and other sources contribute to the amount of nitrogen entering aquatic ecosystems.

Haze is caused when sunlight encounters tiny pollution particles in the air. Haze obscures the clarity, color, texture, and form of what we see. Some haze-causing pollutants (mostly fine particles) are directly emitted to the atmosphere by sources such as power plants, industrial facilities, trucks and automobiles, and construction activities. Others are formed when gases emitted to the air (such as sulfur dioxide and nitrogen oxides) form particles as they are carried downwind. For more information on haze, visit the U.S. Environmental Protection Agency (EPA) Visibility.

Effects on wildlife: Toxic pollutants in the air, or deposited on soils or surface waters, can impact wildlife in a number of ways. Like humans, animals can experience health problems if they are exposed to sufficient concentrations of air toxics over time. Studies show that air toxics are contributing to birth defects, reproductive failure, and disease in animals. Persistent toxic air pollutants (those that break down slowly in the environment) are of particular concern in aquatic ecosystems. These pollutants accumulate in sediments and may biomagnify in tissues of animals at the top of the food chain to concentrations many times higher than in the water or air.

Ozone depletion : Ozone is a gas that occurs both at ground-level and in the Earth's upper atmosphere, known as the stratosphere. At ground level, ozone is a pollutant that can harm human health. In the stratosphere, however, ozone forms a layer that protects life on earth from the sun's harmful ultraviolet (UV) rays. But this "good" ozone is gradually being destroyed by man-made chemicals referred to as ozone-depleting substances, including chlorofluorocarbons, hydrochlorofluorocarbons, and halons. These substances were formerly used and sometimes still are used in coolants, foaming agents, fire extinguishers, solvents, pesticides, and aerosol propellants. Thinning of the protective ozone layer can cause increased amounts of UV radiation to reach the Earth, which can lead to more cases of skin cancer, cataracts, and impaired immune systems. UV can also damage sensitive crops, such as soybeans, and reduce crop yields.

Crop and forest damage: Air pollution can damage crops and trees in a variety of ways.Ground-level ozone can lead to reductions in agricultural crop and commercial forest yields, reduced growth and survivability of tree seedlings, and increased plant susceptibility to disease, pests and other environmental stresses (such as harsh weather). As described above, crop and forest damage can also result from acid rain and from increased UV radiation caused by ozone depletion.

Global climate change: The Earth's atmosphere contains a delicate balance of naturally occurring gases that trap some of the sun's heat near the Earth's surface. This "greenhouse effect" keeps the Earth's temperature stable.

Unfortunately, evidence is mounting that humans have disturbed this natural balance by producing large amounts of some of these greenhouse gases, including carbon dioxide and methane. As a result, the Earth's atmosphere appears to be trapping more of the sun's heat, causing the Earth's average temperature to rise - a phenomenon known as global warming. Many scientists believe that global warming could have significant impacts on human health, agriculture, water resources, forests, wildlife, and coastal areas.

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RESEARCH ARTICLE

Multiparameter Monitoring System for Coal Mine

M.Abdul Wahab Johny[1]* and J.Sudharsan[2]

[1]Department of Nano Science and Technology, Anna University, Tiruchirappalli-620024, Tamil Nadu, India.

[2]Dept. of Electronic and Communication Engineering, Dhaanish Ahmed College of Engineering Padappai, Chennai - 624302, Tamil Nadu, India.

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***Address for correspondence**

M.Abdul Wahab Johny
Department of Nano Science and Technology,
Anna University, Tiruchirappalli-620024,
Tamil Nadu, India
E.Mail : wahabwithyou@gmail.com

ABSTRACT

According to the characteristics of coal mine environment, in this paper we propose a multi-parameter monitoring system based on Zigbee technology for coal mine tunnel using sensors. The systems can real-time monitor the underground environment and production parameters and intelligently give early warning by using a variety of sensors. It is flexible to add wireless sensors and enhance stability of monitoring computer software through RS-232 communication protocol and hardware modular. The system implements the real-time monitoring and displaying for data under mine, query, deletion and maintenance of history data, graphic statistic, report printing, expert diagnosis and decision-making support modules. The intelligent decision-making support module sets in the system, can detect the level of parameters which are affected the production of coal mine. The Research, development and Promote Application will provide the safeguard regarding the mine pit security work. The paper shows that the system is flexible in the architecture of software and hardware, and can be easily extended to other mine safety production fields.

Keywords: Zigbee technology, architecture, support modules, hardware modular, wireless sensors.

INTRODUCTION

In a Coal mine there are two types of security needed one for human being and another for production because coal enterprise is the high-risk profession and technique now is relative backwardness. Security is the most important in the coal mine production. Establishing mine safety production safeguard system is the only way to guarantee the safety in coal mine production. Currently in mine production, there are mainly following two aspects to impact the safety in mine production:

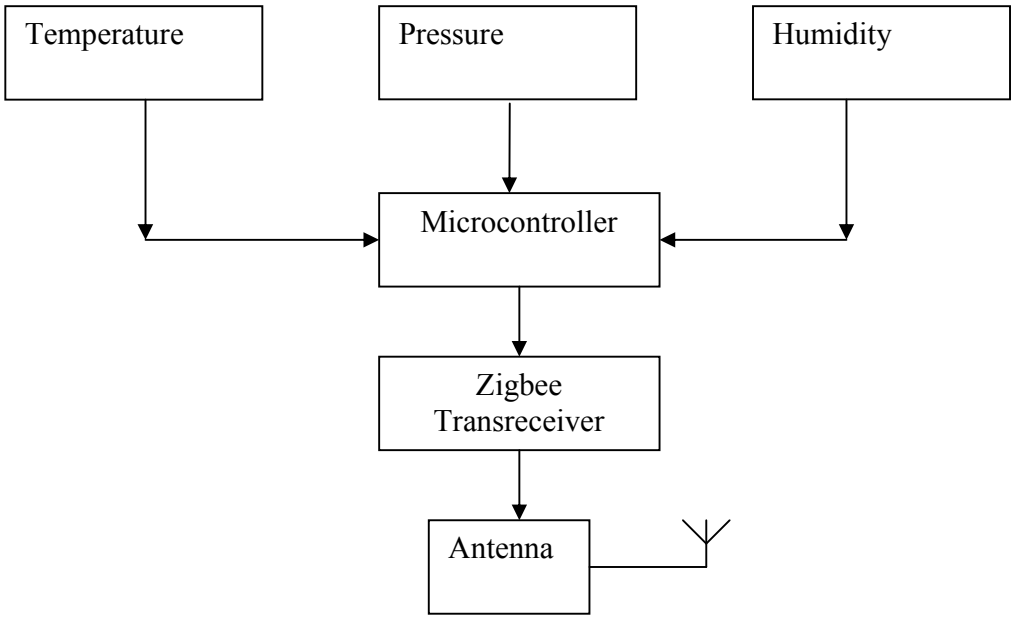
(1)Environment Parameters: methane Gas, Carbon Monoxide, Temperature (Humidity) Degree, Coal Position of the Bunker, Pressure of the roof etc.

(2)Electromechanical Device Running Parameters: transport fix, belt conveyer, Voltage, Electric Current and so on.

Multi parameter monitoring system in coal mine is the significant measure that safeguards the safe production in coal mine. It acts vital role in disaster prevention and reduction in mine, as well as improve the productivity. It also is the significant milestone of implementing the modern management for mine production.

Block diagram of Transmitter section

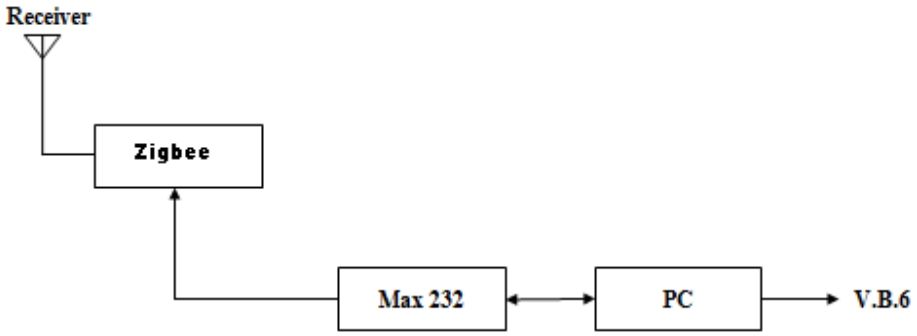
The block diagram of transmitter section consists of Sensors,Microcontroller AT89s52,Max 232,Zigbee Maxstream Power Supply,Analog to Digital Converter O8



Block diagram of receiver section

The block diagram of Receiver section consists of ZigbeeMaxstream,Max 232,PC

Block diagram of receiver section



Hardware Description

Sensors

Temperature Sensor LM35, Pressure sensor MP3V505, Humidity sensor HS220 are used which measures temperature, pressure, humidity and transmits the information to analog to digital converter and then to microcontroller.

Microcontroller AT89s52

The Micro controller used is AT89S52. It has 8k bytes of On-chip programming ROM. It has 256 bytes of RAM. It has 3 Timers. There are 4 ports each consisting of 8 pins thus totaling to 32 input pins. There is one serial port and there are 8 interrupt sources The AT89S52 is a low-power, high-performance CMOS 8-bit microcomputer with 8K bytes of down loadable Flash programmable and erasable read only memory and 2K bytes of EEPROM.

ADC

The ADC0808, ADC0809 data acquisition component is a monolithic CMOS device with an 8-bit analog-to-digital converter, 8-channel multiplexer and microprocessor compatible control logic. The 8-bit A/D converter uses successive approximation as the conversion technique.

MAX 232

The MAX 232 IC is used to communicate between the Microcontroller and the PC. It is connected to port 3 of the microcontroller. It is a driver circuit which includes four capacitors. It is used for voltage doubling and supplying the required voltage to the devices. It is connected to port 3 of the microcontroller.

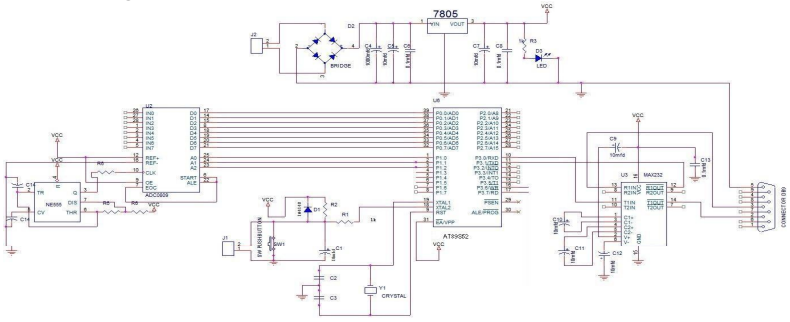
Zigbee Module

It is a transceiver, which is used to transfer data or information from one node to another. The advantages are low cost, low power, high reliability; it is more suitable for this application. It works with the principle of Wireless Mesh Networking.

Power Supply

The circuit is powered by a 12V dc adapter, which is given to LM7805voltage regulator by means of a forward voltage protection diode and is decoupled by means of a 0.1 μf capacitor. The voltage regulator gives an output of exactly 5V dc supply. The 5V dc supply is given to all the components including the Microcontroller, the serial port, and the IR transmitters and sensors.

Circuit Diagram



Working steps

Step 1: The circuit is powered by a 12V dc adapter, which is given to LM7805 voltage regulator. The voltage regulator gives an output of exactly 5V dc supply. The 5V dc supply is given to all the components including the Microcontroller, the serial port, and the IR transmitters and sensors.

Step 2: The temperature sensor measures temperature, the pressure sensors measures the pressure, the humidity sensors measures the humidity occurring in the environment and send to ADC0809.

Step 3: The ADC0809 converts the analog data into digital data, the clock pulse is given to ADC using NE555 TIMER.

Step 4: The ADC is interfaced to microcontroller which stores information temporarily and transmits using Zigbee.

Step 5: In Receiver side we have zigbee which receives the transmitted data and using RS232 we give the information to PC

Step 6: In PC we used visual basic by which the temperature, humidity, pressure values are measured and also graph for each parameter can be seen.

CONCLUSION

In this project we design a multi-parameter monitoring system for coal mine combining the ZigBee wireless sensor network technology with RS485 communication technology. Monitor nodes can easily be added or removed in the system; it is also convenient to expand the network. As an expansion of existing coal mine wired Security systems it can enhance the flexibility of information collecting, while reducing the cost of building safety system communication network in coal mining. So it improves the applied value of coal mine safety monitoring and practical value of coal mine safety monitoring and control information system.

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REVIEW ARTICLE

Management of Garbage and Rubbish at Once by Absorption Technique

Abdul Samad. M. Kamdod*

Department of Civil Engineering, Bharat Institute of Engineering and Technology, Mangalpalli (V), Ibrahimpatnam (M) -501510, R.R.Dist. Andhra Pradesh

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***Address for correspondence**
Abdul Samad. M. Kamdod
Department of Civil Engineering,
Bharat Institute of Engineering and Technology,
Mangalpalli (V), Ibrahimpatnam (M) -501510,
R.R.Dist. Andhra Pradesh
E.Mail: prof.asmk@gmail.com

ABSTRACT

The quantity and generation rate of solid wastes such as garbage and rubbish in the metropolitan cities have increased at an alarming rate over the years with lack of efficient and modern technology for the management of the wastes. The waste from rubbish which can catch the fire easily can be separated first and then burnt in a burning operation. The gases produced from these waste usually contains carbon are sent to the absorption unit operation through pipelines. In the absorption unit operation the carbon particles which separate out by sprinkling out with water and clean gas will be removed out. Next garbage type of waste of many developing nations would theoretically be ideal for reduction through composting, having a much higher composition of organic material than industrialized countries. Hence the carbon particles produced from absorption process are sent for compositing along with garbage as for successful compositing the carbon and nitrogen are added in a proper amount.

Key words: Garbage, rubbish, absorption process and compositing.

INTRODUCTION

It has been known that the anthropogenic carbon dioxide emissions coming out after burning through rubbish and are the major contributors to global climate change. Garbage produce from various sources are now a days supplying for composting plant which act as good fertilizer for the enrichment of soil. Therefore, it is important to develop such a technology that which can treat garbage and rubbish at once in a site. The combustion of fossil fuels produces carbon dioxide (CO2), a green house gas with an increasing potential for by-product end-use in the industrial and energy production sectors. The use of CO2 as a by-product would not only have economic benefits but would simultaneously mitigate global climate change concerns[1].

In absorption (or "scrubbing") flue gas is contacted with a liquid "absorbent" (or "solvent") that has been selected because carbon dioxide dissolves in it more readily than nitrogen – i.e., it is *selective* for CO2. The process takes place in tall columns ("towers") known as scrubbers, in which turbulent flow promotes rapid CO2 transfer from gas to liquid. Differences in density make it easy to separate the emerging gas and liquid [2]. The management of municipal solid waste(MSW) is going through a critical phase, due to the unavailability of suitable facilities to treat and dispose of the larger amount of MSW generated daily in metropolitan cities. Unscientific disposal causes an adverse impact on all components of the environment and human health [3]. The waste generated is consequently released into the nearby environment. Consequently, the management of the MSW needs to be revamped to accommodate the changes in the quantity and quality to ensure the longevity of the environment. Due to several legislative, environmental, economic and social constraints, the identification of most sustainable disposal route for MSW management remains an important issue in almost all industrialized countries[4].

Generally, MSW is disposed of in low lying areas without taking any precautions or operational controls. Therefore, MSW management is one of the major environmental problems of Indian mega cities. One very promising approach to reducing CO2 emissions after burning is removing carbon atoms from it and transferring it to the composting plant. However, if the promise of this approach is to come in to the future, the costs will have to be reduced by treating it in a single place. The separation technique such as absorption technique is used in the industry for recovery/ removal of solute gas component form its mixture with another component gases (called as inert gases with respect to absorption) with the help of suitable liquid solvent in which solute gas absorbed[5,6].

Methods to Dispose Solid Waste

Sanitary Land Filling

Sanitary land filling is one of the cheapest methods for the disposal of solid waste. The dumping of solid waste outside the cities or towns is usually known as sanitary land filling. It is made either on the ground or inside the ground but the thing is that which should not create any damage to the surrounding environment.

Compositing

Decaying organic matter converting in to fertilizer is the compositing, in this case a garbage which is readily degradable waste is to be taken and sent for compositing plant. For successful compositing the carbon and nitrogen are added in a proper ratio, temperature is maintained up to 90^0 F and moisture is maintained up to 40%. After this procedure the waste is being converted in to humus like material which is good for the enrichment of soil that is a fertilizer.

Incineration

When it is very dangerous to dispose the solid waste such as disposing of toxic material and other biological waste the incineration technique is being used in which the toxic waste is heated to a very high temperature to reduce the volume of waste.

Research Method Adopted and Benefits

Managing solid waste includes collecting, transporting and disposing off the solid waste properly to the useful site. In this method (absorption technique) the installation of absorption equipment is the cost effective but this cost is negligible because after separating the garbage a large amount of rubbish left which is usually sending to sanitary land filling. For sanitary land filling the cost required for various purpose at city like Mumbai is mentioned in table.1 below.

From the above table it has been clear that the approximate cost require for sanitary land filling for all processes would be reached to Rs: 2500/Ton/KM and further for composting also there must be requirement of cost. So sending the garbage as well as rubbish to a single site definitely for compositing reduces the cost.

CONCLUSION

In growing metropolitan cities, the waste is generating in a huge quantity. The increasing quantity of waste and limited capacity of the available land area for human being and plants etc. makes solid waste management a critical issue. Managing the garbage and rubbish at once by absorption method clearly reduces the discharge of anthropogenic carbon dioxide to the atmosphere and hence reduces the creation of global warming problem. Incinerators and landfills are very expensive and there are few successful examples in less developed countries. All studies shown that absorption method is found good for the separation of solute particles such as carbon from carbon dioxide that separates oxygen from it which is very essential gas for all living beings. Apart from it this technique also reduces the cost require for sanitary land filling.

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Table: 1. Cost figures for sanitary land filling

Sl.No	Various Processes	Cost required for sanitary land filling in Rs/ Ton/KM
1	Collection	950
2	Transportation	7
3	Operation	328
4	Cost of land	380 also depend on type of city
5	Environment cost	790
6	Revenue from recyclable material	211

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RESEARCH ARTICLE

Land use Changes of Nasuvini Sub Watershed using Remote Sensing and Geographical Information System (GIS) Techniques.

V.Padmanaban*, A.Ganesh, K. Kannadasan.

Department of Geography, Bharathidasan University, Tiruchairappali-620024.TamilNadu,India.

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***Address for correspondence**
Padmanaban.V
Research Scholar,Department of Geography,
Bharathidasan University, Tiruchairappali-620024.TamilNadu, India.
E.Mail :V.Padmanaban@ymail.com

ABSTRACT

Land use change has been a critical issue for planning and management of resources and for modernisation agenda in most developing countries. Significant measure has been implemented to identify the feasible land use activities that could lead to disaster to human and issues to the development of infrastructure. An attempt has been carried out to map the land use and land cover changes of Nasuvini sub watershed, using remote sensing data. The watershed covers an area of 115.39 sq.km, and it is located in the east coast of Tami Nadu. Land use/land cover map were generated and the areas were categorized into built-up land, agricultural land (crop land, fallow/harvested land, agricultural plantation), forest (dense and degraded forests), wastelands, land with scrub, barren rocky areas, sandy areas, saltpan and salt affected areas, waterlogged marsh land, water bodies and other (pasture land) on the basis of NRSA classification. Agricultural land (crop land and agricultural plantation), built-up lands and wastelands were dominant in the watershed. The significance of such a study in the formulation of management plans/developed plans is also discussed.

Keywords: Land use, Land cover, Agenda, Remote Sensing, NRSA.

INTRODUCTION

Knowledge of land use is important for planning and management activities concerned with the surface of the earth. The resource managers and planners for agricultural land use need detailed, timely, accurate and reliable data on the extent, location and quality of land and water resources and climate characteristics. The data on landuse potential and the conservation needs can help in planning for uses that will maintain the quality of land [1] [2].

Development programmes concerning optimum utilisation of natural resources are now increasingly oriented with watershed as an integral unit [3][4]. A watershed is a natural entity conforming to the increasing homogeneity of geomorphic sculpturing process. Watershed management implies rational utilisation of land and water resources for optimal and sustained production with the minimum hazard to natural resources and environment [5][6]. It requires

collection and analysis of great deal of information on physical relationship of vegetation-soil-water to land management to ensure economic and social progress of a watershed [7][8]. The success of planning for developmental activities depends on the quality and quantity of information available on both natural and socio-economic resources. Therefore, accurate and reliable data base generation and management is extremely important for devising ways for optimal planning and management of watersheds [9-15].

Study Area

The Nasuvini sub watershed forms a part of the Cauvery basin and comprises of the catchment of the Nasuvini river. This sub watershed falls in three taluks of the district of Thanjavur namely Thanjavur, Orathanadu and Pattukottai. Nasuvini river, which in its plac of origin flows from northwest to south east to join Bay of Bengal. The sub watershed covers an area of 115.39 sq.km, The Blocks covered by the watershed are Thanjavur, Orathanadu, Thiruvonam, Madukkur, and Pattukottai. The area lies between the latitude 10^0 18′35′′ to 10^0 30′1′′ north and the longitudes 79^0 14′47′′ and 79^0 25′ 33′′ east and contained in the SOI toposheet Nos.58N/6 and 58N/7 of 1:50000. (Fig.1) The sub watershed may be classified as non delta region (west) delta region (east) and coastal region (south east).The general slope of the land is from NW to SE from MSL. Geologically most of the rocks fall under Caddalor sand stone. The Fluvio marine Sediments covers in south eastern part of the sub watershed. The major soil series of the sub watershed is The Madukkur soil series, Pattukottai and Peravurani soil series was the least. The mean annual rainfall of this sub watershed is 685 mm. The sub watershed receiving maximum rainfall during north east monsoon (October to November) and minimum during Winter Monsoon. The intensity and amount of rainfall are unpredictable during south west monsoon period (June to September). The period between January to May is the main dry season.

MATERIALS AND METHODS

The study has made use of various primary and secondary data. These include Survey of India (SOI) topographic maps. (58 J/14, 58 N/2, 58 N/2, 58 N/3, and 58 N/7 on 1:50,000 scale) and IRS LISS-III Geocoded data of 1:50,000 scale for May-1991,2001and April-2009.The Indian Remote Sensing Satellite (IRS) were visually interpreted by using image interpretation elements such as tone, texture, shape, pattern, association etc. Adequate field checks were made before ascertaining/finalizing of the thematic maps.

Land Use/Land Cover Change

Remote sensing technology has made significant contribution in the area of land use change mapping. The land use /land cover changes of the study area are mapped using IRS LISS III data of 1:50,000 scale. The satellite data is visually interpreted and after making a through field check, the map is finalized (Fig.2). The different classes of land use are grouped as builtup land, forest land, agricultural land, waste land, water bodies. Builtup land includes settlements and forest land includes shrub and mangrove forest. Crop land, Fallow/harvested land, plantation, etc. are grouped as agricultural land. Waste land includes barren, Sand/Inland/Coastal, Salt pan & Salt affected land, water logged/marsh land etc. Tanks, rivers, are categorized under water bodies. The areas for the grouped categories are calculated for the respective years and tabulated. The changes in land use area over a period of nineteen years (1991-2001, 2001-2009) are interpreted and the changes in the major categories are analysed. A detailed account of these land use / land cover changes of the study area are described in the following section on the basis of the NRSA standard classification system.

RESULTS AND DISCUSSION

Built up Land

Anderson et al. (1976) define Urban or Built-up Land as "areas of intensive use with much of the land covered by structures. Included in this category are cities, towns, villages, strip developments along highways, transportation, power, communications facilities, and areas such as those occupied by mills, shopping centers, and industrial and commercial complexes".

In the sub watershed, built up land covers nearly 8.44 % (9.75 sqkm) of the total area in 1991. In 2001 which occupied 16.9% and 24.23% in 2009. It showed a gradual increase in the area from 1991 to 2009. This increase is due to population explosion. Adhirampattinam, Veerakurichi Thuvankurichi is the mportant settlements of this watershed. This watershed is comprises of many small villages. Adhirampattinam is a growing town of this sub watershed situated in southern side.

Agricultural Land

Agricultural land is defined by Anderson et al. (1976) as, "arable and regularly tilled for the production of annual field crops, with or without irrigation. It refers to a broad class of resource uses that include all forms of land use for the production of biotic crops, including animal and plants". In a broader sense, agricultural land includes all land that provides direct benefits for mankind through the production of food, fiber, forage and fodder, biofuel, meat, hides, skins, and timber. The major proportion in land use is the agricultural land. Crop land, Fallow/harvested land, plantation, etc. are grouped as agricultural land.

Crop land

Crop land is used for the production of adapted crops, like wheat, paddy and horticultural crops. As such, it is landscape created by humans and is no longer part of the natural ecology. These include all the agricultural areas identified by their characteristic red tone, regular shaped agricultural fields and in association with settlements, water bodies, etc. The crop lands are found well distributed in the new delta region of the sub watershed. The kharif crops (paddy, groundnut, sorghum, red gram, black gram, and green gram horse gram, caster gingerly and sunflower) are cultivated in the months of June, July and August. The rabi crops (paddy, surghm, maize, black gram green gram, groundnut, sunflower, gingerly, sugarcane) are cultivated in the month of October, November and December.The area under crop land category accounts to 52.93 sq km (45.95) during 1991and rose over an area of 63.94 sq km in 2001.This land use cover an area of 62.69 sq km (54.32%) in 2009. Agricultural land is a major source of utilization for other purposes. Nasuvini river is the important source for irrigation in this sub watershed. It is observed that the main occupation of the sub watershed is agriculture.

Plantations

Plantations areas are identified from their dark and red tone, medium texture and are found in the upland and tail end region of the study area. Coconut plantation is the major agricultural plantation seen the watershed. The area under this category accounts to12.08 sq km in 1991 and 5.94 sq km in2009.

Fallow land

These are the lands which remains vacant without crop cultivation. These are identified by their dark greenish tone, smaller size, regular shape and medium texture. These fallow lands are found in the upland and tail end areas of the

study area and in other areas they are scattered. Fallow land is an agricultural land left uncultivated during both Kharif and rabi seasons. Due to infertility and lack of water the land is not brought to cultivation. The fallow land is found over an area of 26.76 sq km, 20.71sq km, and 8.46 sq km in 1991, 2001, and 2009 respectively. This indicates that next only to crop land major part of the land is used for fallow or harvested land.

Forest

The International Panel on Climate Change defines forest as "an ecosystem characterized by more or less dense and extensive tree cover. Typically, the cover is assessed as percent crown cover. Distinctions may be made between open- and closed-canopy forests" (IPCC Technical Paper VI, 2008). Mangrove forest area comprises of 6.29 sq. km, it is considered to be the lowest area in land use found in this sub watershed.

Waste Lands

Waste land may be defined as that land which has been previously used but which been abandoned and for which further use has been found Dudly Stamp (1954). Wasteland survey and reclamation committee Ministry of food and agriculture (1961) has defined waste land as these lands, which are either not available for cultivation or left out without being cultivated, like fallows and culturable waste. Society for promotion of waste land development has defined wasteland, those lands are waste lands which are a) ecologically unstable e) whose top soil has been nearly completely lost and c) which has developed toxicity in the root zones for growth of most plants, both annual crops and trees. National remote sensing Agency (NRSA-1985) defined waste land as that land which is presently lying unused or which is not being used to its optimum potential due to some constraints. Different types of waste land category are identified based on their image characteristics like tone, texture, pattern shape, size, location and association.

This category of land includes all such land, which is practically useless or unproductive to cultivation. These lands are either rugged or sandy wastes or barren rocky; land with scrub or without scrub, waterlogged area, salt affected or gullied or ravenous. The area under this category is given in table. Salt affected land is noticed in southern most part of the watershed. It is considered to be the highest area in land use found in this waste land category.

Sand/Inland/Coast

These types of lands are found in interior as well as coastal area of the sub watershed. This category covers 00.9 sq.km.(0.07 per cent) of the study area in 1991, 0.16 % in 2001 and 1.03% in 2001 respectively. River and tanks is the important source for agricultural activities.

Salt affected Lands

The salt affected lands are generally characterized as the lands that have adverse affect on the growth of most plants. These occur mainly in inland plains as white patches and are can easily identified in the image. The area under this category 5.53sq.km.(4.79 per cent) in 1991,5.13 sq.km 4.44 per cent in 2001 and 5.50 sq.km 4.76 per cent in 2009.

Waterlogged Marsh Land

Marsh lands are observed in the coastal area of the watershed. The area occupies about 0.06 sq.km. (0.05 per cent) in 1991, 2.90 sq.km (2.51 per cent) in 2001 and 0.47 sq.km. (0.4 per cent) in 2009. Waterlogged areas are scattered in this study area.

Water Bodies

Water includes any surface water present including streams, creeks, rivers, ponds, lakes, bays, reservoirs, and estuaries. Water area covers nearly 1.65% of the study area in 1991, 1.60 % in 2001 and 1.03% in 2001 respectively. River and tanks is the important source for agricultural activities.

CONCLUSION

The land use changes of the study area were mapped with help of IRS data. the land use categories were demarcated as built- up land, agricultural land (crop land, fallow land, agricultural plantation, forest (dense and degraded forests) wastelands, land with scrub, barren rocky areas, sandy areas, saltpan and salt affected areas, waterlogged marsh land, water bodies and other (pasture land). The built-up lands in the study area include towns /minor towns and villages; the total area covered under this land use category is about 9.75, 19.53 and 27.96 sq.km.in 1991, 2001 and 2009 respectively. (Table.1).Among the agricultural lands, it was possible to identify the crop lands, fallow lands and plantations. Agricultural areas were found well distributed throughout the study area for the reason that most of the people engaged in agricultural activities. Total area covered by this land use category is about 52.93 sq.km in 1991, 63.94 sq.km in 2001 and 62.69 sq.km in 2009 this changes shows the decline in agricultural land. The forests of the study area are confined to the north western part of the study area. the forests occupy about 6.29 sq.km.

Waste land categories,such as land with scrub, barren rocky areas, sandy areas, saltpan and salt affected areas, waterlogged marsh land areas were demarcated. The lands with scrub or without scrub were found near vallam up land. The salt affected areas found in the inland plains and varies from 5.53 sq.km in1991 to 5.50 in 2009.Salt pan is in the coast of study area. It occupies about an area of 13.15 sq.km.in the study area. The water body category, features such as rivers/streams, tanks and reservoirs were delineated. There are no reservoirs in the study area however, numerous major and minor tanks were identified some of them are dry. The tanks spread entire study area and cover about. The water bodies cover about 1.91sq.km.in1991 and 1.03 sq.km in 2009 reduced due to conversion . The mapping of the land use /land cover is useful for present status of land use analysis, planning and decision making process.

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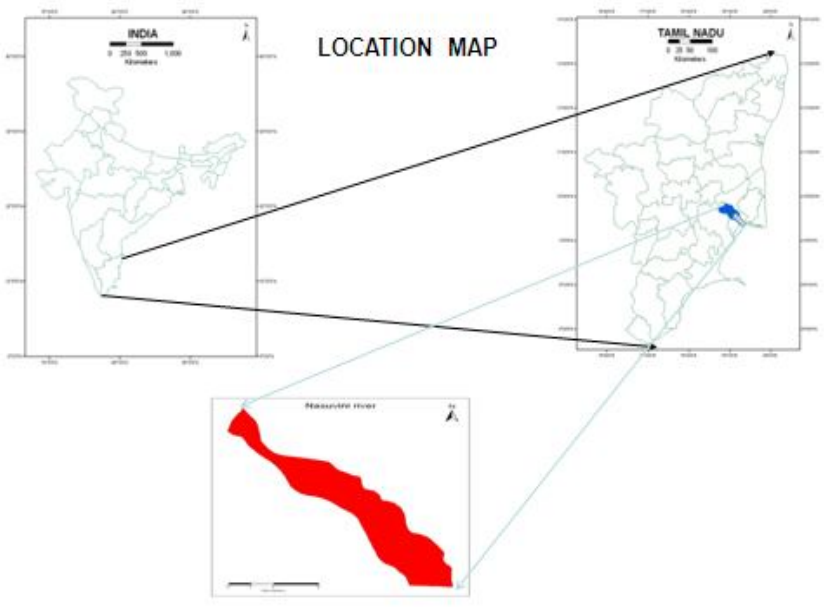

Fig 1: Study area map

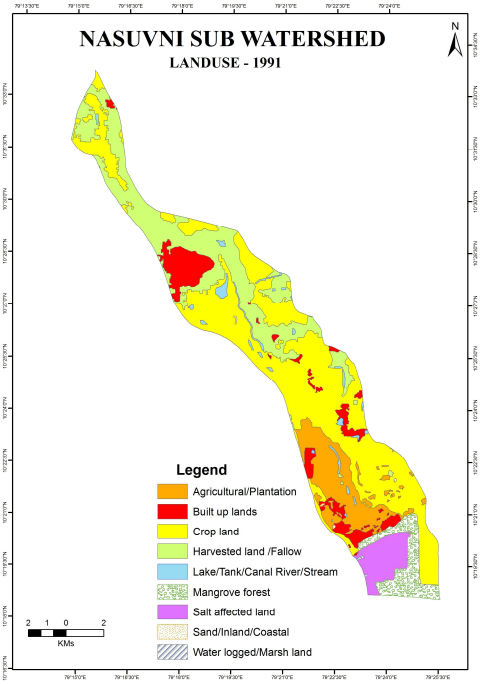

Fig 2:Nasuvni Sub Watershed Land use in 1991

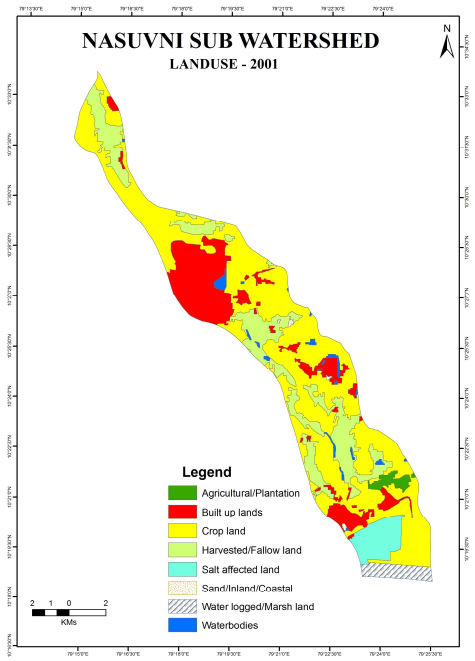

Fig 3 :Nasuvni Sub Watershed Land use in 2001

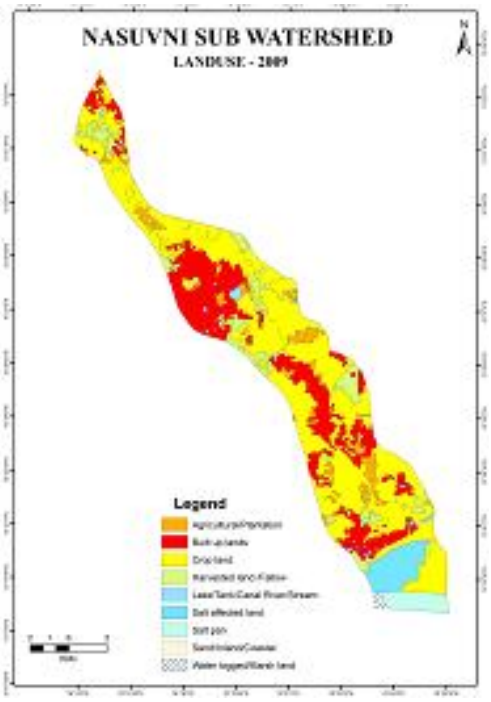

Fig 3 :Nasuvni Sub Watershed Land use in 2009

Table 1: Land use classification of Nasuvini sub watershed

SL.No	landuse	1991 Area in sq.Km	%	2001 Area in sq.Km	%	2009 Area in sq.Km	%
1	Built up lands	9.75	8.44	19.53	16.9	27.96	24.23
2	Crop land	52.93	45.9	63.94	55.4	62.69	54.32
3	Fallow/harvested land	26.76	23.2	20.71	17.9	8.46	7.33
4	Agricultural Plantation	12.08	10.5	1.39	1.2	5.94	5.14
5	Mangrove forest	6.29	5.45	-	-	-	-
6	Sand/Inland/Coastal	0.09	0.07	0.19	0.16	0.03	0.02
7	Salt pan	-	-	-	-	3.15	2.72
8	Salt affected land	5.53	4.79	5.13	4.44	5.50	4.76
9	water logged/marsh land	0.06	0.05	2.90	2.51	0.47	0.4
10	River/strem/lake/tank/canal	1.91	1.65	1.60	1.38	1.19	1.03
	Total	115.39	100	115.39	100	115.39	100

Table 2: Wasteland

Waste land	1991(sq km)	%	2001(sq km)	%	2009 (sq km)	%
Sand/Inland/Coastal	0.09	0.07	0.19	0.16	0.03	0.02
Salt pan	-	-	-	-	3.15	2.72
Salt affected land	5.53	4.79	5.13	4.44	5.50	4.76
water logged/marsh land	0.06	0.05	2.90	2.51	0.47	0.4

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RESEARCH ARTICLE

Effect of Yeast Strain on the Production of Bioethanol from Cheese whey with Sweet Sorghum.

M. Sankar[1], M. Seethalakshmi [1]*, A. Ramanathan[1] and S.Deivendran[2]

[1]Faculty of Agriculture & Animal Husbandry, Gandhigram Rural University, Gandhigram- 624 302, Tamil Nadu, India.

[2]PG and Research Department of Zoology, Thiagarajar College, Madurai-625009, Tamilnadu, India.

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***Address for correspondence**
M. Seethalakshmi
Faculty of Agriculture & Animal Husbandry,
Gandhigram Rural University,
Gandhigram. 624 302, Tamil Nadu, India.
E.Mail:seethagri@gmail.com, sankargri@gmail.com
Tel. 0451-24523721, Fax. 0451-2454466

ABSTRACT

Kluyveromyces marxianus MTCC 242, *Saccharomyces cerevisiae* and *Achaetomiella fusispora* was used for ethanol production from cheese whey with sweet sorghum in batch experiments. Effects of sweet sorghum substrate and different yeast on the rate and extent of ethanol production were investigated. The maximum fermentation time 72 hours, temperature30^{0}C and pH 5 were taken as the experimental conditions. The result indicates that the rate and extent of ethanol production increased by using Saccharomyces cerevisiae in cheese whey with sweet sorghum at the rate of 200gl^{-1} but decreased with other two yeasts due to the yeast inhibition.

Keywords: Bio ethanol, Cheese whey, Sweet sorghum, *Kluyveromyces marxianus. Saccharomyces cerevisiae, Achaetomiella fusispora*

INTRODUCTION

Ethanol production from different raw materials containing carbohydrates has been much interest because of their wide range of applications [1]. Utilization of waste materials for ethanol formation offer special advantages by

providing cheap raw materials and simultaneous waste treatment with ethanol production. The common raw materials for ethanol fermentations are cellulosic materials (straw, baggase and waste paper), starch containing materials (corn, wheat and rice), sugar cane, sugar beet and molasses. Among the raw materials, sweet sorghum and cheese whey are inexpensive and highly available, which are the waste by-products of dairy industries and agricultural fields. In addition, cheese whey is an important source of environmental pollution because of the high organic matter content with biological oxygen demand ranging from 40-50 gl^{-1} and a chemical oxygen demand of 60-80 gl^{-1} [2]. However little attention has been given to production of ethanol from paneer whey, which is an indigenous milk product prepared by combined action of acid coagulation and heat treatment of cow or buffalo milk. In India, the estimated production of whey from cheese is about 4.84 million tons per annum [3]. Hence, the production of ethanol from whey has gained important because not only fuel demand but also reduce the environmental pollution.

Sweet sorghum (Sorghum bicolor*)* is similar to grain sorghum with a sugar-rich stalk, almost similar to sugarcane. Besides having wide adaptability, rapid growth and high sugar accumulation and biomass production potential, sweet sorghum, is tolerant to drought, water logging, soil salinity and acidity toxicity. It has great potential for jaggery, syrup and alcohol (most importantly Gasohol, which is ethanol blended with petrol) production. [4]. *Kluyveromyces* species has been the most widely used yeast strain for ethanol production from cheese whey. Recently, Zafar and Owasis (2006) have been studied production of ethanol from crude cheese whey [5]. Kargi and Ozmichi (2006) reported 1.28% ethanol productions from cheese whey powder [6]. To the best of author's knowledge there is no literature study on ethanol production from cheese whey with sweet sorghum. In the present study, an attempt was made for ethanol production from cheese whey with sweet sorghum in the presence of *Kluyveromyces marxianus. Saccharomyces cerevisiae* and *Achaetomiella fusispora*.

MATERIALS AND METHODS

Preparation of Samples

Cheese whey produced in our laboratory contained 4.5% of lactose Sweet sorghum stalks were collected from the Tamilnadu Agricultural University, Coimbatore, and Tamilnadu, India. The outer skin present in the sweet sorghum stalk removed. Then the stalk was chopped in to very small pieces and crushed nicely using mixer and this preparation was used for further experiment.

Organism

Kluyveromyces marxianus strain MTCC 242, *Saccharomyces cerevisae* MTCC 178 and *Achatomiella fusispora* MTCC 1288 were procured from the Culture Collection Centre of the Institute of Microbial Technology (MTCC) at Chandigarh, India. *Kluyveromyces marxianus* strain MTCC 242 and *Saccharomyces cerevisae* MTCC 178 were maintained in Yeast Peptone Dextrose (YPD). While, *Achatomiella fusispora* MTCC 1288 was maintained in Cornmeal medium.

Substrate composition

Cheese whey containing 4.5% lactose, 0.05 % fat and 0.52 % protein and trace amount of ash and sweet sorghum containing 15% fermentable sugar were used in the present study.

Experimental methods

100 ml of cheese whey enriched with sweet sorghum at the rate of 5, 10, 15 and 20 g were adjusted to pH 5 by using sodium thio-glycolate (98%). This medium was sterilized by autoclaving at 15 psi for 20 min. The autoclaved media were inoculated with 10 ml of *Kluyveromyces marxianus, Saccharomyces cerevisiae* and *Achaetomiella fusispora* in different experimental flasks incubated at 30^0C for 72 h. At the end of 72 hrs, 10 ml of sample from each experimental flask was collected and centrifuged for 15 min at 5,000 rpm and the supernatant liquid was used for estimating ethanol and reducing sugar, all the experiment was repeated ten times. In this study the experimental conditions like the pH 5, temperature 30^0C and fermentation time 72 hours as constant for the whole experiment.

Analytical methods

Yeast cells harvested by centrifugation was weighed after drying at 105 ^{0} C for 24 hrs. Lactose in cheese whey was estimated by the method of Lane-Eynon [7], the total reducing sugar by phenol-acid method [8], and ethanol by the dichromate colorimetric method [9]. Ethanol yield coefficient was calculated in terms of g of ethanol produced per g of substrate consumed. The fermentation efficiency was calculated as below

$$F.E = \frac{\text{Ethanol Produced}}{\text{Theoretical maximum ethanol yield from sugar}} \times 100$$

(Theoretical maximum ethanol yield = 0.54 g ethanol per gram of lactose).
(Theoretical maximum ethanol yield = 0.51 g ethanol per gram of sucrose)

Statistical analysis

All the experimental data were statistically analyzed by using the software AGRES 3.01.

RESULTS AND DISCUSSION

Experiment results are given in Table 1 showed that there was no significant (p=0.05) differences between the treatments for ethanol production, reducing sugar, biomass, ethanol yield co-efficient, fermentation efficiency and conversion efficiency. However the ethanol production of 2.50 % was observed at 72 hrs in the substrate concentration of cheese whey supplemented with 20g sweet sorghum which was higher when compared to other treatments, but ethanol yield coefficient, reducing sugar, conversion efficiency was higher in cheese whey supplemented with 10g sweet sorghum when compared with other three treatments. However conversion efficiency 81.3 % was observed in cheese whey supplemented with 20g sweet sorghum when compared other treatments. Upon the concentration of sweet sorghum increasing ethanol production and biomass production was increased in insignificantly.

Table 2 proved that there was no significant (p=0.05) differences between the treatments for biomass, ethanol, ethanol yield co-efficient, fermentation efficiency and conversion efficiency. However, cheese whey supplemented with 20 g sweet sorghum recorded significantly (p=0.05) higher values of reducing sugar 0.3% compared to other treatments. These results indicated that ethanol production 3.50 % was higher for 72 hrs in cheese whey supplemented with 20g sweet sorghum when compared to other treatments, but there was no significantly difference between ethanol productions from cheese whey supplemented with 5g sweet sorghum to cheese whey supplemented with 20g sweet sorghum. Although ethanol yields coefficient 0.528 and fermentation efficiency was higher in cheese whey supplemented with 5g sweet sorghum when compared other three treatments. However reducing sugar 0.30% was significantly higher and conversion efficiency 96.0 % in cheese whey supplemented with 20g sweet sorghum when

compared other treatments. Upon the concentration of sweet sorghum increasing ethanol production and biomass production was increased in insignificantly.

The diagnostic results are given in Table 3 demonstrate that there was no significant (p=0.05) differences between the treatments for biomass, ethanol, reducing sugar, ethanol yield co-efficient, fermentation efficiency and conversion efficiency. These results indicated that ethanol production 2.10 % was higher for 72 hrs in cheese whey supplemented with 20g sweet sorghum when compared to other treatments, but there was no significantly difference between ethanol productions from cheese whey supplemented with 5g sweet sorghum to cheese whey supplemented with 20g sweet sorghum. Although ethanol yields coefficient 0.350, fermentation efficiency 64.81%, biomass production13.0g/l was higher in cheese whey supplemented with 20g sweet sorghum when compared other three treatments. Upon the concentration of sweet sorghum increasing ethanol production and biomass production was increased in insignificantly.

In our experimental conditions like the pH 5, temperature 30^0C and fermentation time 72 hours as constant for the whole experiment. Hereunder the results are given in fig.1 showed that there was no significant (p=0.05) differences between the treatment of ethanol production in all three yeast. The ethanol production was higher in 100ml of cheese whey enriched with sweet sorghum at the rate of 20g by using *Saccharomyces cerevisiae* sample when compared with other two sample 100ml of cheese whey enriched with sweet sorghum at the rate of 20g by using *Kluyveromyces marxianus* and 100ml of cheese whey enriched with sweet sorghum at the rate of 20g by using *Achaetomiella fusispora*. The decline in ethanol production in 100 ml of cheese whey enriched with sweet sorghum at the rate of 20g by using *Kluyveromyces marxianus* and 100 ml of cheese whey enriched with sweet sorghum at the rate of 20g by using *Achaetomiella fusispora* could be due to inhibitory action of yeast, because *Kluyveromyces marxianus* yeast is best suitable for conversion of lactose to ethanol and *Achaetomiella fusispora* was used in the soil fungus just we are trying the new one species but this is also ethanol produced . Similar findings of increase in ethanol concentration was observed by Ozmichi and Kargi [10] in cheese whey powder solution (2.8 %) fermented by the *K. marxianus* strain (DSMZ-7239). The fermentation efficiency was maximum at 84% in S3 substrate which is higher than that observed by Naresh Sharma *et al.* (2007) 74.11% in kinnow waste and banana peel fermented by *Saccharomyces cerevisiae* [11]. Jianliang Yu 2008 studied that Solid state fermentation of chopped sweet sorghum particles to produce ethanol was studied statically using thermotolerant yeast. On the ethanol yield was investigated maximum ethanol yield of 7.9 g ethanol per 100 g fresh stalks, which was 91% of the theoretic yield [12].

CONCLUSION

From this study it could be concluded that fermentation of cheese whey at the level of 100 ml supplemented with 20 g sweet sorghum by using *Saccharomyces cerevisiae* gives significant results in ethanol production, sugar consumption, biomass concentration, fermentation efficiency and ethanol yield coefficient.

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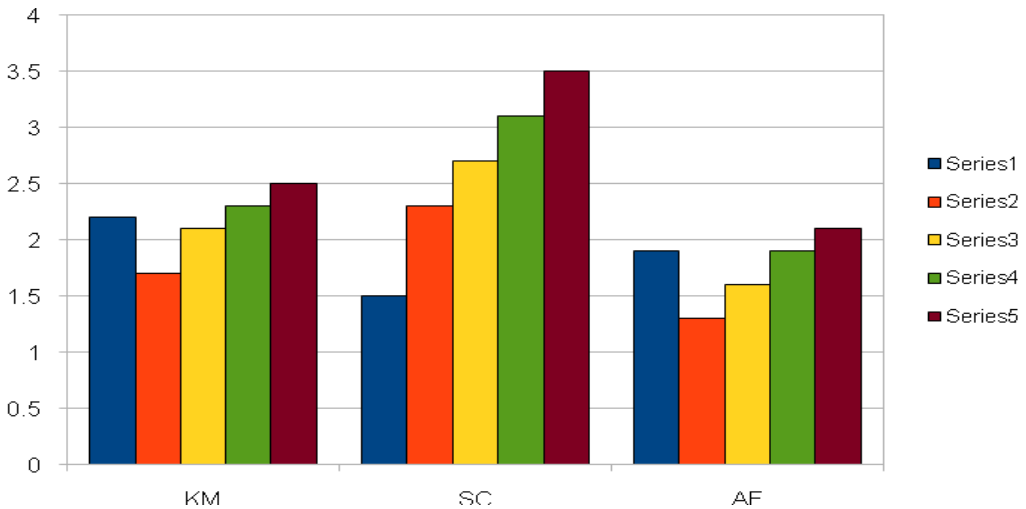

KM-*Kluyveromyces marxianus;* SC-*Saccharomyces cerevisiae;* AF-*Achaetomiella fusispora*

Fig. 1 : Effect of ethanol production from Sweet sorghum in different yeast Species

Table 1. Effect of different levels of sweet sorghum on ethanol production and other parameters by *Kluyveromyces marxianus.*

Treatments	Ethanol (%)	RS (%)	BM (dw-g/l)	Ethanol yield co-efficient	Fermentation efficiency (%)	Conversion efficiency (%)
100 ml of cheese whey (Control)	2.20	0.3 a	11.0	0.523 a	96.8a	93.3
100 ml of cheese whey + Sweet sorghum (5g)	1.70	1.05 b	11.0	0.404b	74.8 b	80.0
100 ml of cheese whey + Sweet sorghum (10g)	2.10	1.10b	12.0	0.446b	82.5b	78.3
100 ml of cheese whey + Sweet sorghum (15g)	2.30	1.30 b	12.5	0.446b	82.5 b	76.2
100 ml of cheese whey + Sweet sorghum (20g)	2.50	1.40 b	13.0	0.409b	75.7b	81.3
S.Ed	**0.23**	**0.13**	**1.29**	**0.05**	**8.4**	**8.3**
CD (p=0.05)	**0.49**	**0.27**	**2.74**	**0.10**	**17.8**	**17.6**

BM-Biomass; RS- Residual reducing sugar; dw- Dry weight; Means bearing different super scripts within a column differ significantly.

Table 2. Effect of Different Levels of Sweet sorghum on Ethanol Production and other Parameters by *Saccharomyces cerevisiae.*

Treatments	Ethanol (%)	RS (%)	BM (dw-g/l)	Ethanol yield co-efficient	Fermentation efficiency (%)	Conversion efficiency (%)
100 ml of cheese whey (Control)	1.50a	0.90 a	10.0	0.416 a	77.0	80.0
100 ml of cheese whey + Sweet sorghum (5g)	2.30 b	0.30 b	12.5	0.528b	97.7	82.8
100 ml of cheese whey + Sweet sorghum (10g)	2.70 b	0.65 b	14.0	0.519 b	96.1	86.6
100 ml of cheese whey + Sweet sorghum (15g)	3.10 b	0.80 b	14.5	0.508 b	94.0	90.3
100 ml of cheese whey + Sweet sorghum (20g)	3.50 c	0.90 c	15.0	0.486 b	90.0	96.0
S.Ed	**0.31**	**0.07**	**1.49**	**0.05**	**10.0**	**9.45**
CD(p=0.05)	**0.67**	**0.15**	**3.17**	**0.11**	**21.1**	**20.0**

BM-Biomass; RS- Residual reducing sugar; dw- Dry weight; Means bearing different super scripts within a column differ significantly.

Table. 3 Effect of Different Levels of Sweet sorghum on Ethanol Production and Other Parameters by *Achaetomiella fusispora.*

Treatments	Ethanol (%)	RS (%)	BM (dw-g/l)	Ethanol yield co-efficient	Fermentation efficiency (%)	Conversion efficiency (%)
100 ml of cheese whey (Control)	1.90a	0.40 a	10.0	0.463 a	85.7 a	91.1
100 ml of cheese whey + Sweet sorghum (5g)	1.30 b	1.10 b	11.0	0.329 b	60.9 b	75.2
100 ml of cheese whey + Sweet sorghum (10g)	1.60 b	1.30 b	11.5	0.326b	60.3 b	81.6
100 ml of cheese whey + Sweet sorghum (15g)	1.90 b	1.30 b	12.0	0.348 c	64.4 b	80.7
100 ml of cheese whey + Sweet sorghum (20g)	2.10	1.50 b	13.0	0.350c	64.8 b	80.0
S.Ed	**0.19**	**0.14**	**1.26**	**0.04**	**6.7**	**8.4**
CD(p=0.05)	**0.40**	**0.29**	**2.68**	**0.08**	**14.0**	**17.8**

BM-Biomass; RS- Residual reducing sugar; dw- Dry weight; Means bearing different super scripts within a column differ significantly.

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RESEARCH ARTICLE

Studies on Biology, Symptoms of Attack, Infestation and Management of Coconut Perianth Mite (*Aceria guerreronis*) in CPCRI, Kasaragod, Kerala.

C. Prathibha, M.Lekeshmanaswamy* and D.Yasotha

PG and Research Department of Zoology, Kongunadu Arts and Science College, G.N. Mills(po) , Coimbatore- 641 029, TamilNadu, India.

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***Address for correspondence**
Dr. M. Lekeshmanaswamy, Associate professor,
PG and Research Department of Zoology,
Kongunadu Arts and Science College,
G.N. mills (PO), Coimbatore-641 029, TamilNadu, India.
E.Mail ID: ml_swamy64@yahoo.co.in. Mobile-07708570555

ABSTRACT

Coconut Eriophyid mite was spotted in India in 1998. The habitat of the mite is floral bracts and the tender portion of 1-5 months old developing nuts. The life cycle of mite lasts for about 7-10 days. The symptoms of attack on nut surface are due to feeding of mite. Mite infestation study was conducted in a randomized block design during the month of October 2010 to July 2011. The infestation of the nut seems to be higher in summer months than in the winter. The dispersal of the mite is mainly through wind.

Keywords: Coconut Eriophyid mite, floral bracts, infestation.

INTRODUCTION

The coconut palm and fruit are regarded as the most important plant. Coconut is grown in more than 93 countries in a total area of 11.84 million ha producing 56390 million nuts annually. India is the third largest producer of coconut in the world. Coconut crop is the heart and soul of the agricultural economy. Besides pathogen, there are several invertebrates and vertebrates attack various stages of the plant [2; 1]. Rhinocerous beetle, red palm weevil, leaf eating caterpillar and white grub are the major pest occurring in various coconut growing tracts of India.

Coconut palms are hosts to at least 12 species of Eriophyid mite in nine genera worldwide. Coconut mite infests the developing young buttons and is seen in the floral bracts and the meristematic portion beneath the perianth. First report of *Aceria guerreronis* was from the Guerrereo states in Mexico. In India its incidence was first reported in 1998 in Amballoor panchayath, Ernakulam district of Kerala [3]. The present study was conducted in Central Plantation Crop Research Institute Kasaragod, kerala.

MATERIALS AND METHODS

Biology

The habitat of the mite is floral bracts and the tender portion of developing nuts (1-5 months old). The 1 – 5 months old nuts were collected and brought to the laboratory to observe the egg , nymph and adult stage. Perianth was removed from the collected nuts. From the nut's inner surface of the inner perianth and nut surface areas below the perianth sliced for taking observation. Observations on the mite were recorded using stereobinocular microscope.

Symptoms of attack

Mite infection is observed by our naked eyes. Mite infection symptoms start in 1-5 months old bunches . Different stages of infected nuts collected and observed the symptoms.

Mite infestation

Mite infection was diagnosed according to the symptoms. We surveyed

Mite infestation in the selected plot in the CPCRI, Kasaragod

Eriophyid mite infestation was laid out in a randomized block design. For this study first we divided the selected plot in to four blocks (ie) A,B,C and D. Ten palms are selected randomly from each block. Counted total number of nuts in these ten palms with the help of workers. Among these total nuts we selected how many nuts are infected and healthy. Among these infestation how many nuts are infected below 25% , between 25 – 50% and above 50% are observed. The percentage of infestation was calculated by using following formula.

$$\text{Percentage of infestation} = \frac{\text{Total number of infested nuts}}{\text{Total number of nuts}} \times 100$$

Management

In the present study we used the following methods for the management of Eriophyid mite.

Cultural method

Destruction of fallen nuts, proper irrigation, appropriate fertilizer application.

Chemical method

In the present study bio pesticide neemoil garlic soap mixture is used for spraying.

Preparation of Spray solution

To prepare one litre of the 2% neem oil garlic soap emulsion, 20ml pure neem oil, 20gm of cleaned garlic pearls and 5 gm washing soap are required. Dissolve the soap in 500ml of water and add the neem oil to this solution and mix it well. Grind garlic pearls well, mix it well in 500ml water and add this to the soap. Neem oil mixture by sieving through a cloth to remove the debris of garlic pearls. The mixture was stirred well and can be used for spraying.

Method of Spraying

The spray solution should be applied as fine droplets on the perianth region with a hand sprayer or rocker sprayer.

RESULTS AND DISCUSSION

Biology

Mites attain sexual maturity winthin a week time and start laying eggs. The eggs are ovoid, translucent and glossy. The first instar nymph is protonymph and second instar is deutonymph and finally to adult. Total life cycle is completed in 7-10 days.

Symptoms of attack

Appearance of elongated white streaks below the perianth is the first external symptom. The later appears as a pale yellow triangular patch turning gradually to brown colour. As the affected nut grows, this injuries form warting and longitudinal fissures on the nut surface.

Mite infestation

Mite infestation was observed in each block during two months intervals are shown in the tables 1-5.In October and November percentage of mite infestation of A block-79%, B block-79%, C-block-74%, D block-71%In December and January percentage of mite infestation of A block-79%, B block-78%, C-block-76%, D block-70%.In February and March percentage of mite infestation of A block-83%, B block-85%, C-block-80%, D block-81%. In April and May percentage of mite infestation of A block-82%, B block-85%, C-block-81%, D block-80%.In June and July percentage of mite infestation of A block-60%, B block-60%, C-block-58%, D block-57%.The result obtained that, mite infestation is more in summer months because dispersal of mite is quick and is mainly through wind.

Managemant

Cultural methods are simple, it can reduce the mite infestation but its effect was lower than the chemical method. In chemical method spraying is more effective because of direct application of chemicals on the perianth region.

Summary

The salient features of the investigation carried out on biology, symptoms of attack, mite infestation and management of coconut perianth mite *Aceria guerreronis* Keifer under laboratory and field conditions are summarized. Studies on biology of Eriophyid mite on coconut palms indicated that the biology consists of egg, nymph and adult. Life cycle is completed in 7-10 days. The symptoms of attack on nut surface were due to feeding of mite. Mite infestation was observed between two months interval, it was shown that the activity of mite was less during rainy months and high during summer months. In management of Eriophyid mite *Aceria guerreronis* , chemical methods are more effective. But they are not continuously used because they have residual effect. Chemical pesticides are effective than bio-pesticides.

CONCLUSION

Colony of Eriophyid mite contains hundreds of egg, larvae nymphs and adults. The pest is fast spreading due to high reproductive potential; the strategy is that it cannot be eradicated completely, but population can be managed below the economic threshold level. Botanicals are preferred thereafter coupled with eco-friendly cultural and nutrient

management practices to keep the palms healthy and minimise the economic loss. No cultivar is resistant to pest, but certain features like round shape of nut, tight petals etc are offer some tolerance.

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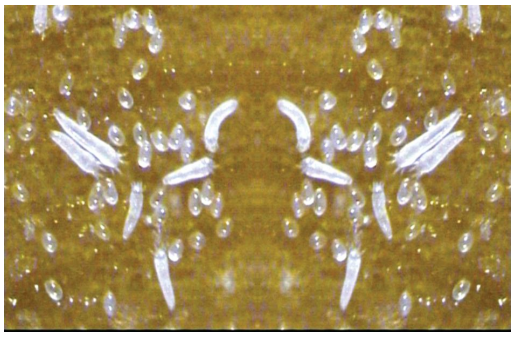

Fig 1: Eggs and different stages of coconut perianth mite

Fig 2: Nut showing first symptom of Eriophyid mite infestation

Fig 3: Different stages of the infestation by the mite

Fig 4: Mature nuts showing warts and fissures

Fig 5: Nut showing reduction in the size of the nut and kernel content

Fig 6: Feeding Injury to the tender tissues covered by the perianth

Table 1: The blockwise Eriophyid mite (*Aceria guerreronis*) infestation duringOctober- November 2010.

Blocks	Total Nuts	Infested	Healthy	% of infected nuts
A	475	375	100	79
B	489	384	105	79
C	493	363	130	74
D	490	350	140	71
Grand total	1947	1472	475	303

Table 2 : The blockwise Eriophyid mite (*Aceria guerreronis*) infestation during December 2010-January 2011.

Blocks	Total Nuts	Infested	Healthy	% of infected nuts
A	478	376	102	79
B	487	382	105	78
C	497	377	120	76
D	499	349	150	70
Grand total	1961	1484	477	303

Table 3 : The blockwise Eriophyid mite (*Aceria guerreronis*) infestation during February- March 2011.

Blocks	Total Nuts	Infested	Healthy	% of infected nuts
A	460	380	80	83
B	472	400	72	85
C	482	388	94	80
D	480	390	90	81
Grand total	1894	1558	336	329

Table 4 : Shows the blockwise Eriophyid mite (*Aceria guerreronis*) infestation during April- May 2011.

Blocks	Total Nuts	Infested	Healthy	% of infected nuts
A	452	371	81	82
B	469	397	72	85
C	480	390	90	81
D	482	388	94	80
Grand total	1883	1546	337	328

Table 5: Shows the blockwise Eriophyid mite (*Aceria guerreronis*) infestation during June-July 2011.

Blocks	Total Nuts	Infested	Healthy	% of infected nuts
A	455	275	180	60
B	470	280	190	60
C	476	276	200	58
D	477	272	205	57
Grand total	1878	1103	775	845

Table 6: Mite infestations in Kerala – Districtwise

No.	Name of District	Number of bearing palms(in lakhs)	Number of palms infected (in lakhs)	Extent of infestation % bearing palms affected
1	Trivandrum	142.77	38.12	26.7
2	Kollam	115.55	36.39	31.5
3	Pathanamthitta	49.81	21.76	44
4	Alappuzha	87.73	54.57	62.2
5	Kottayam	83.78	29.74	35
6	Ernakulam	109.64	89.58	81.7
7	Thrissur	138.3	117.83	85.2
8	Palakkad	63.7	31.53	49.5
9	Malappuram	161.68	73.88	45.7
10	Kozhikode	172.53	75.34	44
11	Kannur	141.59	52.74	37
12	Kasaragode	134.72	70.36	52.2

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RESEARCH ARTICLE

Geoinformatics Based Decision Support System Tools for Water Resources Management in North Karnataka, India.

Basavaraj Hutti* and R. Nijagunappa

Environmental Science Department, Gulbarga University, Gulbarga – 585 106. Karnataka,India

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***Address for correspondence**
Basavaraj Hutti
Environmental Science Department,
Gulbarga University, Gulbarga – 585 106. Karnataka,India.
E.Mail: urbasu@gmail.com; +919945448322; www.urbasu.com

ABSTRACT

Karnataka lack of water resources, especially in its arid and semi-arid regions. So the management of water resources in these areas is very important. The annual average rainfall of 50 cm for the whole country and its totality area, it has been discovered that total water resources in India are of the order of 167 million hectare-meters. It has further been calculated that only 66 million hectare-meters of water resources in India can be employed for irrigation. The population of India as on 2011 stood at 1,210,193,422 (1.21 billion) persons. Thus, India supports about 1/6th of world population, 1/50th of world's land and 1/25th of world's water resources. India also has a livestock population of 500 million, which is about 20 percent of the world's total livestock population. More than half of these are cattle, forming the backbone of Indian agriculture. The total utilizable water resources of the country are assessed as 1086 km3. Geoinformatics technology has its special advantage in this aspect. The paper introduces the applications of Geoinformatics, including remote sensing, geographical information system and global positioning system, in this field, such as surface water resources, groundwater exploration, dynamic monitoring of floods, water environment and drought monitoring, planning of water diversion project between basins and so on. It shows that Geoinformatics technology can play important role for North Karnataka development, especially in India. India is still an agricultural country; with the advent of powerful and high-speed personal computers, efficient techniques for water resource management have evolved, of which Geoinformatics technology includes RS (Remote Sensing), GIS (Geographic Information System) and GPS (Global Positioning System) are of great significance

Keywords: Geoinformatics; water resources; semi-arid; remote sensing.

INTRODUCTION

Karnataka accounts for about six percent of the country's surface water resources of 17 lakh million cubic meters. With rapid growing population and improving living standards the pressure on the water resources is increasing and per capita availability of water resources is reducing day by day, it has become an important and dependable source of water supplies in all climatic regions including both urban and rural areas of developed and developing countries [29]. Of the 37Mkm3 of freshwater estimated to be present on the earth, about 22% exists as groundwater, which constitutes about 97% of all liquid freshwater potentially available for human use [3]. In India, more than 90% of the rural and nearly 30% of the urban population depend on groundwater for meeting their drinking and domestic requirements [15]. The quality of surface water and groundwater resources is also deteriorating because of increasing pollutant loads from point and non-point sources. However, such an approach for water resource investigations is very costly, time-consuming and requires skilled manpower [17]. In contrast, Geoinformatics technology, with its advantages of spatial, spectral and temporal availability of data covering large and inaccessible areas within a short time, has emerged as a very useful tool for the assessment, monitoring and management of water resources [8]. The hydrogeologic interpretation of satellite data has been shown to be a valuable survey tool in areas of the world where little geologic and cartographic information exists or is not accurate, as well as in inaccessible regions of the world [2]. as remote sensors cannot detect groundwater directly, the presence of groundwater is inferred from different surface features derived from satellite imagery such as geology, landforms, soils, land use/ land cover, surface water bodies, etc., which act as indicators of groundwater existence [28]; [9]. Moreover, Geoinformatics have emerged as powerful tools for handling spatial data and decision-making in several areas including engineering and environmental fields [26]; [4]. At present, India is still an agricultural country; the water consumed in agriculture is the most significant one.

In the past, several researchers (from India and abroad) have used Geoinformatics techniques for the clarification of water resource management with successful results [12]; [20]; [11]; [7]; [16]; [21]; [18]; [14]; [22]. On the other hand, some researchers have integrated Geoinformatics techniques to delineate water resources [23]; [19]; [5]; [16]; [24]; [6]; [25]. All the studies have been carried out in India; the majority of which focus on hard-rock terrains. The details about the applications of Geoinformatics technology in water resources, including groundwater prospecting, can be found in [9]; [8].

Indian government always pays more attention to water resources utilization and development; this is why it can support 17.3% population of whole world. Although India occupies only 3.29 million km2 geographical area, which forms 2.4% of the world's land area. The population of India as on 2011 stood at 1,210,193,422 (1.21 billion) persons. Thus, India supports about 1/6th of world population, 1/50th of world's land and 1/25th of world's water resources [30]. India also has a livestock population of 500 million, which is about 20% of the world's total livestock population. More than half of these are cattle, forming the backbone of Indian agriculture. The total utilizable water resources of the country are assessed as 1086 km3. But on the other side, the social-economic development of India would reach the middle level of developed countries in the world. Grain yield will increase 0.14 billion ton, while two third of the increase will be produced in the North India where is lack of water. At that time, the level of urbanization will be raised to 50% water consumed in cities will be increased greatly. So the situation of water shortage will be very serious. The shortage of water resources results from not only water resources itself but also water pollution. Besides, due to the non-uniform distribution of water resources both in space and time, the natural disasters related with water, such as drought, flood and water logging occurs frequently. With the development of society and economy, the loss resulting from these disasters becomes larger and larger. So the continuous and steady development of society and economy is closely related to the susceptible utilization of water resources. It is necessary to set up reliable and safest water supply system, to hold back the worsen tendency of water environment and environment and establish effective system for water resources utilization, development and protection [10].

Water resources in arid and semi-arid regions

The arid and semi-arid regions in Karnataka are mainly located in North Karnataka (Figure 1.), especially in Northeast Karnataka. North Karnataka includes the Krishna River Basin; with the total area of 258,948 kmÂ², which is nearly 8% of total geographical area of the country. The basin covers the states of Andhra Pradesh (113,271 kmÂ²), Maharashtra (69,425 kmÂ²) and Karnataka (76,252 kmÂ²). An average annual surface water potential of 78.1 kmÂ³ has been detected in this basin. Out of this, 58.0 kmÂ³ is utilizable water. Cultivable area in the basin is about 203,000 kmÂ², which is 10.4% of the total cultivable area of the nation. From natural conditions, it is true that arid regions are lack of water resources, but it is also related with human-being activities. The effectiveness of water utilization for agriculture is quite low, the phenomenon of wasting water exists, and the water consumption for unit industrial products is on the higher side, while the repeated-use coefficient on the lower side and the water pollution aggravates the shortage of water resources [10]. Because the increase of population and water consumption for economy development, the water for ecological environment is occupied, resulting in a series of ecological problems related to water, such as river withering, lake tail dead, lowering of groundwater table, secondary salinisation, quick expansion of desertification, increase of desert storm and so on.

The problem with water resources in the more inhabited semi-arid regions is a crucial question for overcoming obstacles to development. It is a fact that the governments of many semi-arid regions of the world are acting with the objective of implanting infra-structures capable of making available sufficient water to assure the supply for humans and animals and make irrigation viable. However, in a global sense this effort still is insufficient to resolve the problems originating from water scarcity, making regions continue to be vulnerable to dry periods, especially when speaking of the diffuse use of water in the rural environment. In any sense, the increase of and strengthening of the water infrastructure, with adequate management, constitute essential prerequisites for solution of the problem, serving as a basic element for the national development.

Applications

Water resource management

Water resources are the basis of suspendable development of society and economy in arid and semi-arid regions. It is recognized from the present situation that the key issue is the management. If powerful engineering and non-engineering measures are adopted, the problem in arid and semi-arid regions in North Karnataka (Figure 1) is really possible to be well solved. In fact, Remote Sensing, Geographic Information System (GIS) and Global Positioning System (GPS) can play important role to water resources management, such as surface water, groundwater, investigation, dynamic monitoring of ecology and estimation of water amount necessary for keeping and recovering ecological environment, existing irrigation area investigation and irrigation planning, soil moisture and drought monitoring, investigation of soil salinisation, planning, monitoring and effect evaluation of returning cultivated and to forest or grassland, dynamic monitoring of desertification and soil erosion, variation of river course and sedimentation in lakes and reservoirs, site selection of water project and its planning, design, construction and management. What follows is relatively detail introduction is several aspects.

Water resources investigation

Discharge in river channel can be accurately controlled by hydrological measurement, while the area of reservoir and lake can be determined by remote sensing. On the basis of that, water storage in lake and reservoir may be determined by means of stage -area-volume curve. This kind of curve can also be worked out on the basis of multi-temporal (flood, middle and dry periods) remote sensing images and corresponding simultaneous water levels in the lake or reservoir under investigation. This method is much economic than under-water topographic measurement. Key problem is obtaining enough multi-temporal remote sensing images.

Groundwater is the most important reproduced natural resources, especially for livelihood, animal husbandry and agriculture in arid regions. Remote sensing can provides the information about geology, hydrogeology geomorphology and urban environment analysis. They are helpful for searching groundwater, provides clue for field investigation and improve successful possibility. For finding groundwater, the penetration of radar is helpful to directly find shallow-layer groundwater in the places with ancient river channel and the plain area in front of mountains. Remote sensing has the ability for the observation in these aspects. With the advantage of high temporal resolution of meteorological satellites, it is possible to distinguish cloud and snow cover due to the movement of cloud.

Drought monitoring and management

Drought is always one of constrained factors for agriculture development; it is also one of natural disasters resulting in most considerable economic loss. According to [1] analysis of 100 years of rainfall data reveals that the frequency of "below-normal rainfall" in arid, semi-arid and sub-humid regions is 54-57%, while severe and rare droughts occurred once every eight to nine years in arid and semi-arid zone. In these zones, rare droughts of severe intensity occurred once in 32 years, with almost every third year being a drought year. Currently, over 10% of blocks classified by the Central Ground Water Board have been identified as 'overexploited'; blocks where the exploitation is beyond the critical level have been growing at a rate of 5.5% every year. It is estimated that 36% of blocks in the country will be on the critical list by the year 2017 [13]. From different considerations, there are agricultural drought, climatic drought and hydrological drought, also with different standard and grades.

Large covering is one of characteristics of drought; the soil moisture content measured in sampling point has certainly the problem of representative, namely, whether the soil moisture content at sampling point can reflect the drought situation over a large area and its spatial distribution. Remote sensing technology has the advantage of macro, objective, rapid and low cost. With its development, it opens a new way for drought monitoring, especially after the combination with GIS. On the basis of combination together with the conventional measurement of soil moisture content on ground and hydrological modeling, remote sensing becomes more practical and approaches the operational purpose for drought monitoring.

Indian Remote sensing Satellite (IRS) series (IRS 1A, IRS 1B, IRS 1C, IRS 1D and IRS P3) have unique payloads to monitor and assess various natural resources available in the country and around globe at different spatial resolutions. Among the payloads available IRS 1C, IRS 1D and IRS P3 have WiFS (Wide Field Sensor) payload (Table 1). WiFS sensor collects data in two spectral bands 0.62-0.68 μm (red) and 0.77-0.86 μm (near infrared) with spatial resolution of 188 m and ground swath of 810 km with a revisit period of 5 days. The combined use of three satellites cover any part of the country once in 3-4 days. The Advanced WiFS (AWiFS) sensor onboard IRS P6 provides data in 56 metres resolution with a swath of about 700 km. Due to higher spatial resolution use of WiFS/AWiFS data enables detailed monitoring at district and sub-district level.

Future missions are planned to meet the requirements of the meteorology community. The INSAT-3D launched in the year 2010 will carry improved VHRR and vertical sounders for temperature/humidity profiles. The imager will have six channels and the sounder will have nineteen channels (Table 2). Radar Imaging Satellite (RISAT), launched in 2009, is expected to boost the utilization of microwave images in the fields of agriculture and disaster management. One of the major constraints of using optical data is persistent cloudy conditions during monsoon season resulting in non-availability of sufficient cloud free data. In this context, microwave remote sensing offers great potential for monitoring crop and soils especially during the monsoon season due to capability of radar systems to acquire data under all weather conditions. The multi mode, multi polarization SAR images of RISAT will be useful to study the crop sown area progression, crop condition and soil moisture during the monsoon season to strengthen the existing drought assessment methodology.

Geoinformatics constitute the geospatial data i.e., mostly available from various satellite platforms and technology available for analysis of such data such as GIS, and other integrative tools like GPS. The ever increasing pressure on

natural resources to meet the requirement of growing population calls for the development of plans that maintains equilibrium environment, ecology and human needs. The use of contemporary technology tools like Geoinformatics to find solutions for sustainable use of land and water resources has been found to be an indispensable management and decision making tool. Geoinformatics facilitate the cost effective, timely, customized and simplified solutions for resource use. Geoinformatics has become a new tool in the hands of modern cartographers and the technology has been proved beyond doubt for its efficiency to generate maps with accuracy and time effectively particularly to depict the physically devastated areas by disasters, impact assessment and quick dissemination of disaster information to people. The application of Geoinformatics for resource management at micro level was successfully demonstrated by integrating both satellite imagery and ground data to generate action plans for land development.

With development of remote sensing sensor, several approaches have been developed. They can directly or indirectly reflect drought regime on the basis of the data obtained from these sensors. The approaches which are relatively practical at present in Karnataka are thermal inertia method, crop water shortage index method, deviation from mean normalized difference vegetation index method, water supply vegetation index method and the method of soil moisture measurement by microwave remote sensing. Karnataka considers regions which received rainfall less than 400 mm during kharif and less than 30 per cent during the cropping season and 20 per cent deficiency of rainfall during critical stage of crop growth as drought affected areas. The criteria followed is production above 75% of normal – no drought, 50 to 75% normal – moderate drought, 25-50 % - severe drought, <25 per cent – disastrous drought. Karnataka state has recently revised its norms for drought declaration in the light of four consecutive years of chronic drought. It was felt that the current norms to define drought affected areas were inadequate and inappropriate. The taluks of the state are divided in to four categories based on annual rainfall. The threshold values for rainfall deviations and number of dry weeks for drought declaration vary across the four groups of taluks. The criteria also differ for year to year, for the taluks experiencing consecutive years of drought. Details are available in the annual report (2005-06) of Revenue Department of Government of Karnataka.

There is lot of confusion still existing between science and policy communities about the characteristics of drought as a result of which drought management practices all over the world is progressing slow. Governments respond to drought through adhoc or crisis management approach rather than through coordinated or strategies. There are two types of management measures namely (short term measures and long term measures). Short term measures include adoption of contingent crop plans, cultivation of drought tolerant crops, mulching, cultivation practices like optimal spacing, rationing, nutrient management and rainwater management. Long term management includes land and water management practices to enhance the productivity in a sustainable manner.

Drought management requires a joint efforts of individuals/institutions originating from multidisciplinary aspects and together should evolve a mechanism to understand the inter relations of various aspects and generate the action plan. For example meteorologists foresee the availability of water through rainfall; natural resource managers or environmental specialists focus on the analysis of the impact of different water availability situations on various interests like agriculture, live stock and people. The major challenge lies in bringing these groups together with inter connectivity and synergy to evolve group actions. The different institutions in the drought management plan should include water institution, meteorology, agriculture, ground water, environment and socio-economic. These groups collectively should address various issues such as identification of human, biological, financial and legal constraints, identification of research needs, integration of science and policy, formulation of drought plan, creation of public awareness, implementation of planned activities either short term or long term etc. A model for institutional participation is shown in Figure 2.

MATERIALS AND METHODS

Thermal inertia method

National Oceanic and Atmospheric Administration (Advanced Very High Resolution Radiometer) is the usual data source for drought monitoring. In thermal inertia method, after atmospheric correction, the fires step is to calculate ground surface temperature, reflectivity, reflectance through atmosphere on the basis of data from CH1, CH2, CH4 and CH5 (spectrum reflectance from various channels), then calculate thermal inertia value. The second step is to establish the correlation between moisture content and thermal inertia value of soil. It is usually a one-dimensional linear equation with two parameters. The thermal inertia values are different for various types of soil. It is affected directly by the soil pattern and property. The spatial structure of soil has also effect on thermal inertia, but it is quite difficult to actually determine this effect.

The thermal inertia method is suitable for drought monitoring (Figure 3) in winter and early spring, namely, under the case of bare soil. In case of land covered with vegetal cover, this method is not so suitable because vegetation may change the thermal conductivity of soil.

Crop water shortage index method

The definition of Crop Water Shortage Index is as follows:

$$CWSI = 1 - \frac{Ea}{Ep} \quad (1)$$

Where Ea is actual evapotranspiration, while Ep evapotranspiration capacity. The smaller the value of Ea, the higher the value of CWSI, indicating less water supply ability, namely land is arid. Because evapotranspiration has close relation with soil moisture content, namely water supply ability, so CWSI also has close relation with soil moisture content. Both CWSI and soil moisture content indicate the degree of soil drought. The analysis for experiment shows [27]; that the relation between them is better to be expressed by the following logarithmic equation:

$$CWSI = A + B * Ln\, W \quad (2)$$

Where W is soil moisture content expressed in percentage. The correlation coefficient between CWSI and the soil moisture content in the soil profile from ground surface to the depth of 50 cm is higher than those for other soil layers. The norm of drought according to CWSI is: heavy drought when CWSI>0.913, middle drought when CWSI is from 0.912 to 0.765, slight drought when CWSI is from 0.764 to 0.617, normal when CWSI is from 0.616 to 0.322, humid when CWSI<0.321.

The infrared temperature Te can be obtained from NOAA meteorological satellite. It has simple linear relation with daily evapotranspiration. Besides, infrared temperature can be used to calculate daily average temperature and then Ep. So CWSI can be calculated from infrared temperature from NOAA meteorological satellite and level of drought can be classified.

Deviation from mean normalized vegetation index method:

Normalized Difference Vegetation Index (NDVI) is expressed as:

$$NDVI = \frac{(CH2 - CH1)}{CH2 + CH1} \quad (3)$$

Where CH1 and CH2 are spectrum reflectance's from first and second channels of NOAA (AVHRR) separately. The vegetation index calculated from remote sensing data can reflect the growth situation of plants, while the normalized one can reduce, to a certain degree, the error from sun elevation, atmosphere and observation for the place not beneath the satellite.

Water supply may affect growth regime, so normalized vegetation index can reflect indirectly drought situation, although there is a lag in time. On the basis of calculated NDVI from NOAA (AVHRR) for many years, the average value of NDVI for each place and each time can be obtained. This average value may indicate the mean situation of water supply from soil. The longer the time series is, the better the representative of these mean values is. The deviation or relative deviation of concurrent NDVI from mean value shows the degree of drought or humidity. The level of drought for different regions can be determined in this way.

This method is simple and easy to be used, also quite objective, but it is necessary to notice what period is the data accumulation term in long time series, namely, normal period, dry period or humid period.

Water supply vegetation index method

When crops are suffered from drought, their leaf apertures are partly closed in order to reduce the loss of water. It makes the increase of temperature of leaf surface. The more severe the drought is, the higher the temperature of leaf surface is. At the same time, the growth of crops is affected by drought, resulting in the decrease of Leaf Area Index (LAI). Besides, leaf will also be withering under high air temperature. All of these may result in reduction of NDVI.

Water Supply Vegetation Index (WSVI) is defined as follows:

$$WSVI = \frac{NDVI}{Ts} \qquad (4)$$

Where Ts is the brightness temperature of the forth channel of NOAA (AVHRR). The smaller this index is, the more severe the drought is.

Irrigation area investigation and development planning

In order to contend water, irrigation area is blindly developed in some places. The irrigation area from statistics way is smaller than the actual one. It results in water shortage in downstream basin. In order to realize comprehensive management of water resources for the whole basin, the irrigation area is important and basic information. The definition of irritable area is as follows: land with leveled ground, conveyance irrigation system and water supply in normal years. At present the major irrigation modes are channel and well irrigation. Drip and sprinkler irrigation are only developed in the area near cities. Grain production increases 4 to 5 times after the construction of irrigation system. From above description, it can be know that land, major channel, land use and water body can be distinguished through Tofts Model (TM) and extended Tofts Model (ETM), Crop growth situation can be learnt from meteorology satellites. On the basis of remote sensing, the irrigation area can be determined and a GIS-based irrigation information system can be established.

In arid regions, it is not suitable to overly develop irrigation area. If there is enough water resources the irrigation planning can be done on the basis of GIS-based database including water body, precipitation, soil, groundwater table, temperature, topography, runoff, water quality of groundwater. After weighting different factors separately, the place suitable for developing irrigation can be optimally selected.

Water environment monitoring

Water pollution results in part of water resources which are already very limited cannot be used, so the monitoring for water environment is also very important at present, remote sensing is very effective for monitoring blue green

alga due to eutrophication in lakes and reservoirs and red tide along the coastal area. With the sampling on water surface, the water quality classification into five grades can be roughly done. In general it is carried out by the multi-band composition of ETM digital images. Which bands would be used is decided according to the major pollutants in the water body under investigation. The quantitative determination of various chemical elements by means of high spectrum is a forward research subject in the world.

Planning, construction and management

Apart from the consideration of hydrological factors and economic evaluation, the site selection of reservoir and key water control project must consider the topography and the geological evaluation which can be done by RS, GIS and GPS. The planning of water diversion project can be performed on the digital platform. Remote sensing is the major source of data and information into GIS-based database, the spatial analysis and on-line virtual reality technology can play their important role on this basis. It is being carried out for the "water diversion project", including route selection, geological investigation, simulation of water transportation, selection of dam and tunnel sites, estimation of cubic meter of earth and stone, as well as the distribution of water after diversion. After the completion of "water diversion project", the arid and semi-arid regions can share more water from the Krishna River. These projects are the relatively thorough solution of water shortage in most of arid and semi-arid regions in North Karnataka.

Real-time monitoring

Due to the importance of water resources and extensity and complexity of its information, it is very necessary to establish a special system for real-time monitoring and information management to provide basis for decision-making on an integrating information platform. The data and information sources from remote sensing and conventional measures. There are real-time data and historical data. Information is managed by GIS and can be used together through network. System consists of four sub-systems, i.e. data acquisition, data transmission, data processing and decision-making support system (DSS).it can automatically acquires real-time data of hydrological data including rainfall, discharge and water elevation in river channel, lakes and reservoir, groundwater table, soil moisture content and so on, as well as water quality of surface water and groundwater. Data is transmitted by communication satellite, microwave, extra-short wave, short-wave radio or computer network to the sub-center or center of information management. In spatial database, there are real time data and historical data. The spatial data includes basic geographic data, such as water body, topography, and land use, land cover, administrative boundary, communication, plant distribution, social-economic data, water resources data concerning utilization and development, such as water supply and demand. Besides, there are banks of maps and remote sensing images.Data processing includes the processing for remote sensing images and other data, also the update of database. In the respect of DSS, there are bank of models and expert knowledge; it can provide comprehensive and synthetic basis for decision making. The functions of the system are in Table 3.

CONCLUSION

Through long-term practice in North Karnataka, it can be seen that Geoinformatics can play an important role to water resources management in arid and semi-arid regions.It is very helpful to apply Geoinformatics technology together with RS-GIS, GPS and conventional measures.Geoinformatics with geo-spatial data from various satellites, Geographic Information System and integrative tools provide immense opportunities to evolve a variety of drought indicators and integrate the same with ground based indicators for objective assessment of drought at different spatial scales.High resolution and high spectrum are necessary for solving some issues concerning water resources management in some key places of arid or semi-arid regions.Geospatial technologies are also useful for hazard and vulnerability mapping to help development of long term strategies of water resource management.Further study is necessary, especially on water demand and quantitative determination of various water bodies by Geoinformatics technology.Institutionalization of contemporary technologies, development of spatial decision support systems, impact assessment and that needs to be addressed to strengthen the water resource management system of the country.

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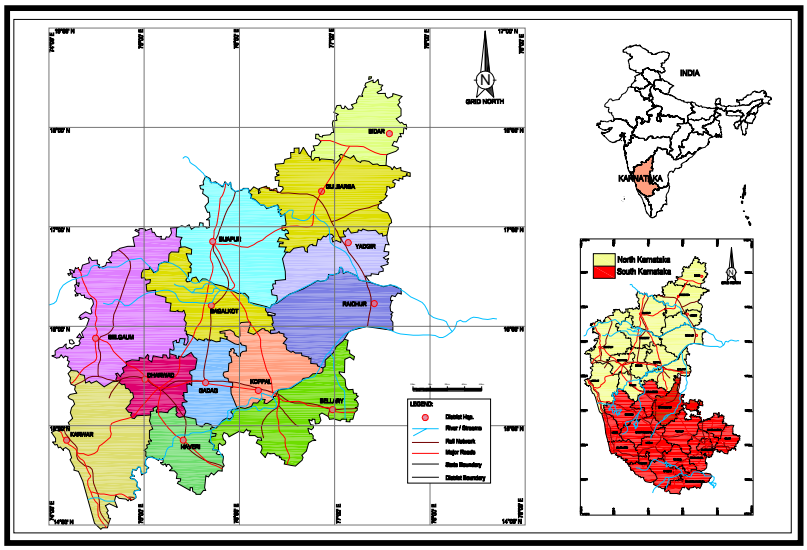

Figure 1 **Index map of the north Karnataka**

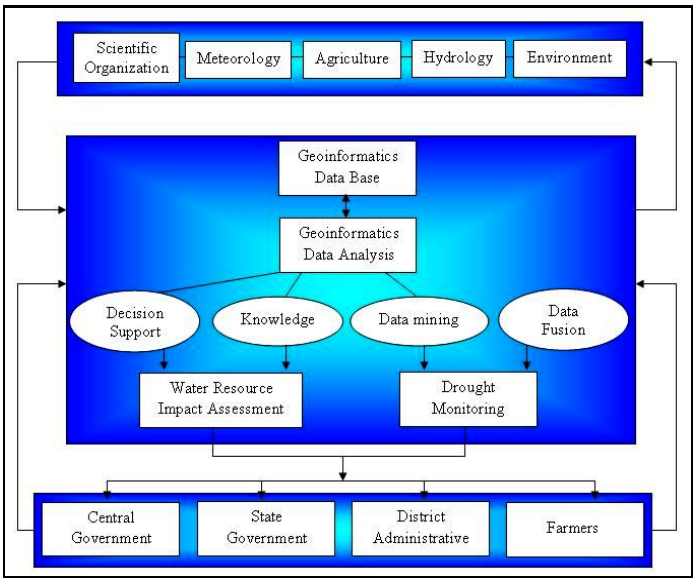

Fig : 2 Data flow applications of Geoinformatics for water resources and Drought management

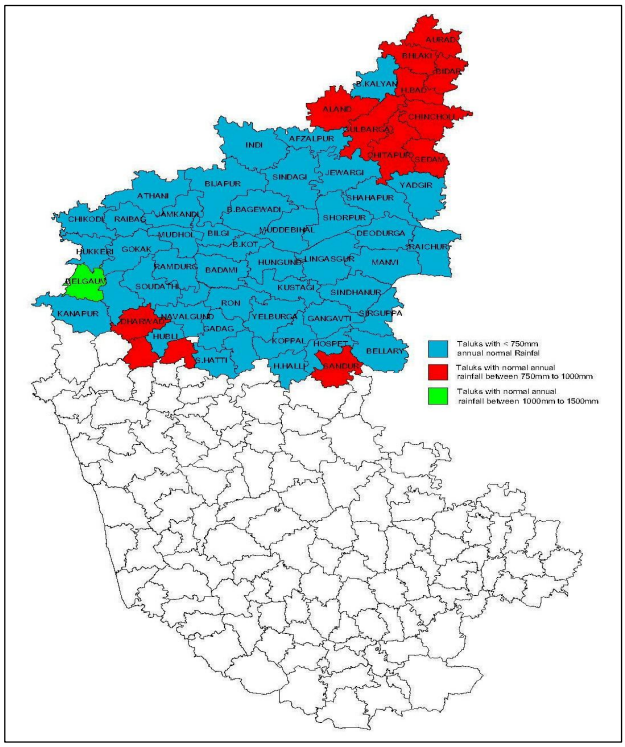

Fig : 3 Drought affected taluks in north Karnataka

Table 1: Satellites/sensors being used for drought monitoring

S.No	Satellite/sensor	Spectral resolution (microns)	Spatial resolution (metres)	Radiometric resolution (bits)	Temporal resolution
1	NOAA-AVHRR (Swath= 2700 km)	0.58-0.68 0.725-1.10 3.55-3.93 10.3-11.3 11.5-12.5	1100	10	Twice a day
2	IRS 1C/1D-WiFS (Swath= 810 km)	0.62-0.68 0.77-0.86	188	7	5 days
3	IRS P3 WiFS (Swath = 810 km)	0.62-0.68 0.77-0.86 1.55-1.75	188	7	5 days
4	Resourcesat-1-AWiFS (Swath= 740 km)	0.53-0.59 0.62-.068 0.77-0.86 1.55-1.70	56	10	5 days

Table 2 :Payload characteristics of and applications of future Indian satellites

Satellite	Payload	Bands/Resolution	Resolution(in Km.)	Applications
INSAT-3D	6 Channel IMAGER	Spectral bands (µm) Visible : 0.55-0.75 Short wave IR:1.55-1.70 Mid wave IR: 3.70-3.95 6.50-7.10 Thermal IR: 10.30-11.30 11.30-12.50	1km 1km 4km 4km 4km 8km	Cloud characterization Mesoscale processes
	19 Channel Vertical SOUNDER	Spectral bands (µm) Short wave infra red : six bands Mid wave infra red: five bands Long wave infra red: seven bands Visible :one band	10×10 for all bands	Atmospheric water vapour/temperature
Megha Tropiques	SAPHIR SCARAB MADRAS GPSROS	Six bands around 183 GHz 4 Channels: Sc-1 (Visible), Sc-2 (Solar), Sc-3 (Total) Sc-4 (IR window) Radiation instrument in short& long wave 89&157GHz radiometer 10,18&37GHz radiometer	10 km Horizontal Resolution 25 km at nadir 40km Horizontal Resolution 10 km Horizontal Resolution	Water vapour profile Six atmospheric layers upto 12 km height Radiation budget Ice particles in cloud tops cloud liquid water and precipitation; sea surface wind speed 23 GHz : integrated water vapour Vertical profile of temperature and humidity

Table 3: The functions of the system

Serial No.	DSS System	Functions
1	Inquiry	Information inquiry can be carried out in two directions, namely to inquire attribute from location on map and to inquire location from attribute or condition, rule and term.
2	Statistics	The statistics can be done both in time and space and according to the condition, rule and term.
3	Prediction and warning	Combining with special models, what is made are water resources prediction, storm-flood forecasting, low flow prediction, soil moisture content forecasting, runoff forecasting and prediction of water supply and demand.
4	WebGIS	WebGIS is adopted for this kind of system in order to realize operation and transfer in distance and in multi terminals including the figures in vector format.
5	Planning.	The planning includes water resources utilization and development, irrigation development, water project, agriculture distribution, returning farmland to forest or grass and so on.
6	Consultation	Consultation is often held for finding a solution concerning water resources management. This system can no doubt provides information, alternatives and corresponding consequence for decision making.

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RESEARCH ARTICLE

Depletion of Diversity and Pigments of Epiphytic Lichens Due to Climate Change.

Naveen Danesh[1]* and Puttaiah.E.T[2]

[1]Department of Environmental Science, Gulbarga University, Gulbarga-585106, India.

[2]Vice-Chancellor, Gulbarga University, Gulbarga-585106, India.

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***Address for correspondence**
Naveen Danesh
Department of Environmental Science,
Gulbarga University, Gulbarga-585106, India
E.Mail: naveeanu@gmail.com

ABSTRACT

Monitoring the effects of air pollutants on vegetation is very important to assess their possible damage to natural vegetation. In this sense, ambient air quality monitoring was carried out during 2006-2010 at industrial area of Bhadravathi town at Karnataka state, India. The concentration of Suspended Particulate Matter (SPM) was higher (41.02-236.56 μgm/m^3) than the concentration of oxides of nitrogen (4.15-19.69 μgm/m^3) and sulfur dioxide (1.90-13.23 μgm/m^3). Preliminary survey was made to determine the effect of air pollution induced climate change on the diversity and pigments composition of the epiphytic lichens on the selected trees; *Mangifera indica,* and *Pongamia pinnata*. The results showed the diversity was greatly reduced at industrial area, Bhadravathi compare to the Kuvempu University campus, Shankaraghatta which is just 15 Kms away. Totally 09 species of lichens were identified among which, *Ramalina* species was found to be most sensitive. The pigments; chlorophyll and carotenoids concentrations in the lichens at industrial area have showed varied response from that of the species present at similar meteorological and geographical conditions (University campus). The correlation was noticed among the effects on lichens and the concentration of air pollutants at the study area. The paper addresses the effect of air pollution induced climate change on the diversity and pigments depletion of epiphytic lichens.

Keywords: Epiphytic lichens, climate change, chlorophyll, carotenoids, Suspended Particulate Matter.

INTRODUCTION

There is unequivocal evidence that the Earth's climate is warming at an unprecedented rate. The majority of informed scientists agree that this is the result of the increase of greenhouse gases in our atmosphere, directly caused by human activities. The effects of climate change are geographically inequitable, varied and unpredictable with potentially devastating and unplanned-for consequences, both for global plant diversity and ultimately for human survival. Lichens are an association of fungi and algae that grow on a variety of substrates including trees. They lack a vascular system and absorb water and nutrients passively from their environment. Because of this, lichens are particularly sensitive to environmental factors such as temperature, water availability and air pollutants. Lichen community composition and changes in composition can provide information about changes in air quality, climate, and biological processes. Since the lichen plots are re-measured periodically, trend analyses can indicate changes in lichen communities brought about by changes in climatic conditions or other environmental stressors such as air pollution or forest fragmentation. Because of their sensitive physiology, lichens are more likely to be affected by environmental changes than other plants. Changes in temperature or water availability will lead to shifts in lichen communities. Such changes, attributed to climate change, have already been reported in Europe.

Repeated re-measurements of the lichen communities' indicator should provide an early warning of response to climate change in US forests [1]. Air quality sensitivity ratings are based on our own field studies and a world-wide literature review emphasizing references from western North America. Lichen sensitivity can vary with climate, the composition and proportion of airborne pollutants in the air, and topographic exposure. About 20,000 species of lichens are so far known from the world, among which the Indian subcontinent harbours 2450 (12.25%) species [2]. In India, a large number of pollution-monitoring studies with higher plants are available [3 and 4]. However, such studies utilizing lichens have been started recently [5]. Since lichens lack roots, surface absorption of rainfall is the only means of obtaining vital nutrients, which are dissolved in rainwater. Lichens act like sponges, taking in everything that is dissolved in the rainwater, and retaining it [6].

MATERIALS AND METHODS

Description of study area

Bhadravathi is an industrial town situated at 13°49 46 N to 75°42 22 E in the Shimoga district, Karnataka state, India. It is situated at a distance of about 255 km from the state capital Bangalore. The study area is notorious for its emissions from two large-scale industries Visweswaraya Iron and Steel Plant and Mysore Paper Mills Ltd.) in addition to numerous small-scale industries of the town. Air quality and lichen diversity of Bhadravathi town were compared with that of a control area (Kuvempu University campus). Secondary additive factors could not have affected the morphology of the identified lichen species, such as soil type and weather of neighbouring areas since both sampling sites (Bhadravathi and University campus) are very similar.

Ambient air quality monitoring to determine air quality

Ambient air quality monitoring followed standard methods of the National Ambient Air Quality Monitoring (NAAQM). Air sampling was carried out using APM-410 and APM-411 high volume air samplers. The sampling frequency was 24 h, twice a week at uniform intervals and for a period of 2 consecutive years (July 2006 to June 2010).

Determination of Suspended Particulate Matter

SPM in ambient air was determined as per the methods prescribed IS 5182-Part IV [7].

Determination of Sulphur Dioxide

A modified West and Gaeke [8] method was used.

Determination of Nitrogen Oxides:

The gas was collected in the absorber of the air sampler and the mixture was analyzed with the sodium arsenite method [9].

Epiphytic Lichen Diversity monitoring

European guidelines [10] were adopted. These are based on the fact that epiphytic lichen diversity is impaired by air pollution and environmental stress. The frequency of occurrence of lichen species on a defined portion of tree bark can be used as an estimate of diversity and as a parameter to estimate the degree of air pollution. Sampling Design: Each sampling unit was selected to represent a certain portion of the survey area and it received equal attention. The size of sampling units depends on the grid size and hence on the geographical scale of the study. With sampling units of 0.25 × 0.25 km, 0.25 km is the maximum grid density. For the same reason, a 1 × 1 unit can be sampled every 1, 2, 3, ...*n* km according to the survey requirements. Sampling units larger than 1 × 1 are not recommended, as they can cause a number of practical problems. Hence, in the present study, the 0.25 × 0.25 km grid density was selected [20]. Identification of distinct perturbation occurrences at point sources were given attention while in control areas, general investigations were adopted. The number of trees per sampling unit was dependent on its dominance.

Selection of tree species

Tree species must be selected, after reconnaissance of the study area, in order to verify the frequency/distribution of suitable trees. Free-standing trees were selected, i.e. those whose trunks received direct solar radiation for at least part of the day. However, the use of both free-standing trees and of those in closed-canopied stands must be avoided in a single survey. After reconnaissance of the study area, on the basis of dominance, two tree species were selected namely, *Mangifera indica* and *Pongamia pinnata*. Sample trees were selected to ensure that they were free standing and also received direct solar radiation at least part of the day.

A monitoring quadrate consisting of four independent quadrate segments of five 10 × 10 cm^2 each (Fig. 1) was attached vertically to the tree trunk in such a manner that the lower edge of each segment was 1 m above the ground. This was adopted as in urban centers where lichen cover is often restricted at the base of trees [10]. All lichen species present within each quadrat segment were recorded using a form and the frequency of occurrence of each species in the 5 squares of each quadrat segment noted. The list of species with their frequency values in one segment constitutes a relevé of lichen vegetation.

All species were suitable for the calculation of lichen diversity value (LDV). However, a few small crustose lichens were particularly difficult to identify and were thus overlooked [11].The four segments of the sampling quadrate were placed to correspond with the four geographic coordinates (NSEW) of the tree trunk with > 2 segments on the trees surveyed. Lichens were identified according to the methods described in the published literature and using European Guidelines [10]. LDVs were calculated. Statistical analysis of the obtained data was done using SPSS software v. 12.0.

Analysis of pigments

The chlorophyll content was calculated from absorbance values at 663 and 645 nm according to the equation of Arnon [12]. The total carotenoid content was calculated according to Parsons [13] from absorbance values at 480 and 510 nm using Systronics 367 spectrophotometer.

RESULTS

The concentration of air pollutants was within the threshold limit prescribed by the Central Pollution Control Board (CPCB 2000), but in comparison with control sites, air pollution was very high in the study area. Among air pollutants, SPM concentration was comparatively higher than the concentrations of NO_x and SO_2 (Table 1). In all, a total of 9 genera of epiphytic lichens were identified: *Pyxine*, *Chrysothrix*, *Parmotrema*, *Teloschistes*, *Dirinaria*, *Graphis*, *Ramalina*, *Lecanora*, and *Heterodermia* (Fig. 2). Their occurrence is listed in Tables 2 and 3.

In comparison, all 9 genera of epiphytic lichens were present in the control site, but lichens belonging to the genera *Ramalina* and *Teloschistes* were absent on *M. indica* in the industrial area. Species of genera *Teloschistes*, *Ramalina* and *Heterodermia* were absent on the bark of *P. pinnata* while only six genera were found. These results suggest that the control site had high diversity of lichens with frequencies of 118 ± 7.23 and 132 ± 5.12 on the barks of *M. indica* and *P. pinnata*, respectively[20]. The genus *Pyxine* (29 ± 5.8) dominated while *Parmotrema* (5 ± 0) showed the least diversity on *M. indica*. *Dirinaria* sp. (21 ± 2.8) was dominant on *P. pinnata*.

At the industrial site *Pyxine* sp. (21 ± 4.4) was most common on the bark of *M. indica*. The total sum of frequencies of lichen genera was 42 ± 3.5, indicating lichen diversity. *Pyxine* sp. (15 ± 3.1) dominated on *P. pinnata*. The industrial area had and LDV of 41.7, about three times less than the control site (122). Further, Results of various pigments (total chlorophyll and carotenoid) analysis are presented in Table 5. The table shows the values of quantified parameters corresponding to the variation of concentrations from industrial site to control site. From the observation it is clear that chlorophyll contents are found to be lesser in Industrial area compared to control area and the percentage of decrease in total chlorophyll ranged from 4% (*Graphis*) to 38.66% (*Heterodermia*). On the other hand, carotenoid also showed decreasing trend from control area to industrial area, the range of percentage decrease was 2-35 % respectively in genera of *Graphis* and *Heterodermia*. Genera of *Ramalina* and *Teloschistes* were found to be absent at industrial area and hence the comparison of the pigments were not made. All values of air pollutants were expressed as the mean of 96 trials for each year and lichen species were expressed as the mean of 12 trials for each year. Data pertaining to the air pollutants were subjected to one-way multifactorial analysis of variance (ANOVA) in order to determine significant differences between means ($P < 0.001$).

DISCUSSION

From the present study it is evident that the diversity of lichens differed between the industrial area and the control site. The variation in response could be directly attributed to the emissions from two large industries and from a number of small-scale industries in the study area. This observation supports what was noted in earlier studies on lichens at Pauri City, Uttaranchal, India by [14]. A qualitative survey of the epiphytic lichens in the surroundings of Ulan Bator in October 2007 also showed similar trends [10]. Gombert *et al*. [16] found lichens and tobacco plants as complementary biomonitors of air pollution in the Grenoble area (Isere, southeast France)**.**The present work on the use of lichens as a litmus for air pollution reveals that air pollution is the main factor affecting lichen distribution in the study area. Lichen communities change as air quality and environmental conditions change. So, by examining the types of lichens on trees in a neighborhood and the amount of bark that they cover, one can obtain a relative measure of local air quality [17] and lichens, therefore, are excellent bioindicators and biomonitors. Some species appear to have different tolerance in different geographical regions and so scales of tolerance must be determined in the area to be surveyed. A scale drawn up in one region cannot consequently be reliably applied to another without prior study. Karunarathna [17] noticed lichens as biomonitors of SO_2 and NO_2 pollution in Colombo and suburbs, further the lowest LDV (0.8374) was recorded from the site located in Colombo Fort. He identified 47 genera of lichens, out of them 10 were sensitive to air pollutants. Dolney *et al*. [18] found more than 20 lichen species among all sample plots with the two (*A. palmulata* and *P. squarrosa*) in southwestern Pennsylvania, USA being sensitive to air pollution. These

results suggest that urban and rural regions show contrasting communities of epiphytic lichen corresponding to air pollution and habitat alteration. Since the LDV varies from site to site it is clear that air pollution has not equally spread throughout the study area. Nayaka *et al*. [19] reported a similar correlation between air pollution and lichen diversity in Bangalore. The present investigation gives strong evidence that lichen growth in the study area was affected by air pollution. Moreover, a relationship existed been between the lichen community existing at sampling sites and the degree of air pollution. The absence of naturally appearing lichens in severely polluted areas limits the spatial differences of polluted areas. Zones based on epiphytic lichen vegetation provide a better indication of air pollution intensity than distribution maps of particular species. Hence, documentation of the lichen species at the study area will be of greater importance in the future.

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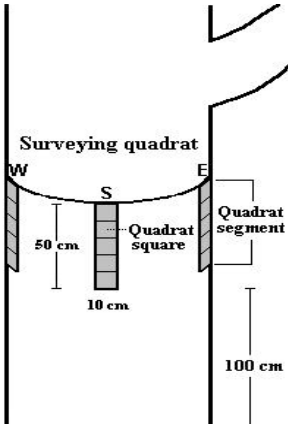

Figure 1 : Recording quadrate composed of four quadrate segments each with five squares.

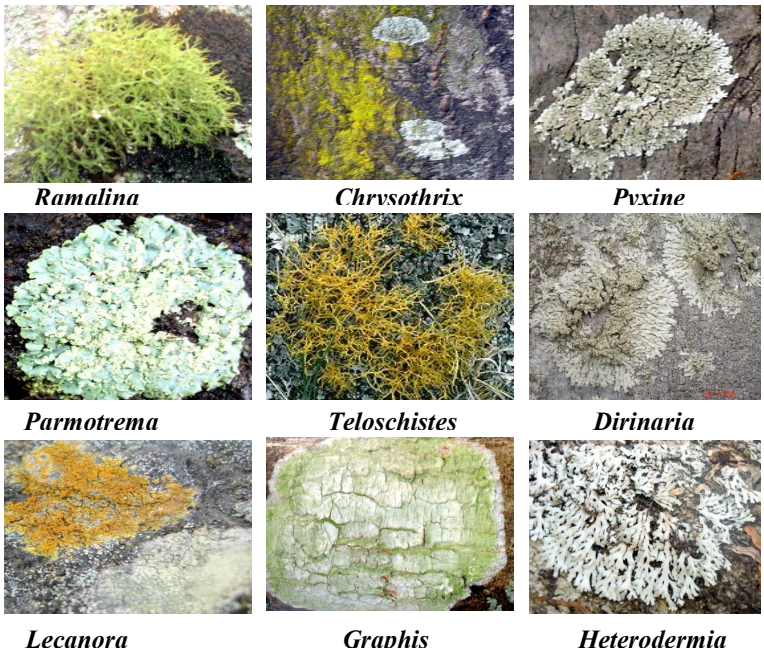

Figure 2: Lichen genera identified during the study period in Bhadravathi town.

Source: Naveen Danesh *et al* [20]

Table 1 :Concentration of air pollutants at Bhadravathi Industrial area contrast to Control area

Study sites	Control area	Industrial area	F-value	p-value
		SPM		
2006-07	20.08 ± 11.91	236.56 ± 83.22	32.16	0.0001
2007-08	20.15± 16.14	232.30 ± 81.63	31.85	0.0001
2008-09	23.08 ± 18.91	286.56 ± 93.22	35.18	0.0001
2009-10	21.15± 10.14	272.30 ± 101.63	33.85	0.0001
		SO_2		
2006-07	0.07± 0.09	13.23 ± 4.75	34.56	0.0001
2007-08	0.033 ± 0.06	13.62 ± 6.09	33.87	0.0001
2008-09	0.1± 0.5	15.83 ± 7.05	39.56	0.0001
2009-10	0.09 ± 0.2	14.50 ± 5.00	37.87	0.0001
		NO_2		
2006-07	0.35 ± 0.36	19.69 ± 7.88	36.21	0.0001
2007-08	0.28 ± 0.31	19.15 ± 6.88	26.01	0.0001
2008-09	0.50 ± 0.60	21.69 ± 7.00	41.21	0.0001
2009-10	0.32 ± 0.65	23.15 ± 5.59	32.01	0.0001

Source: Naveen Danesh *et al.* [20]

Table 2 :Variation in lichens diversity at Bhadravathi industrial area

Lichen genera	***Mangifera indica***				***Pongamia pinnata***			
	North	**East**	**South**	**West**	**North**	**East**	**South**	**West**
Pyxine	16±4.96	04±0.81	04±0.81	05±0.81	04±0.00	12±1.41	02±0.00	02±0.00
Chrysothrix	03±0.00	04±0.00	02±0.81	--	01±0.00	05±0.00	02±0.00	08±1.63
Parmotrema	03±0.00	--	--	02±0.81	06±0.81	03±0.00	04±0.00	--
Teloschistes	02±0.81	--	--	07±2.12	02±0.00	02±0.00	--	01±0.00
Dirineria	04±0.00	02±1.63	06±0.81	--	03±0.00	09±0.00	03±1.41	06±0.00
Graphis	03±0.00	06±0.00	--	04±1.63	--	02±0.00	04±0.00	04±1.63
Ramalina	08±1.63	--	04±0.81	--	05±0.81	07±0.00	01±0.00	--
Lecanora	03±0.81	--	01±0.00	05±0.81	06±1.63	06±0.81	06±0.95	01±0.81
Heterodermia	03±0.00	09±2.16	06±0.81	02±00	02±0.00	07±0.00	01±1.41	05±0.81
Sum of frquencies	**43 ±4.55**	**25 ±3.19**	**23 ±2.45**	**25± 2.63**	**29±2.15**	**53±3.22**	**23±1.96**	**27±2.84**

Source: Naveen Danesh *et al* .[20]

Table 3 :Variation in lichens diversity at Control area

Lichen genera	***Mangifera indica***				***Pongamia pinnata***			
	North	**East**	**South**	**West**	**North**	**East**	**South**	**West**
Pyxine	16±4.96	04±0.81	04±0.81	05±0.81	04±0.00	12±1.41	02±0.00	02±0.00
Chrysothrix	03±0.00	04±0.00	02±0.81	--	01±0.00	05±0.00	02±0.00	08±1.63
Parmotrema	03±0.00	--	--	02±0.81	06±0.81	03±0.00	04±0.00	--
Teloschistes	02±0.81	--	--	07±2.12	02±0.00	02±0.00	--	01±0.00
Dirineria	04±0.00	02±1.63	06±0.81	--	03±0.00	09±0.00	03±1.41	06±0.00
Graphis	03±0.00	06±0.00	--	04±1.63	--	02±0.00	04±0.00	04±1.63
Ramalina	08±1.63	--	04±0.81	--	05±0.81	07±0.00	01±0.00	--
Lecanora	03±0.81	--	01±0.00	05±0.81	06±1.63	06±0.81	06±0.95	01±0.81
Heterodermia	03±0.00	09±2.16	06±0.81	02±00	02±0.00	07±0.00	01±1.41	05±0.81
Sum of frquencies	**43 ±4.55**	**25 ±3.19**	**23 ±2.45**	**25± 2.63**	**29±2.15**	**53±3.22**	**23±1.96**	**27±2.84**

Source: Naveen Danesh *et al.* [20]

Table 4 :Lichen Diversity Values (LDV) for Industrial area and control area

Study area	LDV
Industrial area	62.8
Control area	122.0

Table 5: Variations in concentrations of tested pigments (mg/g of tissue) in nine lichen genera at sampling sites

Sampling Sites	Total Chlorophyll		Carotenoid	
	Control Area	Industrial Area	Control Area	Industrial Area
Pyxine	0.52±0.30	0.36 ±0.20	0.39 ±0.30	0.28±0.20
Chrysothrix	0.60±0.20	0.41±0.10	0.36±0.10	0.29±0.10
Parmotrema	0.90±0.35	0.82±0.00	0.50±0.05	0.39±0.02
Teloschistes	1.20±0.50	Absent	0.65±0.00	Absent
Dirineria	0.55±0.05	0.38±0.00	0.35±0.90	0.28±0.50
Graphis	0.72±0.00	0.69±0.05	0.50±0.20	0.49±0.00
Ramalina	1.25±1.30	Absent	0.66±0.90	Absent
Lecanora	0.59±0.90	0.49±0.94	0.52±0.90	0.49±0.05
Heterodermia	0.75±0.05	0.46±0.00	0.60±0.20	0.39±0.00

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REVIEW ARTICLE

Impact of Treated Paper Board Mill Effluent on Growth, Wood Yield of *Eucalyptus camaldulensis* Dehnh.Clones and Soil Nutrients.

M.Veeramani and R.Mohanraj*

Dept. of Environmental Biotechnology, School of Environmental Sciences, Bharathidasan University, Tiruchirapalli – 620 024. TamilNadu,India.

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***Address for correspondence**
R.Mohanraj
Dept. of Environmental Biotechnology,
School of Environmental Sciences, Bharathidasan University,
Tiruchirapalli – 620 024. TamilNadu,India

ABSTRACT

The influence of paper board mill treated effluent water was investigated on growth (height (ht), diameter breast height (dbh) and wood yield of five different *E. camaldulensis* clones (ITC 3, 7, 105, 285 and 316) at the age of four and the growth was compared with well water irrigated and rainfed plots. Soil properties were also studied annually in all treatments. There was a statistical significant difference was noticed in plant growth (ht, dbh) and wood yield in Eucalyptus clones in treated effluent water irrigated plots. All the five clones' earlier growth (ht and dbh) was better in treated effluent water irrigated plot than well water irrigated and rain fed plots. The clone ITC 3 showed highest growth (ht=12.3 m and dbh = 28.1 cm) and wood yield (122.2 t/ha) in ETP water irrigated plots followed by Well water irrigated and rain fed plots as well clones ITC 316, 285, 105 and 7. The treated effluent water irrigated plot soil nutrients like nitrogen, phosphorus, potassium, calcium, magnesium and boron rate was increased.

Keywords: *Eucalyptus camaldulensis*, treated paperboard effluent, soil nutrients

INTRODUCTION

Fresh water availability to meet the growing needs of mankind has raised serious concerns in recent years. Treated effluent is now considered as potential source of water to supplement the fresh water supplies. Pulp and paper industries are required a good amount of water for processing and releasing effluent water rich in nutrients like Nitrogen, phosphorus, potassium and calcium. Using the treated effluent water as irrigation to non edible species and short rotation pulp wood tree species like eucalyptus is a useful throwaway method for the effluent generating industries. The direct use of sewage as irrigation water would be a useful disposal system (Resende *et al.,* 2000), but a lack of balanced nutrients in the solution could affect plant growth and soil fertility. In reality, wastewater except the

water resource for irrigating the plantations is an enormous nutrient source, too (Rattan *et al.,* 2005). Raising trees under sewage water irrigation has been proved to improve the health and productivity of waste lands. This also minimizes the contamination and poising through food chain. The wood biomasses of trees are good sink for various heavy metal toxicants. Establishment of trees plantation for waste water irrigation has been a common practice for many years. The practice not only defers ecological degradation by the pollutants in the soil, because trees are long-living organisms which can take up trace elements from the soil, water or air and retain them for a long time (Madejo´n *et al.,* 2006). But it also creates opportunities for commercial biomass production and sequestration of excess minerals in the plant system (Sharma and Ashwath, 2006). Therefore, the use of waste water in growing woodlots is a viable option for the economic disposal of waste water (Neilson *et al.,* 1989). Bhati and Singh (2003) reported that seedlings of *E. camaldulensis* irrigated with municipal effluent had 13% greater height and 5% greater collar diameter than those irrigated with good water. The growth and biomass production of seven tree species irrigated with sewage and reported that height and diameter of trees varied significantly. Hassanli *et al.* (2007) reported that the irrigation of different tree species with sewage effluent had no adverse effect on soil properties. Soil nutrient content like N,P,K levels increased/decreased. Four years irrigation with treated effluent caused a slight increase/ decreased in soil properties.

MATERIALS AND METHODS

The study location is situated in Kemmarmpalayam village of Mettupalayam Taluk Coimbatore District, Tamil Nadu, India lies in 11º02' North latitude and in 77 º 03' East longitude. The mean annual rainfall is 700 mm and the soil type is red loamy. Well water is the main irrigation source in this area. The climate of this site is sub tropical with mild cold during the months of November to January and hot in during the months of March to June (Fig.1). The rainfall pattern is scatted and not uniform (Fig 2). Experiments were conducted during the month of November 2006 and each 1 hectare plot was used for conducting trial with split plot design with four replications.*E. camaldulensis* promising clones of ITC 3, 7, 105, 285 and 316 were used for study at the spacing of 3 x 1.5m. The paper board mill treated effluent and well water have been irrigated in the first two hectare of plots once in 15 days after planting and remaining plot was kept as it is after planting without any irrigation(rain fed). The intercultural operations like slash weeding and disc plough were carried out for the all the experimental plots. The plants growth (ht and dbh) was measured annually and the wood yield (tons/ha) was calculated by weighing in sample plot after 4th year. The plant growth data like ht, dbh and wood yield/ha was estimated at the age of 4 in the plots. Treated effluent and well water characters and The soil samples physico-chemical properties like pH, EC, organic carbon (OC), Available N, P, K, Exchangeable cations, Cation Exchange Capacity (CEC), Exchangeable Sodium Percentage (ESP) and Per cent chloride in soil properties and nutrients contents were studied annually in the ITC laboratory.

RESULTS AND DISCUSSION

Growth and wood yield

There was a statistical significant difference was noticed in plant growth (ht, dbh) and wood yield in Eucalyptus clones in treated effluent water irrigated plots. All the five clones' growth (ht and dbh) and wood yield was better in treated effluent water irrigated plot than well water irrigated and rain fed plots (Fig.3). Among the five clones ITC 3 showed highest growth (ht=12.3 m and dbh = 28.1 cm) and wood yield (122.2 t/ha) in ETP water irrigated plots at the age of four. The growth yield of clone ITC 3 is 7% higher in ETP water irrigated plot than well water and 30% higher than rain fed plot. The clones 285, 7 and 105 showed around 40% less growth compared to clone 3 in the ETP irrigated plot. In the rain fed area the clone 3 wood yield is 85 tonns/ha at the age of four the yield is higher than the clones 7, 105 and 285 ETP and well water irrigated plots yield. The experiment reveals that the clone 3 doing better in all experimental plots than the other clones. Howe and Wanger (1996) observed no negative effects in wastewater from pulp mill were applied as irrigation to Populus for six months. Treated effluent water nutrients, such as N, P, K,

Ca, S and B benefit the Eucalyptus clones growth (Malavolta *et al.,* 1997). High nutrient content accumulation was studied in the aerial part of the plant i.e. N>Ca> K >S> Mg> P showed the needs of these elements to growth but in this experiment plant nutrient content was not studied (Silveira *et al.,* 2001).

Chemical and physical properties of treated effluent and well water

Treated effluent water chemical and physical properties were analyzed during the experiment period and results were compared to well water properties. The results were tabulated (Table.1). Treated effluent water pH was ranging from 7.29 to 7.54 but the well water pH was ranging from 8.62 to 8.71. The well water pH was slightly higher than treated effluent water.

Soil chemical and physical properties in treated effluent irrigated areas from the year 2006-2010

The soil samples were analyzed for various physico-chemical properties like pH, EC, organic carbon (OC), Available N, P, K, Exchangeable cations, Cation Exchange Capacity (CEC), Exchangeable Sodium Percentage (ESP) and Per cent chloride and the results are given in table 2. In general, the pH of the soil in all the treatments was near neutral. The pH of the soil in effluent irrigated areas as well as well water irrigated areas were slightly increased over a period, which might be due to the presence of soluble salts in treated effluent as well as well water. However, not much variation in soil pH was observed. Increase in soil pH due to paper mill effluent irrigation was reported by Vasconcelos and Cabrel (1993) and Hameed (1997).

The mean EC of soil samples in the entire field ranged from 0.02 to 0.06 dS m-1 at different stages of sampling periods. In general, the EC of soil in treated effluent irrigated areas comparatively higher than non-effluent irrigated areas. The EC of the soil increased due to soluble salts present in the effluent and continuous effluent irrigation which could have added up the soluble salt content in the soil (Prasanthrajan, 2001). Besides, the effluent also contained organic polyelectrolytes which bind divalent cations, increasing the EC of water and soil (Metzgr *et al.,* 1983). The organic carbon content of the soil is showed an increasing trend in all the treatments. The initial soil test showed that lowest organic carbon content of 0.40 % and slowly organic carbon content has been increased to 0.50% in rainfed condition and huge increment is noticed in the treated effluent irrigated condition i.e. 0.70% followed by well water irrigated soil. The raise of organic content in effluent irrigated soil might be due to the accumulation of suspended and dissolved organics present in the effluent which in turn contribute to build up of organic matter in soil. The perennial species acted as surface mulch that replenish nutrients, conserve soil moisture and supplies organic matter to the soil (Young, 1990; Sharma *et al*., 1998)

During the study period, the available nutrients like nitrogen, phosphorus and potassium content of soil ranged from 145 to 153, 16.0 to 21.03 and 145 to 154 kg ha^{-1}, respectively in effluent irrigated areas. In general, the available nutrients content of soil was lower in non-effluent irrigated areas when compared to effluent irrigated areas. An increasing trend on available nutrient status was observed invariably at all the treated effluent irrigated areas, due to the impact of continuous application of treated effluent to the soil. In general, the increase in soil fertility status in all the treatments could be attributed to the release of root exudates, which ultimately stimulated the microbial population in the soil. These microbes might be responsible for the mineralization of various nutrients present in the litter fall. Palaniswamy and Sree Ramulu (1994) reported increased available Nitrogen content of soil due to effluent irrigation. The difference in respect of available P and K also followed the same trend as that of available N in all the crops. This corroborates with the findings of Udayasoorian *et al.,* (1999a).

The mean soil exchangeable cations like calcium, magnesium, sodium and potassium varied from 17.0 to 18.2, 7.6 to 8.2, 6.5 to 7.5 and 0.3 to 0.5 c mol (p^+) kg^{-1}, respectively in treated effluent irrigated soil. The level of exchangeable cations increased with duration of the effluent irrigation and fast movement of soluble ions present in the effluent resulted in an increase soil exchangeable cations. In general the exchangeable cations were comparatively lower in non-effluent irrigated areas when compared to effluent irrigated areas. This in accordance with the findings of

Kannan (1988) and Oblisamy and Palanisami (1991). Higher concentration of exchangeable cations under effluent irrigation was reported by Gomathi and Oblisamy (1992) and Udayasoorian *et al.* (1999c).

The lowest soil CEC of 27.1 c mol (p^+) kg^{-1} was observed in rainfed condition and the highest soil CEC of 34.4 c mol (p^+) kg^{-1} was recorded in treated effluent irrigated areas over the four years of duration. This might be due to accumulation of more organics, which inturn retained the cations in soil Elayarajan (2002). In general, ESP of soil in well water irrigated areas was comparatively higher than treated effluent irrigated areas. The results revealed that the continuous irrigation with well water had increased the ESP of the soil in well water irrigated areas, which might be due to large amount of soluble salts especially sodium present in the well water.

The mean per cent chloride content of soil in experiment site premises varied from 0.16 to 0.22 in effluent irrigated areas. The lowest per cent chloride content of 0.008 was recorded in rainfed condition. In general, over a period of 4 years the soil properties in effluent irrigated areas was slightly get increased than non-effluent irrigated areas which might be due to continuous application of treated effluent irrigation to soil.

CONCLUSION

The eucalyptus wood yield was increased in effluent water irrigated conditions. Among the five clones, ITC 3 showed highest growth and wood yield (122.2 t/ha) in ETP water irrigated plots at the age of four. The growth yield of clone ITC 3 is 7% higher in ETP water irrigated plot than well water and 30% higher than rain fed plot. The clones 285, 7 and 105 showed around 40% less growth compared to clone 3 in the ETP irrigated plot. In the rain fed area, the clone 3 wood yield is 85 t/ha at the age of four the yield is higher than the clones 7, 105 and 285 ETP and well water irrigated plots yield. The experiment reveals that the clone 3 doing better in all experimental plots than the other clones. Soil nutrient was increased over the period in effluent water irrigated plot. An increasing trend on available nutrient status was observed invariably at all the treated effluent irrigated areas, due to the impact of continuous application of treated effluent to the soil. Irrigating the effluent water to the short rotation tree species like eucalyptus (non edible) is found to be a good disposable method.

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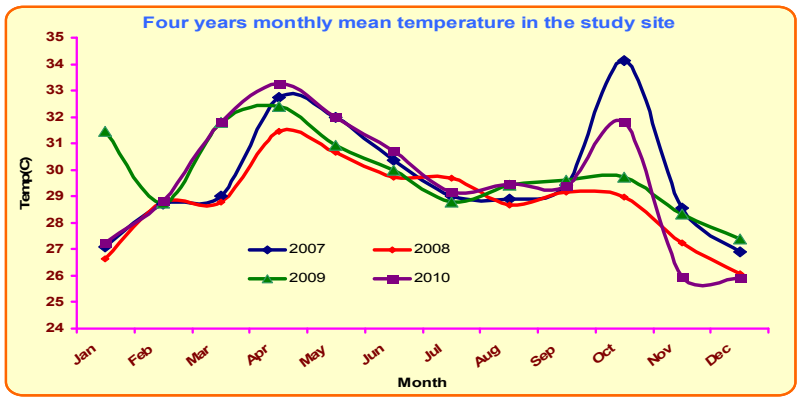

Fig 1. Annual temperature (monthly mean) status in the study site

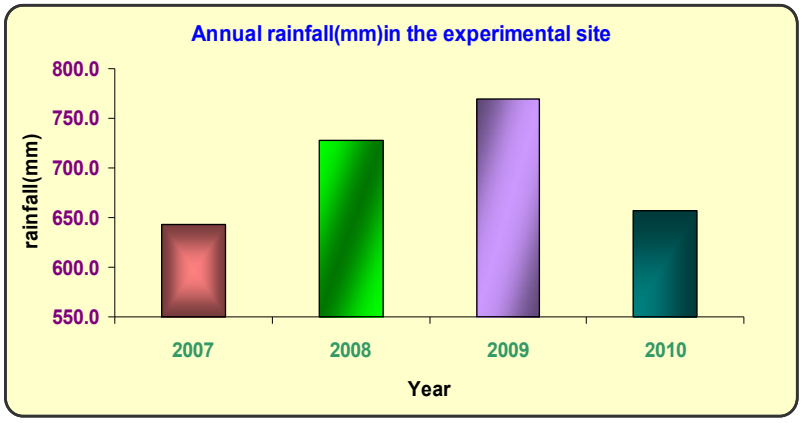

Fig 2: Annual rainfall status in the experimental location

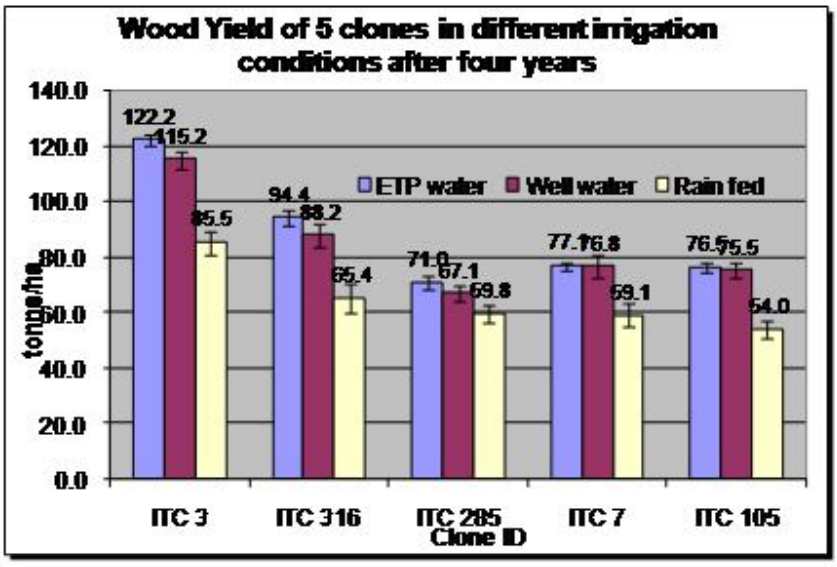

Fig 3: Five different clones wood yield in different irrigation plots at the age of four

Table 1. Treated effluent water and well water chemical properties during the experiment

Parameters	Unit	2007		2008		2009		2010	
		Treated effluent	Well water	Treated effluent	Well water	Treated effluent	Well water	Treated effluent	Well water
pH	**-**	7.54	8.71	7.295	8.62	7.405	8.67	7.485	8.76
EC	dS m^{-1}	2.53	1.05	2.51	1.16	2.36	1.02	2.39	1.19
TDS	mg L^{-1}	1217.5	654	1326	739	1462.5	660	1500	704
BOD	mg L^{-1}	19	13.4	20.5	14.1	21.15	13.3	22.05	15.6
COD	mg L^{-1}	180.5	55.7	182.55	57.6	178.5	53.6	175.5	70.5
HCO_3	mg L^{-1}	117.5	-	133.7	-	127.5	-	135	-
Ammonical-N	mg L^{-1}	24.75	-	25.95	-	25.4	-	25.9	-
Phosphorus	mg L^{-1}	1.54	-	10.5	-	2.435	-	2.4	-
Potassium	mg L^{-1}	18.4	16.4	18.245	16.7	18.4	16	18.15	18.5
Calcium	mg L^{-1}	240	97	273	101	264	96.3	270.5	100
Magnesium	mg L^{-1}	181.5	71	182	55.1	170.5	32.4	178.5	35.7
Chloride	mg L^{-1}	344	192.6	390	197	366	186	371.5	195
Sulphate	mg L^{-1}	128	102	228.5	104	161.5	97.1	158.5	101.8
Sodium	mg L^{-1}	394	-	381	-	376.5	-	380.5	-
Percent Sodium	%	47.245	47.1	44.645	46.7	45.385	47.3	44.89	49.9

Table 2. Soil physico-chemical properties and nutrient status in different irrigation condition during study period

Parameters	Unit		2007			2008			2009			2010		
		Initial	A	B	C	A	B	C	A	B	C	A	B	C
pH		7.1	7.5	7.6	7.2	7.6	7.7	7.1	7.6	7.7	7.3	7.5	7.8	7.2
EC		0.2	0.4	0.3	0.2	0.4	0.3	0.2	0.5	0.4	0.3	0.6	0.5	0.3
Organic carbon	%	0.4	0.5	0.4	0.3	0.5	0.4	0.4	0.6	0.4	0.4	0.7	0.6	0.5
Available Nitrogen	Kg/ha	126.5	145.0	145.0	127.0	147.0	147.0	128.0	151.0	149.0	129.0	153.0	151.0	129.0
Available Phosphorus	Kg/ha	12.0	16.0	16.0	12.8	18.0	20.1	12.9	20.8	20.4	13.1	21.3	20.9	13.8
Available Potassium	Kg/ha	110.0	145.0	136.0	120.0	148.0	139.0	118.0	151.0	143.0	126.0	154.0	147.0	126.0
Exchangeable Calcium	cmol(p+) Kg/ha	13.0	17.0	13.7	14.2	17.6	14.2	14.5	17.9	14.5	14.2	18.2	14.8	14.5
Exchangeable Magnesium	cmol(p+) Kg/ha	6.9	7.6	7.2	7.0	7.8	7.3	7.1	8.0	7.8	7.2	8.2	8.1	7.2
Exchangeable Sodium	cmol(p+) Kg/ha	5.2	6.5	5.8	4.8	6.7	5.9	5.1	7.2	6.9	5.3	7.5	7.2	5.1
Exchangeable Potasssium	cmol(p+) Kg/ha	0.2	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.5	0.4	0.3
CEC	cmol(p+) Kg/ha	25.3	31.4	27.0	26.3	32.5	27.7	27.0	33.5	29.6	27.0	34.4	30.5	27.1
ESP	%	20.5	20.7	21.5	18.2	20.6	21.3	18.9	21.5	23.3	19.6	21.8	23.6	18.8
Chloride	%	0.006	0.016	0.012	0.006	0.018	0.014	0.006	0.016	0.014	0.008	0.022	0.016	0.008

A: Treated effluent water irrigated soil, B: Well water irrigated soil, C: Rain fed soil

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RESEARCH ARTICLE

Degradation of Simulated Dye Wastewater by Electrochemical Method on Carbon Electrodes.

Prakash Kariyajjanavar[1], J. Narayana[1] , Y. Arthoba Nayaka [2*]

[1]Department of P.G. Studies & Research in Environmental Science, Kuvempu University, Jnana Sahyadri, Shankaraghatta, 577 451 Karnataka, India.

[2]Department of P.G. Studies & Research in Chemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, 577 451 Karnataka, India.

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***Address for correspondence**
Dr.Y.Arthoba Nayaka
Associate Professor
Department of P.G. Studies & Research in Chemistry,
Kuvempu University,
Jnana Sahyadri, Shankaraghatta, 577 451
Karnataka, India
E.Mail: drarthoba@yahoo.co.in

ABSTRACT

The electrochemical degradation of industrial wastewater has become an attractive method in recent years. In this work simulated dye wastewater containing vat dye C.I. Vat Green 1 is degraded from electrochemical method using graphite carbon electrodes. The experimental results indicated that initial *p*H, current density and supporting electrolytes were played an important role in the degradation of dye. Electrochemical behavior of dye has been studied with cyclic voltammetry in basic medium using glassy carbon as working electrode. The potentials selected for the dye was in the range 0.0 to –1.5 V. The UV-Vis and chemical oxygen demand (COD) studies were selected to evaluate the degradation efficiency. The maximum colour removal efficiency of 99% and chemical oxygen demand (COD) removal of 75.43% could be achieved for dye, at 25 g L^{-1} of NaCl concentration. The LC-MS and FTIR studies revealed the degradation of dye and confirmed that aromatic rings were destroyed. The results revealed the suitability of the present process for the effective degradation of dye C.I. Vat Green 1.

Key words: Carbon electrodes, cyclic voltammetry, electrochemical degradation, FTIR, LC-MS, UV-vis, vat dye.

INTRODUCTION

Textile wastewater has a high content of pollutants, the sources of which are the natural impurities extracted from the cotton fiber, the processing chemicals and the dyes. The discharge of this wastewater to the environment causes aesthetic problems due to the color and also damages the quality of the receiving water. The color impedes light penetration and the dyes and their degradation derivatives can prove toxic to aquatic life [1]. Vat dyes account for about 15% of total consumption of textile dyes [2]. They exhibit good fastness to light, acid, alkali, and solvents, and they mainly used in dyeing cotton fibres [3]. Vat dyes cause environmental concerns when released in industrial wastewaters due to their carcinogenic health effects [4]. Vat dyes are practically insoluble in water, but can be reduced in the presence of an alkali and a reducing agent to form a soluble dye known as the leuco dye [5-6], which have a certain affinity to cellulosic fibers. It needs to be reduced to its water soluble leuco-form before dying.

The treatment of textile dye effluent is difficult and ineffective with conventional biological processes and several physico-chemical methods viz., adsorption, photo degradation, chemical oxidation, membrane processes, coagulation, flocculation etc. because many synthetic dyes are very stable to light, temperature and are non-biodegradable nature of most dyes [7, 8]. In this context, electrochemical technique is considered to be a powerful means for the treatment of dyeing wastewater. Indeed, electrochemical method has been successfully tested [9] and it has certain significant advantages such as simple equipment, easy operation and lower operating cost [10-12]. The process requires significantly less equipment than conventional biological treatment processes [13, 14]. Graphite electrodes were used as anode and cathode by many researchers for the application in organic oxidation [15, 16]. Hence, there is an interest in electrochemical methods to develop an efficient, cost-effective and eco-friendly alternative for the degradation of dyestuffs [17]. In the past, graphite was frequently used as an anode for the electrochemical degradation of textile dye as it is relatively cheaper and gives satisfactory results [18]. The aim of this work was to test the feasibility of electrochemical method for the degradation of C.I. VAT GREEN 1 using graphite carbon electrodes.

MATERIALS AND METHODS

The commercial vat dye, Indanthren Brilliant green FFB Coll. (C.I .Vat Green 1, CAS No. 128–58–5) and was obtained from textile industry Himatsingka Linens, Hassan, India. All other chemicals used for the experiments were of analytical grade reagents and obtained from s d fine chem-limited, Mumbai, India. Cylindrical carbon electrodes (Chemical composition: graphite carbon + coke: 85% and ash 15%) were obtained from Power Cell Battery India Limited. A digital DC power supply (AD 302S: 30V, 2A) was used as an electrical source. Double distilled water was used to prepare the desired concentration of dye solutions and the reagents.

Instrumentation

Electrochemical measurements

The electrochemical measurements were carried out using CHI660D electrochemical workstation (CH Instruments Austin, USA) controlled by electrochemical software. A three electrodes system was used for the cyclic voltammetric experiments. The working electrode was highly polished, glassy carbon disc with an effective surface area of 0.06 cm^2. A platinum wire and saturated calomel were used as counter and reference electrodes, respectively.

Electrochemical degradation studies

Figure 1 shows the experimental setup for the electrochemical degradation studies. Graphite carbon electrodes of 4.5cm length and 0.8cm diameter were used as anode and cathode. The effective electrode area was 11.82cm^2. The supporting electrolytes such as NaCl and Na_2SO_4 were added to the electrolytic solution, which increases the conductivity of the solution and reduces the electrolysis time. The solution was kept under agitation using magnetic stirrer.

UV-Visible studies

A UV-Vis Spectrophotometer (ELICO, SL-159) was employed to measure the optical density of dye solution (λ_{max}=630) before and after electrolysis. The decolourisation efficiency was calculated using the relation:

$$\%E = \frac{A_i - A_f}{A_i} \times 100 \qquad (1)$$

where, A_i and A_f are absorbance values of dyes solutions before and after treatment with respect to their λ_{max}, respectively or A_i and A_f are initial and final COD values of the dyes solutions, respectively.

pH and conductivity measurement

A water analyser (Systronics, Model-371) was used to measure the *p*H and conductivity of the dye solution before and after electrolysis under different electrolysis conditions.

Liquid Chromatography-Mass Spectrometry studies (LC-MS)

The extent of degradation of dye samples were analyzed by LC-MS studies (LCMS-2010A, Shimadzu, Japan). The LC-MS was fitted with column C18. The mobile phase was methanol: water (90:10). The flow rate was 0.2 mL min^{-1} and the injection volume of dye was 5μL. The dye solutions were injected into LC column before and after electrolysis. Analyses using ESI (electron spray ionization) interface were done under the same chromatographic conditions as described for the APCI (atmospheric pressure chemical ionization) analysis, except the guard column, which was not used in the ESI analysis.

FTIR studies

To study the structural changes of dye before and after electrolysis the dye sample was characterized by using Fourier Transform Infrared Spectrometry (FTIR) spectrometer (model 3010 JASCO, JAPAN). The scan range of the wave number was set from 400 to 4600 cm^{-1}. The dye samples was fitted with sample holder mixed with Potassium Bromide (KBr) in the ratio of 1:5 and grind uniformly, the powder is distributed in 5 mm pellet making die and apply 10 tonnes of pressure and liquid samples without solvent, was kept in between two transparent sodium chloride cells and kept in the sample holder and scanned to acquire the FTIR spectra.

RESULTS AND DISCUSSION

The structure of C.I. Vat Green 1 is shows in figure 2. C.I. Vat Green 1 is best known to polycyclic aromatic carbonyl dyes cover the entire color range of green [19].

Voltammetry

The cyclic voltammagrams of C.I. Vat Green 1 (50 ppm, w/v) was recorded in *p*H 9 using glassy carbon as working electrode. The potential range selected was 0.0 to –1.5 V. The voltammetric curve of C.I. Vat Green 1shows a cathodic peak at –1.11 V (I_{pc}), and there was no anodic peak found, indicating the irreversible nature of the dye (Figure 3).The cathodic peak currents observed for C.I. Vat Green 1attributed to the reduction of ketones to alcohols. These data are very much important to assess the feasibility of the electrochemical process for the degradation of the dye VG 1.

Figure 4 shows the effect of scan rate on the cyclic voltammograms of C.I. Vat Green 1. The reduction peak current (I_{pc}) increased linearly with square root of the scan rate ($\upsilon^{1/2}$) over the range 0.025 to 0.150 V s^{-1}. Inset plots show a linear relationship (Figure 4 inset) between $\upsilon^{1/2}$ and current. The correlation co–efficient (r^2) of C.I. Vat Green 1 was found to be 0.9924 for cathodic peak current (I_{pc}) and indicated the diffusion controlled electrode process.

Influence of electrolysis conditions on dye degradation

Effect of supporting electrolytes

In UV–Vis spectra (Figure 5a), it can be seen that, the addition of NaCl to the dye solution increased the decolourisation efficiency. From this observation it could be concluded that the introduction of NaCl can enhance the degradation efficiency, which may be attributed to the reaction between the electrogenerated chlorine/hypochlorite, hydroxyl radicals and the dye molecule. Figure 5b demonstrates the effect of Na_2SO_4 on the degradation of dye. The decolourisation efficiency in presence of Na_2SO_4 is attributed to the generation of persulphate ions, which can oxidize organic dyes [20].

Moreover, the increased concentration of supporting electrolytes results in a decrease in the operating voltage at the given current density (Figure. 6a). An increase in the concentration of NaCl up to 25 g L^{-1} accelerated the degradation rate, enabling the decolourisation efficiency of C.I. Vat Green 1 of 99.38% (Figure 6b).

Effect of initial pH

Solution *p*H is one of the important factors that affect the performance of electrochemical process. Hence experiments were conducted to study the effect of *p*H on the degradation efficiency of C.I. Vat Green 1. A significant difference in the extent of decolourisation was noted when the concentration of NaCl was at 25 g L^{-1}. The initial *p*H of the solution (3-11) was adjusted using 1M H_2SO_4 or NaOH [21-22]. The electrolysis was carried out at the current density of 170 A m^{-2} for 240 min. with a dye concentration of 50 ppm (w/v) at room temperature. From the UV-Vis spectra, it was clear that, the absorbance in the visible region is attributed to the presence of anthrquinone and carbonyl groups (C=O). In basic *p*H, during electrochemical degradation, the reduction of C=O bonds and cleavage of aromatic rings has taken place, which results in the decrease of optical density of the dye solution. Also the absorption band has been shifted from visible to UV region (Figure. 7), which indicated the degradation of larger dye molecules into smaller fragments [23]. However, the hypochlorite can lead the partial mineralisation of dyes [20]. From figure 7, it is clear that the decolouration efficiency of C.I. Vat Green 1 was higher in both neutral and basic *p*H (99%) and slightly lower in acidic *p*H. After electrolysis the final *p*H was found to be slightly increased to basic (Figure 8).

Effect of current density

The electrolysis of dye solution was carried out at different current densities (85, 170, 255, 340 and 425 A m^{-2}) at graphite carbon electrodes to investigate the influence of current density on the degradation efficiency of C.I. Vat Green 1 keeping NaCl concentration at 25 g L^{-1}, dye concentration at 50 ppm (w/v), *p*H at 9 and temperature at 300 K. It can be found that, the decolourisation and COD removal efficiencies increased (Figure 9) with increasing the applied current density [24]. This is because of the increased rate of generation of oxidants, such as chlorine/hypochlorite and hydroxyl radicals at higher current densities. Up to a current density of 170 A m^{-2}, the degradation efficiency of the dye was increased almost linearly. At higher current densities (>170 A m^{-2}) the degradation efficiency was attained almost constant. Also the energy consumption was found to be more at higher

current densities with a subsequent stripping of electrodes [25]. Therefore, the optimal current density for the successive electrochemical degradation was fixed at 170 A m^{-2}.

Analysis of COD

The electrolysis was carried out at a current density of 170 A m^{-2}. At this current density, hypochlorite ($HOCl^-$) and •OH generated in the solution drives the oxidation process at basic *p*H. The maximum COD removal efficiency of 75.43 % was observed at *p*H 9 (Figure 10a). The percent removal of COD increased with increase in the concentration of NaCl (Figure 10b). This confirmed that the electrogenerated chlorine/hypochlorite will play an important role in the electrochemical degradation process of the dyestuffs.

LC–MS studies

LC–MS studies were employed to monitor the diminution in mass of the fragments of Vat Green 1 dye before and after electrolysis. MS spectrum of the dye C.I. Vat Green 1 recorded before electrolysis shows more number of peaks at higher m/z values due to the presence of dye and other impurities (Figure 11a). The MS spectrum of the dye residue (during electrolysis) shows newer peaks (Figure 11b) than the earlier peaks indicating partially degradation of dye and formation of intermediates. The MS spectrum of the filtrate solution after complete electrolysis shows the absence of majority of the peaks (Figure 11c). This clearly indicated that almost all dye was coagulated and removed in the form of sludge. The remaining peaks at low m/z values in the mass spectra may be due to the presence of substituted simple aromatic compounds.

FT–IR

The FT–IR spectra of dye C.I Vat Green 1 was obtained before electrochemical treatment showed several bands at fingerprint region. After the electrochemical treatment a significant reduction of bands were observed in fingerprint region (Figure 12). This indicated the disappearance of functional groups during electrolysis.

Electrical energy consumption

The major operating cost is associated with the electrical energy consumption during electrochemical degradation process. The electrical energy consumption (E) is required to decompose 50 ppm (w/v) C.I Vat Green 1 dye solution at various current densities was calculated using the relation:

$$E = \frac{VIt_E}{V_s} \times 10^{-3} \qquad (2)$$

where E is the electrical energy consumption (k W h m^{-3}), V is the applied voltage (V), I is the applied current (A), t_E is the electrolysis time (h) and V_s is the volume of dye solution (m^3). As per the results, the minimum electrical energy consumption was 15.60 k W h m^{-3} at 170 A m^{-2} current density. At higher current densities, the energy consumption was found to be increased and it may be attributed to the increased hydrogen and oxygen evolution reaction (Table 1).

CONCLUSIONS

In the present work the electrochemical degradation of C.I Vat Green 1 was carried out using graphite carbon as anode and cathode, in the optimal operating conditions (current density 170 A m^{-2}, NaCl concentration 25 g L^{-1} and at room temperature). Increasing the initial *p*H will lead to corresponding decrease in the degradation efficiency of C.I Vat Green 1 dye. The effect of the hypochlorite at *p*H 9 can lead the degradation efficiency of the dye. Cyclic voltammograms of C.I. Vat Green 1 shows irreversible electrochemical natures. UV-Vis, MS spectral studies and FT-IR studies confirmed that the proposed electrochemical degradation process is an effective method for the degradation of C.I Vat Green 1 dye.

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Table 1: The electrical energy consumed during decolourisation of C.I. Vat Green 1 dye solution (50 ppm, w/v)

Current (A)	Current density (A m^{-2})	Electrolysis time (min.)	Energy Consumption (k Wh m^{-3})
0.10	085	240	07.20
0.20	170	240	15.60
0.30	255	240	31.20
0.40	340	240	44.00
0.50	425	240	58.00

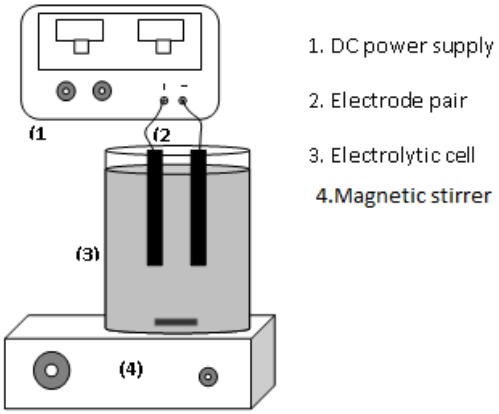

Figure 1: Schematic diagram of the experimental setup for the electrochemical degradation

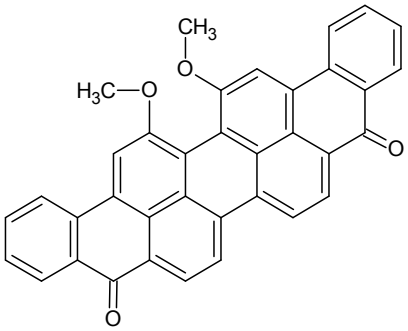

Figure 2 : Molecular structures of the vat dye, C.I. Vat Green 1.

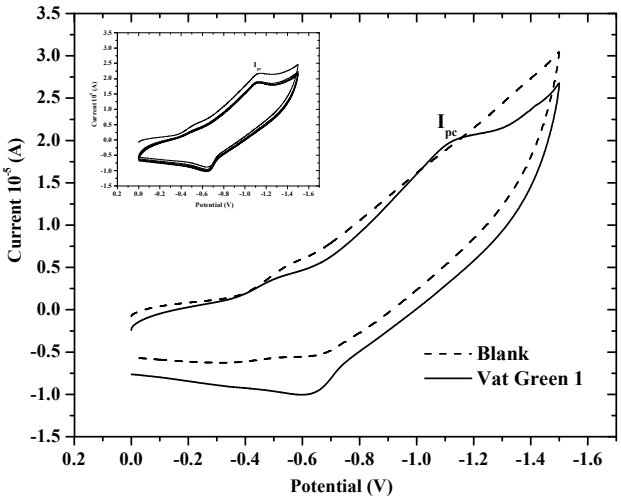

Figure 3 : Cyclic voltammograms of dye C.I. Vat Green 1 on glassy carbon. Scan rate: 100 Vs^{-1}; *p*H: 9; concentration of dye: 50 ppm (w/v). Inset plot: multiple scan (segments: 10).

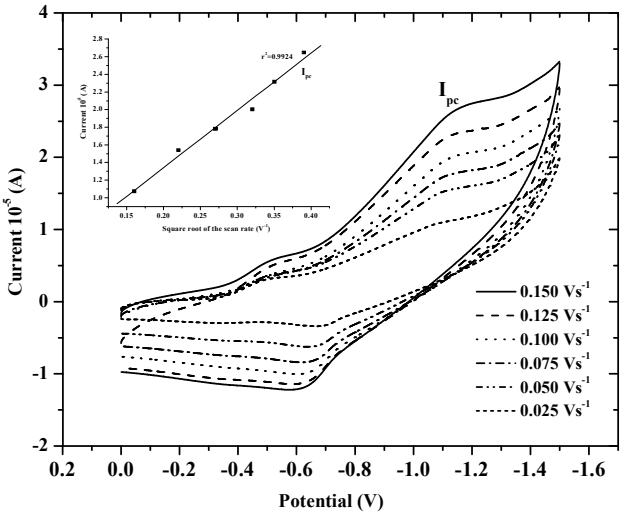

Figure 4: Cyclic voltammograms of dye C.I. Vat Green 1 on glassy carbon at different scan rate. *p*H: 9; concentration of dye: 50 ppm (w/v). Inset plots: linear relationship between the current and $v^{1/2}$.

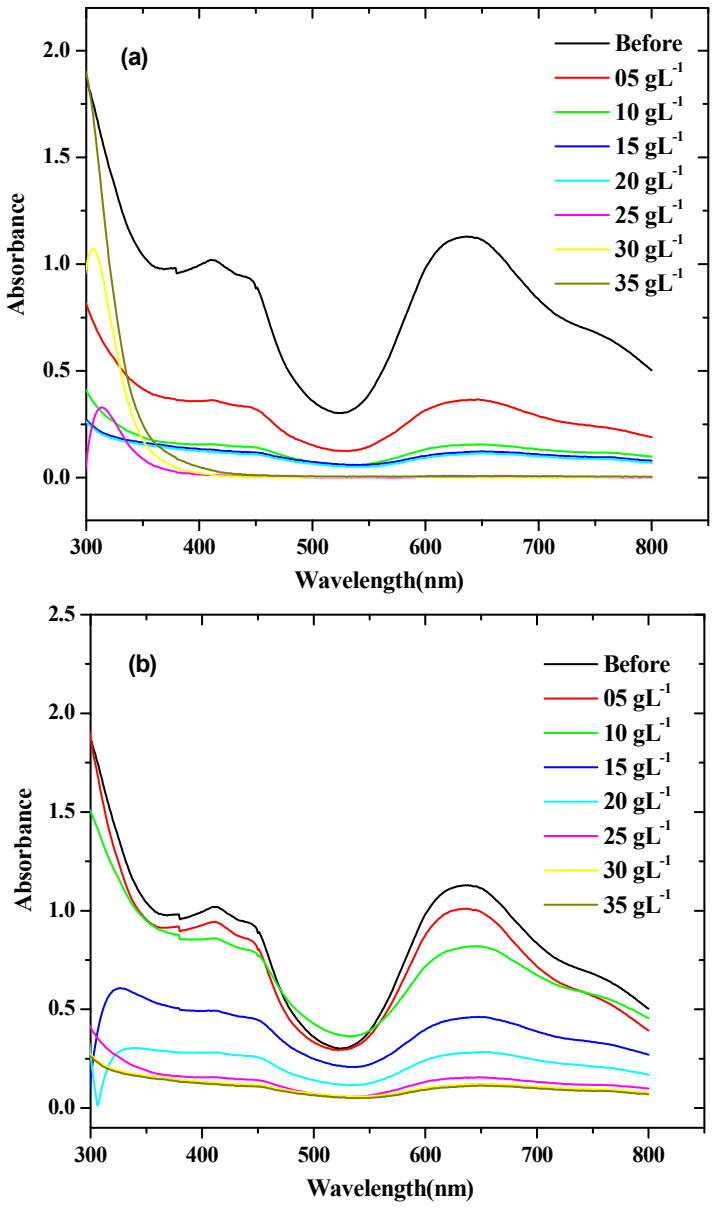

Figure 5 : Effect of supporting electrolytes (a) NaCl and (b) Na_2SO_4 on decolourisation efficiency of the dye C.I. Vat Green 1. Electrolysis condition: concentration of the dye solution: 50 ppm (w/v); electrodes: graphite carbon; pH: 9; current density: 170 A m^{-2}; time: 240 min.

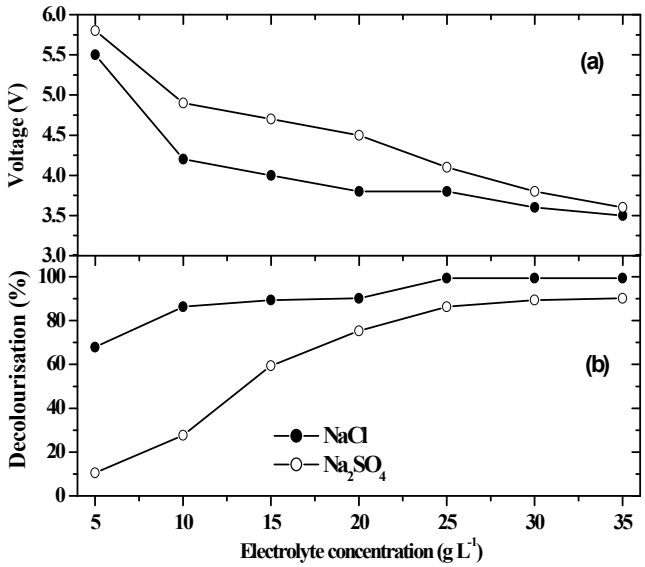

Figure 6 : Effect of supporting electrolytes on (a) voltage variation (b) decolourisation efficiency. Electrolysis condition: concentration of the dye solution: 50 ppm (w/v); electrodes: graphite carbon; current density: 170 A m^{-2}; time: 240 min.

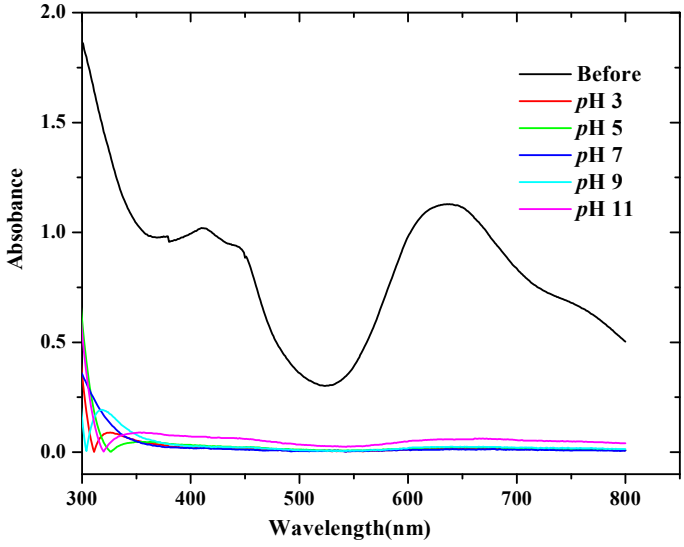

Figure 7 : Absorption spectra for C.I .Vat green 1 dye solution before and after electrolysis at different *p*H. Electrolysis condition: concentration of the dye solution: 50 ppm (w/v); electrodes: graphite carbon; NaCl: 25 g L^{-1}; current density: 170 A m^{-2}; time: 240 min.

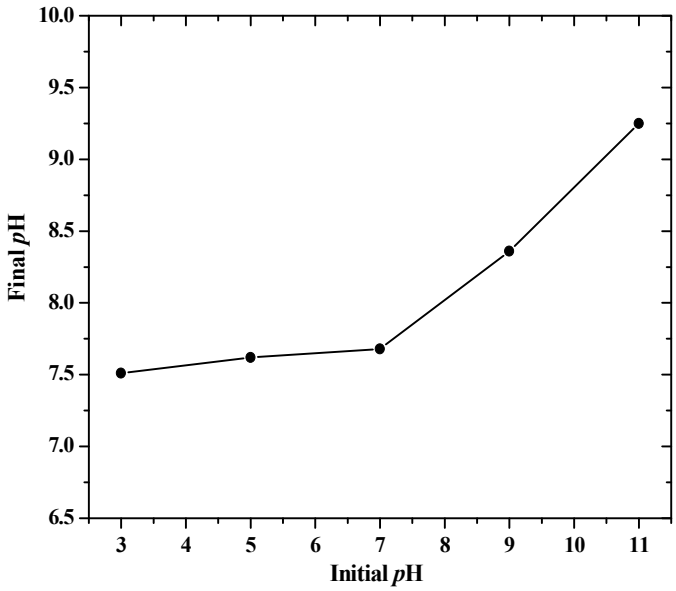

Figure 8 : Effect of *p*H on electrochemical degradation of C.I. Vat Green 1 dye solution before and after electrolysis at different *p*H Electrolysis condition: concentration of the dye solution: 50 ppm (w/v); electrodes: graphite carbon; NaCl: 25 g L^{-1}; current density: 170 A m^{-2}; time: 240 min.

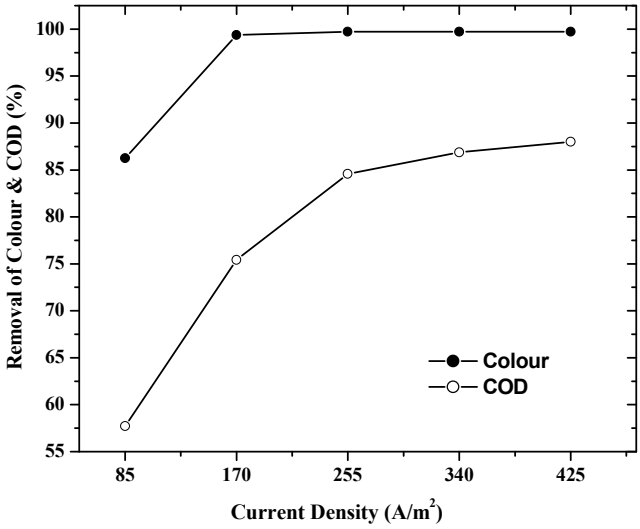

Figure 9 : Effect of current densities on decolourisation and COD removal efficiencies of dye C.I. Vat Green 1. Electrolysis condition: concentration of the dye solution: 50 ppm (w/v); *p*H: 9; NaCl: 25 g L^{-1}; current density 170 A m^{-2}; time: 240 min.

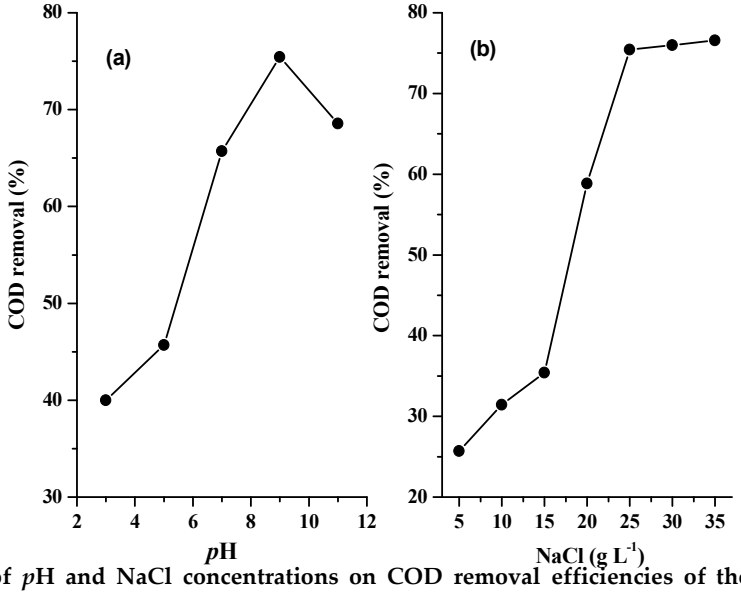

Figure 10 : Effect of *p*H and NaCl concentrations on COD removal efficiencies of the dye C.I. Vat Green 1. Electrolysis condition: concentration of the dye solution: 50 ppm (w/v); electrodes: graphite carbon; current density: 170 A m^{-2}; time: 240 min.

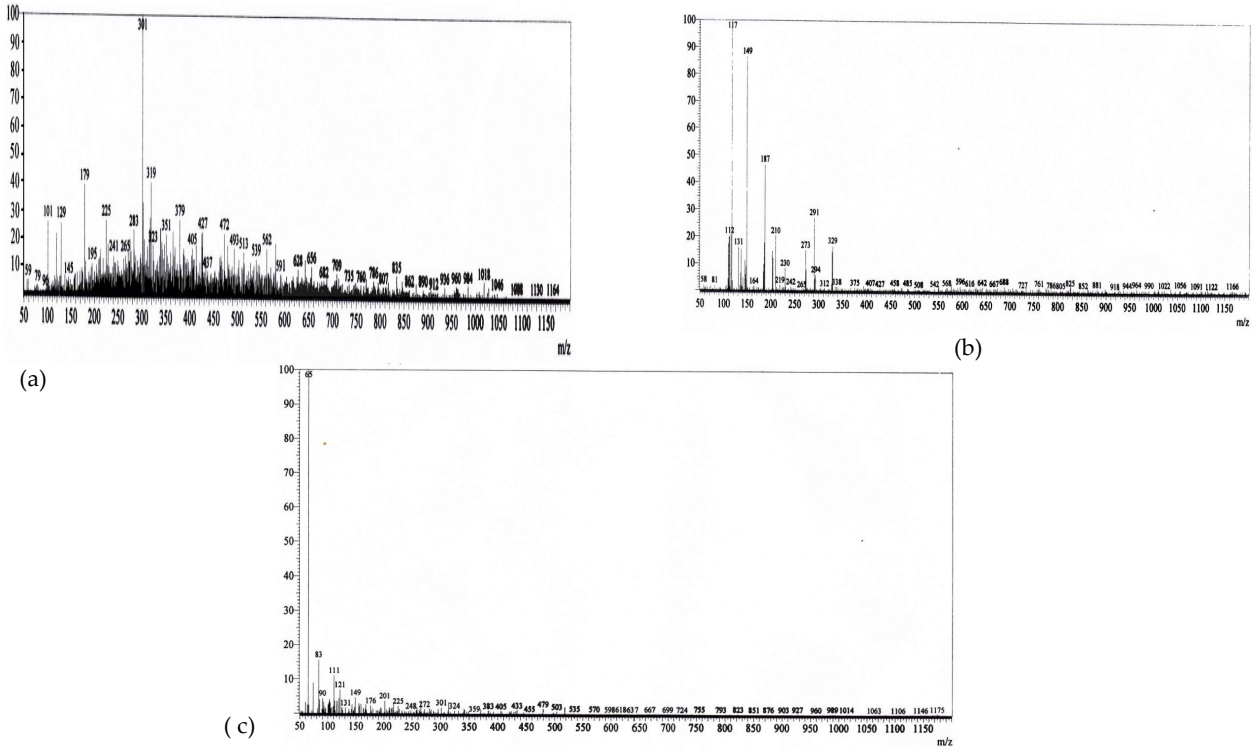

Figure 11 : Mass spectrum of C.I. Vat Green 1: (a) before electrolysis, (b) dye residue (c) clear filtrate after complete electrolysis. Electrolysis condition: concentration of the dye solution: 50 ppm (w/v); electrodes: graphite carbon; *p*H: 9; current density 170 A m^{-2}; time: 240 min.

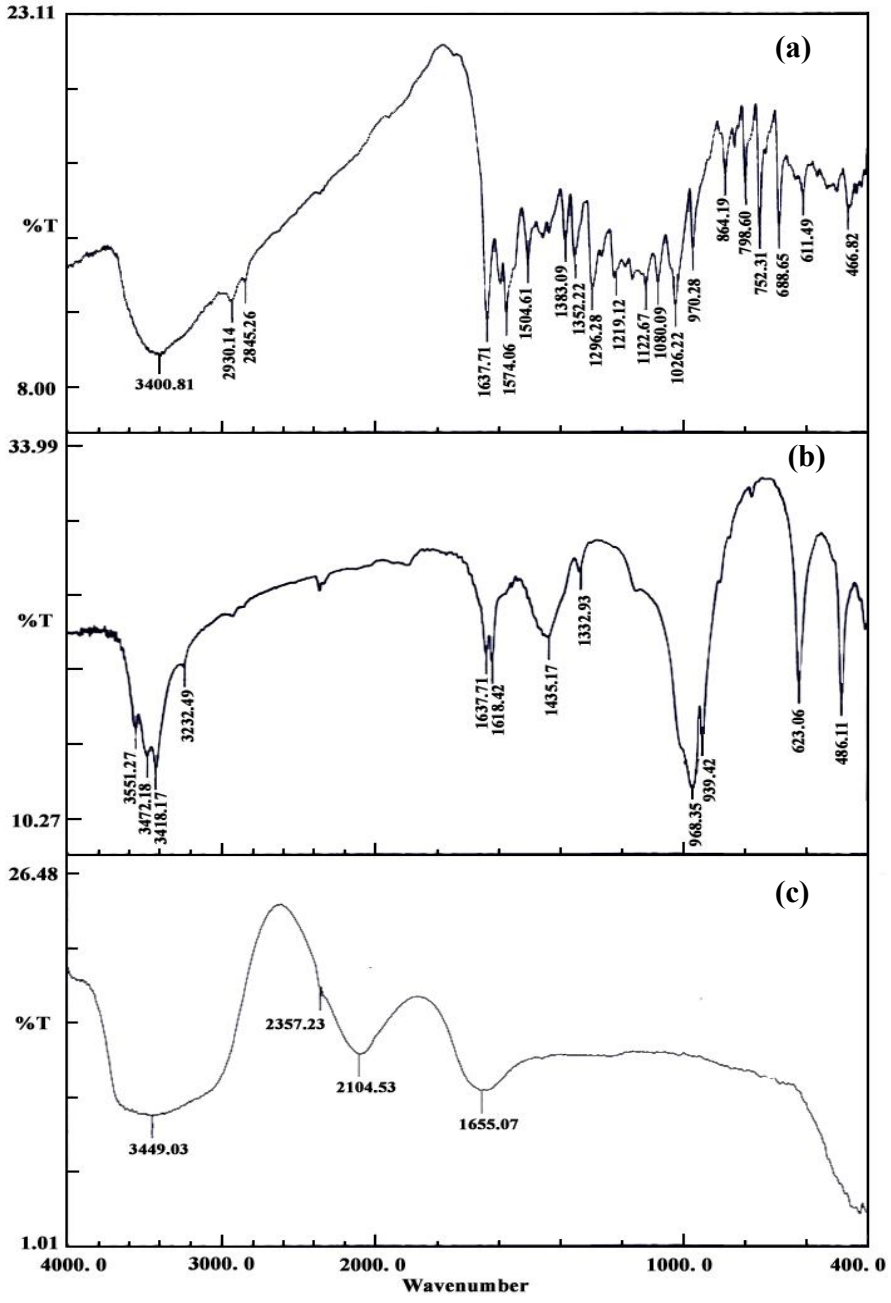

Figure 12: FT–IR spectrum of Vat Green 1 (a) before electrolysis (b) intermediate dye molecule (c) after electrolysis, Electrolysis condition: concentration of the dye solution: 50 ppm (w/v); electrodes: graphite carbon; *p*H: 9; current density: 170 A m^{-2}; time: 240 min.

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RESEARCH ARTICLE

Comparative Studies on Dye Decolourisation and Bioremediation of Paper Mill Effluent using *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

R.Anu alias Meena[1]* and K.Kalimuthu[2]

[1]Department of Environmental Sciences,Bharathiyar University, Coimbatore-641046. TamilNadu,India.

[2]Department of Botany, Govt Arts College, Coimbatore-641018.TamilNadu,India.

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***Address for correspondence**
R.Anu alias Meena
Department of Environmental Sciences,
Bharathiyar University, Coimbatore-641046.
TamilNadu,India.
E.Mail : anupremanand@gmail.com

ABSTRACT

The large scale production of a variety of chemical compound has global deterioration of environmental quality. The growth of paper and pulp industry is an index of the social, cultural, technical, industrial and economic development of nation. The effluent from the pulp mill is nearly black due to high concentration of lignins in a strong alkaline medium. Depletion of dissolved oxygen is the most serious effect of paper waste. Revival of cultures has been made for two organisms' viz., *Pseudomonas aeruginosa* and *Staphylococcus aureus*. PH, Total Suspened Solids, Total Dissolved Solids, Biochemical oxygen demand and Chemical oxygen demand like parameters are determined. From the above study, it is inferred that effluent treated with *P.aeruginosa* was found to degrade the components of a faster rate when compared to the effluent treated with *S.aureus.*

Key words: Paper mill effluent, Bioremediation, Dye decolourisation, *Pseudomonas aeruginosa* , *Staphylococcus aureus.*

INTRODUCTION

The problem of environmental pollution is so grave due to industrialization. The volume of water consumed by paper industry is next to agriculture, being highly water intensive it becomes a major polluter of the water resources. The high water usage between 20,000 and 60,000 gallons per ton of product, released enormous amounts of waste water [1]. Anyway, the average water used in the pulp and paper mills in India was still 200-259 m^3per ton of paper production [2]. A large amount of lignocellulosic parts of plants and the chemicals used during manufacturing in the

pulp and paper industry are regarded as polluting industries, because they release huge amounts of waste into the environment [3, 4, 5]. About 175m³ of waste water generated by the pulp and paper industry per ton of paper produced [6]. In addition to this, the pollution load per day contributed by the Indian pulp and paper industry is equivalent to that contributed by 7.12 million people [7]. The pulp and paper industry released pollutants of various types such as suspended solids because of fibres, foam producing materials colours due to lignin and its derivatives, BOD, COD, talc, rosin inorganic and organic sulphur compounds, phenolic derivatives.

Discharge of waste water prior to treatment will create serious water pollution problems resulting in toxicity of aquatic life, deterioration in water quality and increase in the cost of waste water treatment. Pulping and bleaching processes released effluents are amongst the most polluter and are characterized by parameters unique to these waters such as color and organic halides [8, 9, 10]. Lignin and its derivatives and polymerized tannins are highly responsible for colour in pulp and paper mills, which are mostly discharged from the pulping, bleaching and recovery sections [11, 12, 13, 14]. In the past, colour was not considered to be a major problem, being classified as a non-conventional pollutant. Recently, its importance has been realized that the coloured effluent released from pulp and paper mills is not only a aesthetic problem, but also has other ramifications, since here is a marked change in the algal and aquatic plant productivity caused by the reduced penetration of solar radiation [15]. Colour due to lignin compounds persist in water body for long distance [16]. Now a days, coloured water is thought to be mutagenic, carcinogenic and toxic [17, 18]. Colour not only poses aesthetic problems but also contributes to BOD [19].

Pulp and Paper industry discharges water which usually contains halogenated organic materials that can pose environmental problems [20, 21]. During bleaching, chromophoric and highly oxidized, polymeric lignin derivatives are formed that give rise to a dark colourisation in the waste water. Pulp and Paper effluents have been found to contain more than 200-300 different organic compounds [22, 23, 24, 25]. All these are toxic to aquatic organisms and resistant to microbial degradation resulting in a decrease of the ecological value of natural systems surrounding the paper mill. The presence of various pollutants produced during Pulp and Paper manufacturing necessitates the need for waste water pretreatment prior to discharge [26]. Several physical and chemical processes like coagulation, adsorption, chemical oxidation and membrane filtration techniques are expensive and none of these have been found to be industrially applicable and commercially viable due to one or other drawback [27, 28].

Compared to physical and chemical methods of effluent treatment, biological methods have the advantage of being cost effective and in addition to colour removal, they can also reduce both the Biological Oxygen Demand (BOD) and Chemical oxygen Demand (COD) of the waste water [29]. However, the most widely used biological treatment system is activated sludge process; it is also ineffective in total removal of colour and toxicity of the effluents [30]. Greater decolourisation and reduction in COD, lignin content and total phenols was observed at PH 5.5 [31] . Combinations of anaerobic and aerobic treatment processes are found to be efficient in the removal of soluble biodegradable organic pollutants [32]. Colour was primarily removed by adsorption with little depolymerisation of lignin derivatives [15].

Bioremediation refers to site restoration through the removal of organic contaminants by micro organisms. It is a process that exploits the natural metabolic versatility of micro organisms to degrade environmental contamintants. Of all the methods investigated, bioremediation specifically holds promise in solving environmental problems in a cost effective way [25].

MATERIALS AND METHODS

The Pulp and Paper mill effluent was collected in sterilized brown bottles from the main outlet of the effluent from a Paper Mill, Erode. After collecting the samples, the effluent was brought to the laboratory and stored at 4° C in a refrigerator until analysis for its physico- chemical properties and further use.

For the revival of cultures, 50 ml of nutrient broth was prepared and studied. The broth was then distributed equally in 2 different sterile conical flasks. Two organisms' viz., *Pseudomonas aeruginosa* (MTCC 424) and *Staphylococcus aureus* (MTCC 96) was inoculated one into each flask. They were left for incubation at 37°C for 24 hrs. Serial dilution up to 10^{-7} was made with the sample. Nutrient agar media and Petriplates were kept under aseptic conditions. After solidification, 0.1 ml from sample from each dilution (10^{-4} to 10^{-7}) was taken and spread uniformly on the media using L-rod. The plates were then incubated at 37° C for 12- 24 hours and colony morphology and staining techniques carried out. Parameters like P^H, TSS, TDS, BOD and COD were determined for both the raw effluent and treated effluent as described by APHA [33]. Colour of the raw and treated effluent was measured by simple visual observation method. Sigmastat (3.1) version is used for statistical analysis and One way Anova is used as a statistical method to find out the standard deviation and level of significance.

RESULTS AND DISCUSSION

The effluent under study was found to be complex in nature. The effluent was checked for various physico-chemical factors such as P^H, TSS, TDS, BOD and COD. Both the raw effluent and the treated effluent were subjected to above treatments and recorded. Data represented in Table shows the physico- chemical parameters of raw effluent and effluent treated with both, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Dye decolourisation of the effluent with both *Pseudomonas aeruginosa* and *Staphylococcus aureus* was carried out and after incubation for 5 days the amount of dye decolourised was noted. The effluent inoculated with *Pseudomonas aeruginosa* showed good amount of reduction when compared to the one that was inoculated with *Staphylococcus aureus.*

Pulp and Paper mill effluent treated with *Rhizopus oryzae* was less effective in terms of colour removal and COD reduction but proved to be the most promising in reducing toxicity [34]. Dye decolourisation of the effluent after inoculation of the organisms with 15 days of incubation was noted and it was seen that the effluent inoculated *Pseudomonas aeruginosa* showed higher percentage of decolourization, i.e., high amount of dye was reduced when compared with the raw effluent. The effluent inoculated with Staphylococcus *aureus* showed an increase in the percentage of dye decolourized. The similar observations of dye decolourisation of the effluents were observed in *Pleurotus florida* and *Trametes hirsute* by [35].

The treatment of Pulp and Paper mill effluent with two basidiomyctes fungi (*Merulius aureus* syn.Phelbia sp. and *Fusarim sambucinum* Fuckel MTCC3788) resulted in the reduction of colour, lignin and COD of the effluent within first 24 hr of the treatment which was also characterized by a steep decline in the P^H of the effluent [36].From the results obtained, it is inferred that the bioremediation was found to be effective in the treatment of the effluent. The P^H was found to be decreased in the treated effluent and considerable reduction in TDS, TSS, BOD and COD values were also noted in the treated effluent. To confirm that, *Pseudomonas aeruginosa* was capable of reducing kraft mill effluent colour by 26-54 % or more under aerobic conditions [37]. Bourbannais and Paice [38] tested *Bacillus cereus* and two strains of *Pseudomonas aeruginosa* for the decolourisation of bleach kraft effluent.

From the above analysis, it is inferred that effluent treated with *Pseudomonas aeruginosa* was found to degrade the components at a faster rate when compared to the effluent treated with *Staphylococcus aureus*. There was considerable difference in the recorded values of both raw and treated effluent, with *Pseudomonas* treated effluent showing high percentage of difference.

The dye decolourisation studies also infer the same. It was found that the effluent treated with *Pseudomonas aeruginosa* showed a high percentage of dye decolourisation when compared to the effluent treated with *Staphylococcus aureus*. From the above results obtained, it was found that the organism *Pseudomonas aeruginosa* has a good potent to degrade the organic and inorganic matter in the effluent and so serves as a useful tool in biodegradation of effluents.

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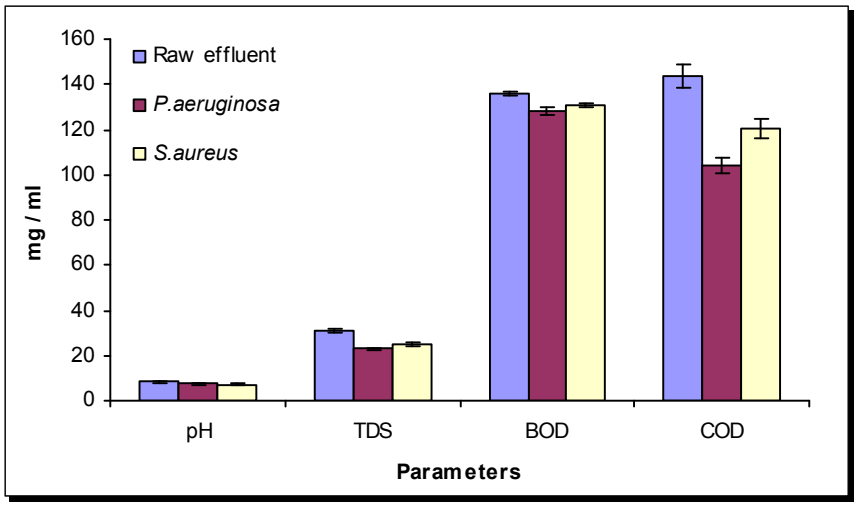

Figure 1: **Physico- chemical parameters of raw effluent and effluent treated with *Pseudomonas aeruginosa* and *Staphylococcus aureus***

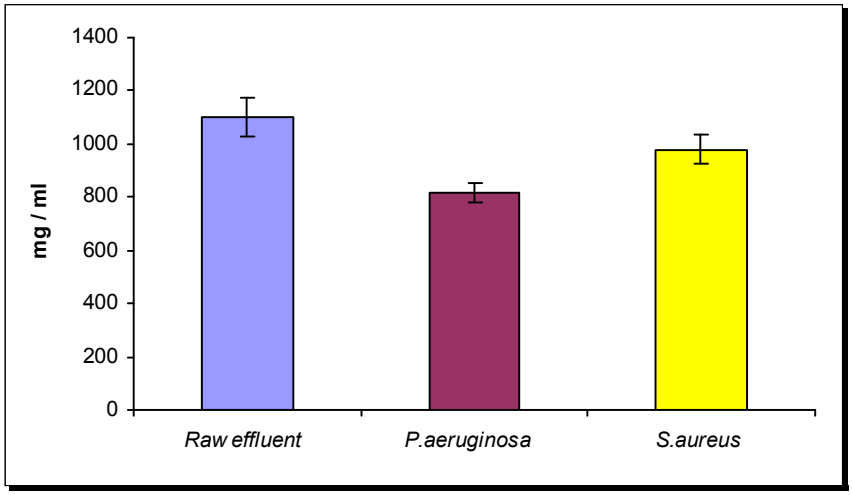

Figure 2: **Total dissolved solids observed in the raw and treated effluents**

Table 1: Physico- chemical parameters of raw effluent and effluent treated with *Pseudomonas aeruginosa* and *Staphylococcus aureus*

S.No	Parameters	Raw effluent mg/l (G1)	treated with *P.aeruginosa* mg/l (G2)	Treated with *S.aureus* mg/l (G3)
1.	PH	8.2 ± 0.26a**	7.4 ± 0.12b*	7.2 ± 0.15c**
2.	TSS	1100 ± 74.36**	819 ± 36.29**	979 ± 53.16**
3.	TDS	31 ± 0.94**	23 ± 0.62*	25 ± 0.55**
4.	BOD	136 ± 1.23**	128.4 ± 1.73*	131 ± 1.04**
5.	COD	144 ± 5.28**	104 ± 3.15**	120.7 ± 4.27**

Values are mean ± SD if three samples in each group
Group comparison: a – G1 vs G2; b – G2 – vs G3; c – G3 vs G1
Statistical significance: * - $p<0.05$; ** - $p<0.01$

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REVIEW ARTICLE

Scientific Validation of *Madhuca indica* J.F.Gmel.-an Overview.

Priyadarshini.S[1] *,Rathika.M[1], Selvasundaram.R[2], A.Vinoth[2] ,S.Arun[2] and S.Vijikumar[3]

[1] Department of Biotechnology,Bharathidasan University,Trichirappalli, TamilNadu,India.
[2] Department of Biotechnology, Thanthai Hans Roever College. Perambalur - 621 212, Tamilnadu.India
[3] Director,Tamil Nadu Scientific Research Organisation,Arimalam-622201,TamilNadu,India.

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***Address for correspondence**
Priyadarshini.S,
Department of of Biotechnology,
Bharathidasan University,Trichirappalli,TamilNadu,India.
E.Mail: priyadarshinibiot@gmail.com

ABSTRACT

Madhuca indica J. F. Gmel. (Sapotaceae) Synonym: *Madhuca longifolia* (J.Konig) J.F.Macbr.) plant is economically important because of the role it plays in yielding country liquor, edible succulent corollas and oil from the seeds. Flower is traditionally considered as tonic, both nutritional and cooling and also in treatment of helminthes, acute and chronic tonsilitis, pharyngitis as well as bronchitis. The bark is good remedy for itch, swelling, fracture and snake bite poisoning, internally employed in diabetes mellitus. Its flowers are prepared to relieve coughs and heart-trouble, while its fruits are given in cases of blood diseases. Previous phytochemical studies on *Madhuca indica* included characterization of sapogenins, triterpenoids, steroids, saponins, flavonoids and glycosides. The in-vitro digestibility of mahua seed flour after treatment with isopropanol was found to be 81%. Polyacrylamide gel electrophoresis showed five bands with different relative mobilities and they contained both high and low molecular weight protein fractions. Methanolic extract of *Madhuca longifolia* to be a potential antidiabetic agent, lending scientific support for its use in folk medicine. Graded doses of both aqueous and alcoholic extract of *M.longifolia* (4.0 to 64.0 mg/kg, *i.m.* X 3 days) produced dose dependent analgesic effect in all the three nociceptive methods carried out either in rats or mice. The heart wood extract of *Madhuca longifolia* was investigated for anticonvulsant activity and the possible mechanism of action involved in this activity.

Key words: *Madhuca indica* J. F. Gmel., tonsilitis, pharyngitis, phytochemical, anticonvulsant activity.

INTRODUCTION

Madhuca indica J. F. Gmel. (English Name: Indian Butter Tree, Family: Sapotaceae, Synonym: *Madhuca longifolia* (J.Konig) J.F.Macbr.) locally known as 'Mahua or Maul' in Bangladesh. It is also known as Mahua (Hindi), Madhuka (Sanskrit), Mahwa (Marathi), Illuppai(Tamil), Yappa (Telugu). It is a large, shady deciduous tree both wild and cultivated, found in different parts of Bangladesh. It is also distributed more or less throughout India especially in the states of Jharkhand, Uttar Pradesh, Bihar, Madhya Pradesh, Kerala, Gujarat and Orissa[5].

The tree reaching 20 meters in height with a spreading crown. This plant is economically important because of the role it plays in yielding country liquor, edible succulent corollas and oil from the seeds. Kernels of green- color egg size fruits of Mahua (*Madhuca indica Gmel.*) contained 55-65 % of soft yellow oil that is widely used locally for cooking and tallow. Mahua flowers are regarded as cooling tonic and demulcent and are used in coughs, colds and bronchitis .*Madhuca indica* flower is traditionally considered as tonic, both nutritional and cooling and also in treatment of helminthes, acute and chronic tonsilitis, pharyngitis as well as bronchitis. The medicinal properties attributed to this plant are stimulant, demulcent, laxative and astringent. The bark is good remedy for itch, swelling, fracture and snake bite poisoning, internally employed in diabetes mellitus. Its bark is used to cure leprosy and wounds. Its flowers are prepared to relieve coughs and heart-trouble while its fruits are given in cases of blood diseases [6].

This plant each part has medicinal value have to use in traditional knowledge, like leaves, bark, flower, stem, root, fruit.The fruit contains high amount of sugars. But this plant name under the Indian Union of Concervation Nature (IUCN) list .so, we have to conserve the plant in this stage. In this review study on derived from various research paper knowledge based on the ethnomedicinal uses.

REVIEW STUDY

Evaluation of Leaf Extract

Madhuca longifolia leaves are expectorant and also used for chronic bronchitis and Cushing's disease.The distilled juice of the flower is considered a tonic, both nutritional and cooling and also in treatment of helminthes, acute and tonsillitis, as well as bronchitis. The leaves are applied as a poultice to relieve eczema. The aerial parts are used for treatment of inflammation. The bark is a good remedy for itch, swelling, fractures and snake-bite poisoning, internally employed in diabetes mellitus. Previous phytochemical studies on *Madhuca indica* included characterization of sapogenins, triterpenoids, steroids, saponins, flavonoids and glycosides[10].

To explore the cytotoxic activity of acetone and ethanol extracts from the leaves of *Madhuca longifolia* against Ehrlich Ascites Carcinoma (EAC) cell lines using different Invitro cytotoxic assay at 200μg/ml. Results found that both extracts exhibited significant cytotoxic activity, but higher cytotoxic activity was found in ethanol extract [1]. The hydroethanolic extract of the leaves of *Madhuca longifolia* was administered orally to alloxan–induced diabetic rats and investigated for its antidiabetic properties. Administration of 150 mg/kg and 300 mg/kg extract (once a day, for thirty consecutive days) significantly lowered blood glucose levels. Furthermore, the activity of glucose-6-phosphate dehydrogenase, serum triglycerides, HDL and total cholesterol levels showed marked improvement which indicates that the hydroethanolic extract possesses antihyperglycemic activity [2].

The leaf extract of *Madhuca indica*, when administered to mice at dose levels of 50, 100, 250, and 500 mg/kg body weight demonstrated dose-dependent and significant reductions in serum glucose levels at the three higher doses. Serum glucose levels were reduced by 22.2, 25.8, and 36.3%, respectively, at doses of 100, 250, and 500 mg extract/kg body weight. In comparison, the standard antihyperglycemic drug, glibenclamide, reduced serum glucose levels by 35.9%, which is approximately equivalent to that obtained with the highest dose of *Madhuca indica* leaf extract [7].

Evaluation of Bark Extract

Another one study was carried out to assess the antihyperglycemic effects of methanolic extract of Madhuca longifolia bark in normal, glucose loaded and streptozotocin induced diabetic rats. All three animal groups were administered the methanolic extract of Madhuca longifolia at a dose of 100 and 200 mg/kg body weight (p.o.) and the standard drug glibenclamide at a dose of 500 μg/kg. Serum glucose level was determined on days 0, 7, 14 and 21 of treatment. The extract exhibited a dose dependent hypoglycemic activity in all three animal models as compared with the standard antidiabetic agent glibenclamide. The hypoglycemia produced by the extract may be due to the increased glucose uptake at the tissue level and/or an increase in pancreatic β-cell function, or due to inhibition of intestinal glucose absorption. The study indicated the methanolic extract of Madhuca longifolia to be a potential antidiabetic agent, lending scientific support for its use in folk medicine [3,11].

The ethanol extract of the dried bark of *Madhuca* was investigated for its possible antinociceptive and antidiarrhoeal activities in animal models. The extract produced significant (P<0.001) writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250 and 500 mg/kg of body weight comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The extract also showed antidiarrhoeal activity on castor oil induced diarrhoea in mice, it increased mean latent period and decreased the frequency of defecation significantly (P<0.001, P<0.01) at the oral dose of 500 mg/kg body weight comparable to the standard drug Loperamide at the dose of 50 mg/kg of body weight. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation [5].

The antioxidant and hepatoprotective effects of 70% ethanolic extract of bark of *Madhuca longifolia* (koenig) (EEMLK) were studied. The antioxidant property of 70% EEMLK was tested by using reducing power and free radical (hydroxyl and superoxide) scavenging models (*invitro*); the *in-vivo* antioxidant activity was assessed by determining the tissue GSH and lipid peroxidation levels. The 70% EEMLK at the doses of 200 and 400 mg/kg and silymarin 100mg/kg were administered to the CCl4 challenged rats. The effect of 70% EEMLK and silymarin on wet liver weight, liver volume, serum biomarkers like SGOT, SGPT, ALP, direct and total Bilirubin were measured in CCl4 induced hepatotoxicity in rats. Similarly hepatic tissues were subjected to histopathological observations. The test extract has shown dose dependant antioxidant activity in all the models. The altered biochemical and physical markers by the CCl4 induced rats brought back to near normal level by the 70% ethanolic extract of MLK in a dose dependent manner [13].

Evaluation of Seeds Extract

Seeds of mahua fruits contain 16·9 and 51·5% protein and oil, respectively. Fatty acid composition of oil revealed the presence of oleic acid (46·3%) and linoleic acid (17·9%) as the major unsaturated and palmitic acid (17·8%) and stearic acid (14·0%) as major saturated fatty acids. The defatted mahua seed meal contains 29·4% protein and 9·8% saponins which are toxic at this level. However, the levels of saponins could be reduced by treatment with isopropanol. The defatted flour showed good oil absorption and emulsification properties. The solubility of protein was high at both acidic and alkaline pH with a minimum at 4·0. The in-vitro digestibility of mahua seed flour after treatment with isopropanol was found to be 81%. Polyacrylamide gel electrophoresis showed five bands with different relative mobilities and they contained both high and low molecular weight protein fractions. Detoxified mahua seed flour appears to be a good source of protein for food and feed products [4].

Evaluation of Flower Extract

Aqueous, ether, acetone and methanolic extracts of fresh flowers and fruits of *Madhuca indica* were used for phytochemical screening. In the present study eight principle bioactive compounds were investigated. Out of all,

carbohydrates, proteins, flavanoids and tannins were found to be positive in all four extracts while alkaloids were positive in aqueous and ether extract, saponins were positive only in methanolic extract. Sterol is positive in ether, acetone and methanol extract where as lipid was found to be positive in aqueous, ether and methanolic extracts. Flowers of *Madhuca indica* showed antibacterial activity against *Bacillus subtilis* and *Klebsiella pneumonia*. Aqueous and methanolic extract of flowers were used. Aqueous extract showed more activity than methanolic extract for both bacteria [4].

Pharmacognostic evaluation and preliminary phytochemical investigation on flower of *Madhuca indica* J. F. Gmel was carried out using macroscopy, fluorescence analysis, extractive value, successive solvent extraction and preliminary phytochemical screening by chemical tests. The ethanol and methanol extract of the flowers of *Madhuca indica* J. F. Gmel (Sapotaceae) was investigated for its possible anthelmintic activity in *Pheretima posthuma (*Indian Earth Worm). Three concentrations (20, 40 and 60 mg/ml) of each extract were studied in a bioassay, which involved the determination of time of paralysis and time of death of the worms. Both extracts showed significant anthelmintic activity but methanolic extract demonstrated the best anthelmintic activity in both the parameters. Mebendazole was included in the assay as standard reference drug[5].

The research study was undertaken to evaluate the effect of *Madhuca latifolia* on immunomodulatory activity that comprises of screening to identify the activity of ethanolic extract of *Madhuca latifolia* on humoral and cell mediated immunity (specific immune response). Experiments were conducted in vivo in Swiss albino mice. Madhuca latifolia ethanolic extract was found to enhance humoral immune response on 7th day by 13 % as compared to the standard control cyclophosphamide that exhibited 54% humoral immune response, where as cell mediated immune response was observed with an enhancement in the values (20.27%) in comparison with control cyclosporine (37.63%)[8]. To screen the analgesic effect of aqueous and alcoholic extract of *Madhuka longifolia* and elucidate its probable mechanism of action. The analgesic effect was screened through tail flick, hot plate and chemical writhing methods. The probable mechanism of action through opioid receptors was elucidated by *i.m.* administration of naloxone - specific antagonists 30 min before the last dose of aqueous or alcoholic extract of *M.longifolia*. Graded doses of both aqueous and alcoholic extract of *M.longifolia* (4.0 to 64.0 mg/kg, *i.m.* X 3 days) produced dose dependent analgesic effect in all the three nociceptive methods carried out either in rats or mice. The analgesic effect exhibited by both the extracts was not antagonized by naloxone in rats only. The analgesic effect exhibited by both aqueous and alcoholic extracts does not mediate through opioid receptors [9].

Oral administration of methanolic extract of *Madhuca indica* showed significant regression of the diabetic state and restored the deranged serum glucose, cholesterol, triglycerides and HDL parameters in STZ and STZ-NIC induced diabetes. This observation may indicate that *Madhuca indica* enhances insulin release from destroyed pancreatic β-cells. Diabetic rats treated with methanolic extract of *Madhuca indica* significantly decreased the serum cholesterol and triglyceride level since insulin is a major hormone regulating lipid metabolism. *Madhuca indica* facilitated stimulation of insulin secretion in STZ induced rats will help to overcome lipid metabolism abnormalities and increase of glucose uptake in the presence of insulin suggests the possibility of increased binding of insulin to receptors in STZ-NIC induced rats. From the above results, it can be conclude that methanolic extract of *Madhuca indica* has significantly decreased the elevated blood glucose, cholesterol, TG's and increased the HDL levels in diabetic rats, and hence *Madhuca indica* may be effectively active against Diabetes mellitus[7].

Evaluation of Wood Extract

Heart wood of *Madhuca longifolia* J.F. Macbr. (Sapotaceae) is used in traditional medicine of India to treat seizure. The heart wood extract of *Madhuca longifolia* was investigated for anticonvulsant activity and the possible mechanism of action involved in this activity. The anticonvulsant activity of the methanol extract of heart wood of *Madhuca longifolia* was assessed in pentylenetetrazole (PTZ) – induced convulsion in mice with benzodiazepine as standard drug. Mechanistic studies were conducted using both flumazenil, a GABAA-benzodiazepine receptor complex site

antagonist, and naloxone a non-specific opioid receptor antagonist. *Madhuca longifolia* at the dose of 400 mg/kg prolonged the onset time of seizure and decreased the duration of seizures compared to saline group ($p < 0.001$). Flumazenil and naloxone suppressed anticonvulsant effects of *Madhuca longifolia.* It appears that *Madhuca longifolia* may be useful for the treatment of absence seizures and that these effects may be related to its effect on GABAergic and opioid systems. This result suggests that *Madhuca longifolia* possesses biological active constituents which may contribute to the anti-convulsant activity of *Madhuca longifolia.* This supports the ethnomedical claims of the use of plant in the management of epilepsy [12].

CONCLUSION

From this study we suggested that *Madhuca longifolia* has long history of medicinal valued for its treatment of helminthes,acute and chronic tonsillitis, pharyngitis, bronchitis, relieve eczema itch, swelling, fracture ,snake bite poisoning, diabetes mellitus and cure leprosy and wounds.Advance scientific research will urgently need for make healthy india through this beneficial ethnomedicinal plant.

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RESEARCH ARTICLE

In vitro Regeneration of Multiple Shoots from Cotyledon Explants of *Trichosanthes anguina* L. (Snake Gourd).

A.Ambethkar[1], C. Uma Maheswari[1], Shantha Margaret[1], Ganeshan Sivanandhan[2] and Natesan Selvaraj[1]*

[1]Plant Tissue Culture Laboratory, Department of Botany, Periyar E. V. R College, Tiruchirappalli – 620 023, Tamil Nadu, India.

[2]Department of Biotechnology, Bharathidasan University, Tiruchirappalli – 620 024, Tamil Nadu, India

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***Address for correspondence**
Natesan Selvaraj,
Plant Tissue Culture Laboratory, Department of Botany,
Periyar E. V. R College, Tiruchirappalli – 620 023, Tamil Nadu, India.
E.Mail : selvasarayu@gmail.com.

ABSTRACT

Successful regeneration of multiple shoots via direct organogenesis was established in *Trichosanthes anguina* L. (Snake Gourd) from cotyledon explants derived from 7 day-old *in vitro* seedlings. About 82.5% cotyledon explants produced 18 shoots/explant on MS medium containing BA (0.6 mg/l), Zeatin (0.4 mg/l) and AdS (20 mg/l). Multiple shoots (2-3 cm. length) excised from cotyledons were elongated in MS medium fortified with GA_3 (0.5 mg/l).Elongated shoots were rooted in MS medium supplemented with IBA (0. 6 mg/l). Rooted plants were subsequently hardened and acclimatized. This protocol yielded higher number of shoots in a culture period of 130-140 days.

Key words: *Cotyledon, Cucurbitaceae, Direct regeneration, Plant growth regulators, Trichosanthes anguina*L.
Abbreviations: MS- Murashige and Skoog Medium; BA – Benzylaminopurine; AdS – Adenine suplhate; NAA - 1-Naphthaleneacetic acid; IBA - Indole-3-butyric acid

INTRODUCTION

Trichosanthes anguina L. is an important vegetable crop of the *Cucurbitaceae* family. Over 20 species are recorded in India of which two species namely *T.anguina* and *T.dioica* are cultivated as vegetables. Fruits of *T.anguina* are usually consumed as vegetable due to their good nutritional value constituted with proteins, carbohydrates, fibre, fat,

vitamins A and E and minerals such as potassium, phosphorous, sodium, zinc and magnesium in trace. The whole plant is richly constituted with a series of secondary metabolites such as flavonoids, carotenoids, phenolic acids (Sandhya *et al.,* 2010). The nutritional value makes the plant pharmacologically and therapeutically as one of the important plants of the family. It has a prominent place in alternative systems of Ayurvedic and Siddha medicines due to its various pharmacological activities like antidiabetic, hepatoprotective, cytotoxis, antiinflammatory and larvicidal effects (Longman, 2002).

Many important crop plants are propagated vegetatively and grown as clones, but in *Trichosanthes,* the propagation is usually done by stem or root cuttings to maintain favored female plants, and parthenocarpy can be induced in some cases with growth regulators. However, for the development of transgenic plants, *in vitro* regeration is required at least for the initial establishment of the plant irrespective of the mode of propagation (Mohiuddin *et al*., 1997). Conventional crosses and transfer of desirable traits, especially disease resistance from wild species have not been accessible so far in *T. anguina.* Tissue culture techniques and genetic engineering are therefore of special value for this genus. In vitro shoot/root cultures could provide an alternative to field-grown plants for the production of therapeutically valuable compounds. In vitro cultures can thus be applied to produce plants for secondary metabolites accumulation as well as commercial micropropagation (Sivanandhan *et al.,* 2011). *In vitro* cloning is achieved through enhanced axillary shoot, adventitious bud formation and regeneration from callus or somatic cell embryogenesis. Direct organogenesis is less time consuming with less abnormally observed in the regenerants (Mohiuddin *et al*., 1997). The present investigation was therefore aimed to establish an efficient and reproducible protocol for *in vitro* regeneration of multiple shoots from de-embryonated cotyledon explants *T. anguina* by direct organogenesis.

MATERIALS AND METHODS

Explant source and preparation

Mature seeds of *T. anguina* (cv. UKB200561) were procured from Mahyco (Maharastra Hybrid Seed Company, Maharashtra, India) for the present study. After removal of seed coat, the seeds (decoated) were surface sterilized by washing in 3-5 drops of Teepol® (5.25% sodium hypochlorite; commercial bleach solution) and 2% (w/v) Bavistin for 5-minutes each and then rinsed with distilled water for 3-4 times. Then, the rinsed seeds were surface sterilized in 0.1% mercuric chloride for 2 min. and rinsed again with sterile water 3-4 times and left to air dry in a sterile environment. Sterilized seeds were then inoculated on Murashige Skoog (MS) basal medium (Murashige and Skoog, 1962) with 30g/l sucrose and 8g/l agar. The culture tubes were kept in dark for 3 days for germination and later on transferred to light (18/6 photoperiod) with the intensity of 50 μmol $m^{-2}s^{-1}$ provided by cool white tube light. The pH of the medium was adjusted to 5.8 prior to autoclaving with a pressure of 1.05 Kg cm^2 at 121 ºC. Cotyledons were separated from 7 day-old seedlings and the embryo axis was removed. The proximal half of de-embryonated cotyledon explants (0.5 cm) was used as explants. The cultures were maintained at 25±2 ºC.

Multiple shoot bud induction and proliferation

The cotyledon explants were aseptically inoculated vertically with the proximal end facing upward and cut end/distal end touching the medium in culture tubes (one cotyledon explant per tube) containing 20 ml of MS medium, 3% (w/v) sucrose, 0.8% agar fortified with different concentrations of BA (0.2-1.0 mg/l) and Zeatin (0.2-1.0 mg/l) with AdS (20 mg/l) (Shoot Bud Induction Medium; SBIM). The cultures were maintained at 25±2 ºC for 16 h photo period with light intensity of 50 μmol $m^{-2}s^{-1}$ provided by cool white tube light. After two weeks of culture in the shoot bud induction medium (SBIM), numerous nodules like protuberances formed at the proximal region of the cotyledon explants. Then, the explants were subjected to two subcultures at an interval of four weeks each in MS medium supplemented with the optimal concentration of BA (0.6 mg/l) and Zeatin (0.4 mg/l) fortified with the AdS (20 mg/l) (Shoot Proliferation Medium; SPM). The cultures were maintained as mentioned above.

Shoot elongation, rooting and acclimatization

The proliferated multiple shoots with an average length of 2-3 cm were carefully excised from the explants and they were transferred to shoot elongation medium (SEM) containing GA_3 (0.2-0.8 mg/l). After 4 weeks, shoots longer than 6-8 cm were selected and transferred to root induction medium (RIM) containing NAA (0.2-1.0 mg/l) and IBA (0.2-1.0mg/l) individually and in combination. The cultures were maintained as mentioned above. After 3-weeks, the rooted plants were transferred to paper cups containing soil, sand, vermiculite (1:1:1, v/v/v) and were supplied with sterile water daily. After 3-4 weeks, the plants were transplanted to earthen pots, grown in shade house and finally to field.

Statistical analysis

In all the experiments each single treatment consisted of 20 replicates and all experiments were repeated three times. Data on multiple shoot regeneration, elongation and rooting were statistically analyzed using one-way analysis of variance (SPSS, version 10) and comparison of means were performed at the 5% level of significance using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Multiple shoot bud induction and proliferation

Multiple shoot bud induction ability of cotyledon explants (proximal half) derived from 7-day old *in vitro* seedlings of Snake Gourd was studied on MS medium supplemented with two cytokinins, BA and Zeatin individually and in combinations (Table 1). After two weeks of culture, the proximal region of the cotyledon explants produced numerous nodule like protruberances (Fig.1b). In individual BA (0.6 mg/l) or Zeatin (0.4 mg/l) treatment, 75.3% and 73.4% of cotyledon explants responded and produced 15 shoots/explant and 16 shoots/expant respectively. However, the highest percentage (82.5%) of explants response and production of higher number of multiple shoots (18 shoots/explant) were recorded with the combined addition of BA (0.6 mg/l) and Zeatin (0.4 mg/l) along with AdS (20 mg/l) (Table 1). Presence of BA either alone or in combination with other cytokinins proved essential for direct as well as indirect shoot regeneration in cucurbits (Chaturvedi *et al.,* 2010). Gambley and Dodd (1990) observed that the cytokinin induced activation of totipotent cells present in the proximal end of the cotyledons of cucumber. Previous studies reported that *in vitro* regeneration of plants from cotyledons of matured seeds and young seedlings has received considerable attention (Chaturvedi and Bhatnagar, 2001). Studies on the morphogenetic potential of *Cucurbitaceae* in *in vitro* have demonstrated that cucurbit tissue could regenerate via organogenesis with the application of cytokinins (Chaturvedi *et al.,* 2010). Punja *et al*., (1990) and Selvaraj *et al.,* (2006) used Zeatin to induce adventitious shoots from cucumber cotyledon explants. Earlier reports recorded the formation of multiple shoots in cucurbits in *in vitro* by using different explants either with cytokinins alone (BA/ Kn) (Mahzabin, *et al.,* (2008) or combination of cytokinins (BA/Kn) with auxins (NAA, IAA and IBA).

BA (90.45%) and Kn (50.37%) induced multiple shoots from shoot tip explants of Pumpkin with the production of 14 and 6 shoots/explant respectively (Mahzabin *et al.,* 2008). In Pumpkin and Ash gourd the nodal explants produced considerable percentage (85% and 90%) of multiple shoots (Haque, *et al.,* 2008). Devendra *et al*., (2008) observed the maximum percentage of (76.6%) multiple shoot regeneration from BA and NAA combination in shoot tip explants of *T. cucumerina*. Multiple shoots were induced from shoot tip explants of two genotypes of Pointed Gourd by BA alone and in combination with NAA (Malek 2007). Khatun *et al.,* (2010) reported that the combination of BA and NAA resulted in the maximum shoot induction from the nodal explants of water melon. In melon cotyledonary node explants, the combined effect of BA and IAA resulted in higher frequency of multiple shoots regeneration (Zhang *et al.,* 2011). Nodal explants of Pointed Gourd produced multiple shoot in BA, Kn and NAA combination (Komal 2011).

In the present study, maximum shoot induction (82.5%) was observed from proximal half of the cotyledon explants in BA, Zeatin and AdS combination. The regulatory role of AdS was first demonstrated by Skoog and Tsui (1948) and Miller and Skoog (1953). The effect of AdS on induction of multiple shoots in cotyledons, stem and callus was due to its promotive effect and the released organic nitrogen sources from AdS were easily taken up by cells (Thom *et al.,* 1981). In our study, AdS at 20 mg/l was very effective along with BA and Zeatin in inducing multiple shoots from the proximal half of the cotyledon explants. Muruganandam *et al.* (2002) also reported that BA (1.0 mg/l) and AdS (15 mg/l) combination promoted multiple shoot induction from cotyledon explants of *Cucumis melo*. After two transfers in SBIM at an interval of four weeks, maximum number of shoots (18 shoots/explant) was recorded. The shoot buds (adventitious buds) emerged from the proximal region of cotyledon explants after two weeks of culture and then after two successive transfers at 4 weeks interval in the same medium, the explants produced maximum proliferation of multiple shoots. This is the first report on the high frequency of multiple shoot production from cotyledon explants of *T. anguina* cv. UKB 2005612.

Shoot elongation, rooting and acclimatization

Emerging shoots (2-3 cm long) were harvested and transferred to SEM. After 4 weeks, the shoots attained an average height of 8.5 cm with 95% of response in MS medium containing GA_3 (0.5 mg/l) (Table 2; Fig.1e). GA_3 appears to be effective in shoot elongation in cucumber (Selvaraj *et al.*, 2006). The elongated shoots appeared normal and healthy. The rooting of elongated shoots varied with concentration of auxins present in RIM (Table 3). NAA responded poorly with the production of weak and slender roots when compared to IBA at 0.6 mg/l. Maximum frequency (97.3%) of rooting, number (28 roots/shoot) and mean length of the roots (4.5 cm) were observed in RIM after 3 weeks of culture (Table 3; Fig.1f). This was in accordance with earlier reports of Devendra *et al.,* (2008) in *T.cucumerina var. cucumerina;* Khatun *et al.,* (2010) in water melon, Komal (2011) in *T. cucumerina.* The present study revealed that IBA was an effective plant growth regulator on root induction ability from the elongated shoots. Agarwal *et al.,* (2004) obtained similar results in *Momordica charantia* and Hoque *et al.,* (2007) in *Momordica* dioica, while NAA was also used for rooting in Squash (Kathiravan *et al.,* 2006). Efficient rooting was achieved in *Trichosanthes dioica* in combination of IBA (0.5 mg/l) and NAA (2.0 mg/l) (Kumar *et al.,* 2003). The rooted shoots were subsequently hardened. After 3-4 weeks of hardening, the plantlets were transferred to field with 90% survival.

CONCLUSION

In the present study, maximum number of shoots (18 shoots/explant) was regenerated directly on cotyledon explants derived from 7 day-old in vitro grown seedlings. Combination of BA (0.6 mg/l) and Zeatin (0.4 mg/l) favoured multiple shoot bud induction and AdS (20 mg/l) enhanced multiple shoot proliferation. Elongation of shoots was achieved in GA_3 (0.5 mg/l) and elongated shoots were rooted in IBA (0.6 mg/l). This simple yet repeatable protocol would be useful for mass multiplication of *Trichosanthes anguina* in a shorter period and for genetic transformation studies.

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Table 1: Effect of cytokinins (BA, Zeatin) with AdS (20 mg/l) on multiple shoot induction from cotyledon explants derived from 7 day-old *in vitro* seedlings of *T. anguina*.

Plant growth regulators (mg/l)		Percentage of explants responding	Number of shoots per explant
BA		-	-
0.2		56.02±0.64^{c}	10.52±0.07^{d}
0.4		62.45±0.60^{bb}	14.47±0.07^{b}
0.6		**75.31±0.48^{a}**	**15.35±0.11^{a}**
0.8		61.66±0.44^{ba}	13.47±0.05^{c}
1.0		52.40±0.44^{d}	9.51±0.10^{e}
Zn		-	-
0.2		58.57±0.07^{c}	11.59±0.06^{a}
0.4		**73.46±0.07^{a}**	**16.60±0.07^{a}**
0.6		60.42±0.09^{b}	13.50±0.07^{b}
0.8		52.55±0.07^{d}	10.49±0.06^{b}
1.0		45.55±0.08^{e}	8.54±0.07^{c}
BA	**Zn**	-	-
0.6	0.2	63.36±0.27^{ca}	9.45±0.05^{d}
0.6	**0.4**	**82.52±0.41^{a}**	**18.32±0.03^{a}**
0.6	0.6	66.55±0.37^{b}	13.43±0.09^{b}
0.6	0.8	62.42±0.37^{cb}	10.46±0.05^{c}
0.6	1.0	58.12±0.34^{d}	8.54±0.04^{e}

Data presented as means ± SE from 20 explants for each treatment and repeated three times. Means followed by same letters within a column are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level

Table 2: Effect of GA_3 on elongation of regenerated shoots from cotyledon explants of *T. anguina* cultured on MS medium.

Concentration of GA_3 (mg/l)	Percentage of explants responding	Mean shoot length (cm)
0.2	68.12±0.27^{e}	4.220.09^{e}
0.3	75.47±0.38^{c}	5.28±0.10^{d}
0.4	83.04±0.23^{b}	6.59±0.18^{b}
0.5	**95.73±0.34^{a}**	**8.50±0.14^{a}**
0.6	72.97±0.29^{d}	5.59±0.21^{e}
0.8	56.16±0.50^{f}	3.43±0.13^{f}

Data presented as means ± SE from 20 explants for each treatment and repeated three times. Means followed by same letters within a column are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level

Table 3: Effect of auxins on root induction from the elongated shoots derived from cotyledon explants of *T. anguina* on MS medium

Plant growth regulators (mg/l)		Percentage of rooting response	Mean number of roots per shoot	Mean root length (cm)
NAA	IBA			
0.2	-	38.65±0.20^{c}	4.34±0.10^{c}	1.15±0.54^{ca}
0.4	-	47.57±0.16^{b}	5.52±0.15^{b}	2.13±0.07^{b}
0.6	-	72.76±0.23^{a}	8.51±0.16^{a}	3.09±0.08^{a}
0.8	-	32.55±0.28^{d}	3.59±0.17^{d}	1.02±0.05^{cc}
1.0	-	21.62±0.16^{e}	2.20±0.09^{e}	1.04±0.07^{cb}
-	0.2	62.52±0.18^{d}	11.72±0.14^{e}	2.11±0.07^{ca}
-	0.4	74.66±0.29^{c}	20.54±0.17^{c}	3.13±0.08^{b}
-	**0.6**	**97.30±0.20^{a}**	**28.76±0.16^{a}**	**4.48±0.11^{a}**
-	0.8	76.66±0.20^{b}	23.12±0.16^{b}	2.07±0.05^{cb}
-	1.0	51.64±0.20^{e}	18.56±0.50^{f}	1.04±0.06^{d}

Data presented as means ± SE from 20 explants for each treatment and repeated three times. Means followed by same letters within a column are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level

Fig. 1

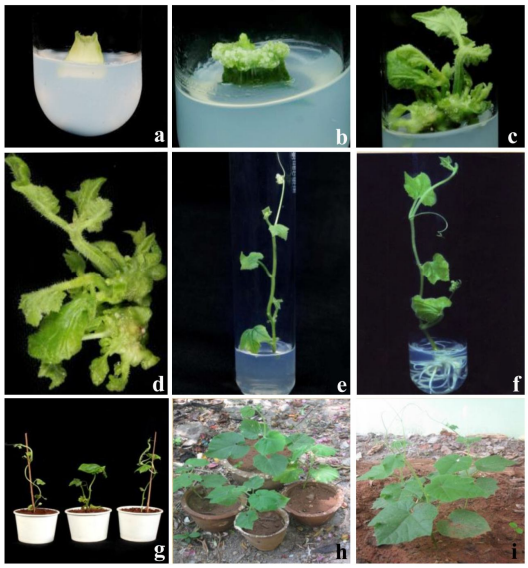

Fig. 1: Direct regeneration of *T. anguina* from cotyledon explants. (a) Cotyledon explant on MS medium. Induction (b) and proliferation of shoots (c, d) on MS medium containing BA (0.6 mg/l), Zeatin (0.4 mg/l) and AdS (20 mg/l). (e) Elongated shoot on MS medium containing GA_3 (0.5 mg/l). (f) A rooted shoot on MS medium fortified with IBA (0.6 mg/l). (g) Hardened plants in paper cups. (h) Hardened plants in earthen pot i) Field performance of plants in shade.

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REVIEW ARTICLE

In vitro propagation of *Centella asiatica* L.– an Overview

Suganya.A[1] , A.Reginamary[1], S.Sindhu[1] , C.Balusami[2]* and S.Vijikumar[3]

[1] Department of Biotechnology,Bharathidasan University,Trichirappalli, TamilNadu,India.
[2] Dept.of Livestock Production Management, College of Veterianry and Animal Sciences ,Pookode, Wayanad-673 576,Kerala,India.
[3] Director,Tamil Nadu Scientific Research Organisation,Arimalam-622201,TamilNadu,India.

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***Address for correspondence**
Dr.C.Balusami
Assistant Professor (SS),
Deparment of Livestock Production Management,
College of Veterianry and Animal sciences,Pookode,Wayanad-673 576,Kerala,India.
Contact No:09446510821
E.Mail : balusamic@gmail.com

ABSTRACT

Centella asiatica L. is valuable medicinal plants and sources of medicinal and many other pharmaceutical products. The conventional propagation method is the principal means of propagation and takes a long time for multiplication because of a low rate of fruit set, and/or poor germination and also sometimes clonal uniformity is not maintained through seeds. The plants used in the phyto-pharmaceutical preparations are obtained mainly from the natural growing areas. With the increase in the demand for the crude drugs, the plants are being overexploited, threatening the survival of many rare species. Also, many medicinal plant species are disappearing at an alarming rate due to rapid agricultural and urban development, uncontrolled deforestation, and indiscriminate collection. Advanced biotechnological methods of culturing plant cells and tissues should provide new means for conserving and rapidly propagating valuable *Centella asiatica* L. medicinal plants. The purpose of the present review is to focus the application of tissue culture technology for in vitro propagation via somatic embryogenesis and organogenesis and the cell suspension culture with suitable examples reported earlier. An overview of in -vitro propagation studies on *Centella asiatica* L. medicinal plants and related species is presented.

Key words: *Centella asiatica*L. deforestation, biotechnological, organogenesis, phyto-pharmaceutical.

INTRODUCTION

India is one of the twelve mega diversity countries of the world with a rich diversity of biotic resources 2]. Out of 34 hotspots recognized, India has two major hotspots - the Eastern Himalayas and the Western Ghats. India harbours about 47 000 species of plants of which 17 000 are angiosperms[2]. Centella asiatica L.(Fig.1) is important herbal medicinalplant used for various applications [9] and used in Indian Ayurvedic medicine as a nerve tonic [15]. Utilization of Centella asiatica (Centella) have been known for many years in treating all kind of diseases such as gastrointestinal disease, gastric ulcer, asthma, wound healing and eczema [4] .The use of Centella in food and beverages has increased over the years basically due to its health benefits such as antioxidant [8] ; [20]; [19]; [14], as anti-inflammatory[5], wound healing[12] memory enhancing property [17]; 15] and many others.

The potential of Centella as an alternative natural antioxidant especially of plant origin and its protection against age-related changes in brain antioxidant defense system, have notably increased in recent years[17]. Free radicals have been claimed to play an important role in ageing process and capable of damaging many cellular components. These changes will affect the brain as it is particularly vulnerable to oxidative damage; as such many studies on its neuroprotection activity have been reported.

The Centella asiatica L. belongs to the family Apiaceae or Umbelliferae, a small creeping perennial herbal plant that flourishes in wet areas of Malaysia, Indonesia, India, and other parts of Asia including China. The herb is also known as pegaga in Malaysia, Indian pennywort and Gotu Kola in Europe and America, mandookaparni in India, pegagan or kaki kuda in Indonesia, Luei Gong Gen or Tung Chain in China[18].There are several types of Centella asiatica that can be found in Malaysia such as Pegaga Cina or Nyonya, Pegaga Daun Lebar, Pegaga Salad and Pegaga Renek [1]; [2]. Centella asiatica is used in Indian Ayurvedic medicine and in herbal medicine in Malaysia and China, and other part of Asia for hundreds of years[4].

Besides being used as a traditional and alternative medicine, Centella is commonly used in these countries as vegetables and drinks as in tea or juice[9].Centella asiatica (also known as vallarai in tamil and Hydrocotyle asiatica) is a perennial, herbaceous creeper with kidney-shaped leaves, found in India, Sri Lanka, Madagascar,South Africa, Australia, China, and Japan. Centella prefers to grow in shady, moist, or marshy areas. Centella contains several active constituents, of which the most important are the triterpenoid saponins, including asiaticoside, centelloside, madecassoside, and asiatic acid. In addition, Centella contains other components, including volatile oils, flavonoids, tannins, phytosterols, amino acids, and sugars.

Theraputic uses

Improves the mind's receptive capacity, Capable of improving a person's memory power,Used in the treatment of skin diseases such as leprosy,Exhibits significant wound healing activity in normal as well as delayed healing models, Used in the commercial production of face creams and anti wrinkle creams.

Used in various mental disorders, it is regarded as one of the best psychotropic drugs.Six week treatments in patients of anxiety neurosis reduced anxiety levels and showed improvement in the mental fatigue rate and immediate memory span.Significant improvement in both general ability and behavioral pattern was obtained in 30 mentally retarded children within 12 weeks. Significant improvement was observed in the intelligence quotient in children treated ith a dose of 0.5g / day of the powder for one year. In mentally retarded children, a significant increase in the general mental ability, over all general adjustment and mental concentration at the end of 6 months was observed.Constituents Contributing to use the plant contains many types of active ingredients like are Asiaticosides,Brahmoside,Thiamine,Riboflavin,Pyridoxine,Vitamin K,Asparate,Glutamate,Alanine,Histidine,Calcium and sodium.

The active ingredient saponins affect collagen inhibiting energy and vitality.Due to high concentration of thiamine, riboflavin and pyridoxine, this assists inconverting carbohydrates into glucose as well as for normal nervous system functioning. The asiaticoside content dissolves the waxy coating of the leprosy bacteria allowing the immune system to destroy the bacteria.Phytopreparations containing terpenes, flavonoids and coumarins have a good therapeutic potential as venoactive drugs and are widely used in thetreatment of symptomatology associated with chronic venous insufficiency of the lower limbs.

This illness causes severe injuries in the venous wall, metabolic alterations of the vascular and perivascular tissue and morphological and functional changes in the microcirculatory system.1,2 It is reported that an abnormally increased capillary permeability, due to damage to the endothelium, causes tissue edema and the leakage of plasma proteins such as fibrinogen.Fibrinogen, turned into fibrin, induces severe alterations in the metabolism and in the structure of the perivascular connective tissue, which may lead to the onset of venous ulcers and to a worsening of the illness.An increase in resting blood flow, an impairment of the venoarteriolar reflex, and a decreased lymphatic clearance are the main functional changes in microcirculatory system.

Plant description

Morphological features

CLASS : Dicotyledenae
SUB –CLASS : Polypetalae
SERIES : Calyciflorae
ORDER : Umbellales
FAMILY : Umbelliferae (Apiaceae)
GENUS : Centella
SPECIES :asiatica

Vernacular Names

ENGLISH : Indian penny wort
TAMIL : Vallarai,Yoshanavalli,Chandaki,Pindeeri
SANSKRIT : Mandookaparni
HINDI : Brahmi
BENGALI : Tholkari
ARABI : Artniya –e- hindi
MALAYALAM : Kudakam

Methods for Conservation

Conventionally, *in situ* conservation allows evolution to continue within the area of natural occurrence, and *ex situ* conservation provides a better degree of protection to germplasm compared to *in situ* conservation. However, both *ex situ* and *in situ* conservation are complementary and should not be viewed as alternatives. *Ex situ* conservation includes germplasm banks, common garden archives, seed banks, DNA banks and techniques involving tissue culture, cryopreservation; incorporation of disease, pest and stress tolerance traits through genetic transformation and ecological restoration of rare plant species and their populations.

***In vitro* propagation**

In vitro propagation or micro propagation is a viable alternative for species which are difficult to regenerate by conventional methods; where populations have decreased due to over exploitation by destructive harvesting and can effectively be used to meet the growing demand for clonally uniform elite plants. When species have been over collected by hobbyists for medicine, food or fragrance, *in vitro* propagation can provide an alternate source of plants and alleviate pressures on wild populations.

The present review summarizes the protocols reported for propagation and conservation of *centella asiatica* and the feasibility of their large-scale propagation.A protocol is described for rapid and large-scale *in vitro* clonal propagation of the valuable medicinal herb *Centella asiatica* (L.) by enhanced axillary bud proliferation in nodal segments isolated from mature plants. Although bud break was dependent on BA supply, the synergistic combination of 22.2 μM BA and 2.68 μM NAA induced the optimum frequency (91%) of shoot formation as well as shoot number (4 to 5 shoots per node). Sub culturing of nodal segments harvested from the *in vitro* derived axenic shoots on the multiplication medium enabled continuous production of healthy shoots with similar frequency. MS medium supplemented with 6.7 μM BA and 2.88 μM IAA was found most suitable for shoot elongation. Rooting was highest (90%) on full-strength MS medium containing 2.46 μM IBA. Micropropagated plants established in garden soil were uniform and identical to the donor plant with respect to growth characteristics. This micropropagation procedure could be useful for raising a stock of genetically homogenous plant material for field cultivation [11].

Hossain *et al*., (2000) [7] reported that Stem node explants of naturally grown *Centella asiatica* L. were used for *in vitro* regeneration of multiple shoots. Various combinations of BAP and NAA in different concentrations were used in the regeneration of multiple shoots and a concentration of 1.0 mg/1 BAP and 0.5 mg/1 NAA was found superior in the optimum production of multiple shoots. Among the three auxins used in different concentrations, 0.2 mg/1 IBA was found effective in the production of roots. Eighty per cent of the plantlets produced from *in vitro* culture method survived in the *ex vitro* condition. Measurement of chlorophyll *a*, chlorophyll *b*, and carotenoid and soluble protein content of fresh leaf of both *in vitro* regenerated and natural plant represented non significant differences.

Hong *et al*. (2007)[6] studied that the nodular stem segment of *Centella asiatica* L. was taken as the explant to induce fascicular bud. They observed that MT culture medium with 0.5mg/L thidiazuron(TDZ) added was effective to induce the bud formation,and the shooting rate was 87.6%.As for the induction of rooting,MT culture medium with 0.2mg/L indoleacetic acid(IAA) added was optimal,the rooting rate being 100% and the plant growing well.The survival rate of the transplanted test-tube plantlet arrived 90%. They concluded that MT culture medium with TDZ added can increase the shooting rate of nodular stem segment of Centella asiatica and MT culture medium with 0.2mg/L IAA can increase the rooting rate of the bud, indicating that this method can serve as the preliminary in-vitro propagation system for *Centella asiatica* L.

Nguyen H. L and Nguyen T .T. A (2010)[13] found that the petiole explants of centella plants (*Centella asiatica* L. Urban) were cultured on Murashige and Skoog (MS) solid medium containing 20 g/L sucrose, supplemented with 1.0 mg/L benzylaminopurine and 1.0 mg/L naphthaleneacetic acid for callus production. To establish a cell suspension culture, 2 g of fresh callus was cultured in 50 mL of the same medium but without solid agent at a 100 rpm agitation speed. Every 2 g of culture was subcultured in fresh MS liquid medium for maintenance. After 24 days of culture at a 120 rpm agitation speed, the centella cell biomass reached a maximum of 9.03 g/50 mL on the same MS medium with 30 g/L sucrose and a 3 g inoculum size. A high performance liquid chromatography analysis showed that asiaticoside content in 24-day old suspension cultured cells (45.35 mg/g dry weight) was significantly higher (4.5 fold) than that of *in planta* leaves (10.55 mg/g dry weight)[13]. A protocol has been established for clonal propagation of *Centella asiatica* (L.) Urban, a medicinal plant recommended for wound healing. A shoot induction rate of 8.9 shoots/responding explant was obtained when shoot tip explants were cultured in liquid Murashige and Skoog (MS) medium with 4.54 μM thidiazuron (TDZ) for 15 days before being transferred to semisolid MS without plant growth

regulator for 10 weeks. Rooting spontaneously occurred. The regenerated plants were successfully transplanted to soil under greenhouse conditions. The regenerates showed uniformity in triterpenoids and ploidy level with their mother plant.

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CONCLUSION

In Asian countries, especially in India this *Centella asiatica* plant is well found. Nowadays the plant has been using Indian system of medicines. Over exploitation of *Centella asiatica* today this plant has noted endangered species by IUCN (International Union for Conservation Nature) .So invitro propagation techniques and its procedures were widely used for large quantity production . Also our review study is find out further research is need for this area.

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Fig.1: Centella asiatica L.

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RESEARCH ARTICLE

Drinking Water Quality Assessment of Bhaskar Rao Kunta Watershed, Dhamaracherla Mandal, Nalgonda Disrict, Andhrapradesh, India.

Karee.Srinivasa Reddy[1]* ,K.S.Reddy[2] , B.V.Rao[3] and Umarfarooque Momin[4]

[1] Research Associate, CRIDA, Santoshnagar, Hyderabad-500059,Andhrapradesh ,India.
[2] Principal Scientist, CRIDA, Santoshnagar, Hyderabad -500059, Andhrapradesh ,India.
[3]Professor, CWR, IST, JNTUH University, Hyderabad-500085, Andhrapradesh ,India.
[4] Senior Research Fellow, CRIDA, Santoshnagar, Hyderabad -500059, Andhrapradesh ,India.

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***Address for correspondence**
Karee.Srinivasa Reddy,
Research Associate, CRIDA,
Santoshnagar, Hyderabad -500059,
Andhrapradesh ,India.
E.Mail: oshoyogi@yahoo.com
Contact No.: 09866673218

ABSTRACT

This paper presents study, semi-arid region of Bhaskar Rao kunta watershed is under drinking water quality aspect, where 20 groundwater samples were collected from open and bore wells during pre and post monsoon seasons and analyzed physicochemical parameters (pH, EC, TDS, TH, Ca^{+2}, Mg^{+2}, Na^{+}, K^{+}, CO^{-2}_{3}, HCO^{-}_{3}, SO^{-2}_{4}, Cl^{-} and F) in the laboratory to measure the concentration of the quality using (APHA) standard methods. It orders to understand the hydro geochemistry of the water; the results of analysis were interpreted with geochemical tables are presented. Suitability of this water for its utility was verified using by Indian standards specification: IS:10500(Reaffirmed 1993), as a result the groundwater is not suitable for drinking purpose.

Key words : Physiochemical, Hydrogeochemistry, Drinking water quality

INTRODUCTION

The diversity of the groundwater quality changes with respective space and time, with various ages of geological formation, varies from place to place with respective depth. Water quality is required in emergency situations to determine whether water is safe to drinking/irrigation/industrial purpose, people who are traumatized by an emergency event and in poor health to water borne diseases through drinking poor quality water in India, and which is the major problem facing in India. The groundwater in shallow aquifers is generally suitable for use for different purposes and is mainly of calcium bicarbonate and mixed type. Only in some cases, groundwater has been found unsuitable for specific use due to various contaminations, mainly because of geogenic reasons. It is essential to classify the groundwater on the basis of chemical analyze to know the type of water, compositions and concentration of various constituent present it.

Study area

The study area, semi-arid region of Bhaskar Rao kunta watershed located at the Krishna lower basin and Survey of India (SOI) toposheet No: 56 P/6 & 56 P/10 (1:50000 scale), geographically lies between Northern Latitudes from 16^0 42' 25" to 16^0 37' 58" and Eastern Longitudes from 79^0 28' 15" to 79^0 32' 30" and politically located in Damaracherla Mandal, Nalgonda districts of Andhra Pradesh (Fig.1).

It's an area is covered 40.25 sq.km. The watershed elevation ranges between 80 to 140m above the Mean Sea Level (MSL), slightly undulating terrain with slight to moderate slopes (2 to 3%) and annual normal rain fall is 737mm. The average maximum and minimum temperature is being 40^oC and 28^oC respectively, but December is the coldest month with the maximum and minimum temperature being 35^oC and 20^oC respectively. Soils are consisted with red, red sandy and black soils.

Geology and Soils

The study area geologically consisted with the Kurnool group of Palnadu sub basin and partially covered by Srisailam succession of Kadapa Super Group. General sequence of sub-surface strata encountered the top soil, weathered/semi weathered, and shale/quartzite. Srisailam sub basin rocks are exposed with Quartzites. The Quartzites is inter bedded with thin siltstone units and is usually thick bedded, dense and fine to medium grained. Palnadu sub-basin rocks are exposed with Calcareous (chemical precipitates) sediments like quartzites, shales and flaggy-massive limestones.

Drainage and Topography

The Bhaskar Rao kunta watershed is showing dendritic to sub-dendritic pattern of the drainage system, governed by regional slope, homogenous lithology and relief, exhibited by some streams, which could be either due to structural or topographic control. Dendritic pattern is characterized by irregular branching of tributary streams in many directions and at almost any angle although usually at considerably less than a right angle (Fig.2). They develop upon rocks of uniform resistance and imply a notable lack of structural control. Dendritic patterns are most likely to be found upon nearly horizontal sedimentary rocks. Totally 146 streams are curved with contributes the flow of mostly dry except for seasonal run-off.

MATERIALS AND METHODS

Twenty groundwater samples were collected from bore wells (18 samples, depth 60m) and open wells (2 samples, depth 25m) in pre and post monsoon seasons at identical locations. Locations of sampling points were determined using a Global Positioning System (GPS) (Fig.2). Collected samples were analyzed in the laboratory to measure the

concentration of the quality parameters using (APHA) standard methods. Correlation of geochemical data has been attempted through presented with tables.

RESULTS AND DISCUSSION

In the study area, mean pH is varies from 8.25 to 7.96 in pre and post-monsoon seasons, its indicating slightly basic nature with little seasonal fluctuations. The mean electrical conductivity is varies ranges between 981 to 9867 μS/cm in pre and post and monsoon seasons. Among the alkaline earths, the concentration of mean calcium and magnesium contents varies from about 107 to 110pp and 81 to 84ppm in pre and post and monsoon seasons respectively. Among the alkaline, the concentration of mean sodium and potassium contents varies from about 87 to 89ppm and 0.92 to 0.84ppm respectively. Hardness is varies 445 to 449ppm in pre and post monsoon, the hardness is caused by divalent metallic ions dissolved in water; calcium and magnesium are principally the most important cation associated with HCO_3, SO_4, Cl and NO_3 ect. The hardness is effect causes more consumption of detergents at the time of cleansing. The mean sulfate and nitrates concentration of contents varies 30 to 32ppm and 30 to 32ppm respectively in pre-post-monsoon seasons. As a result are in both seasons fluoride, hardness, calcium, magnesium, sodium and salinity, contents are more than permissible limits as for the Indian standards specification: IS:10500(Reaffirmed 1993), however the chloride sulfates and nitrates are within the permissible limits, accordingly as a result the groundwater is not suitable for drinking purpose.

Fluoride

Apatite menials are principle sources for fluoride content in the groundwater. Natural concentration of fluoride commonly varies from about 0.01 to 10ppm. In India is facing one of the major problems in groundwater quality like fluoride. Fluoride in excessive concentration may case dental defects, affect bone structure and in acute causes fluorosis. In the study area, mean fluoride is varies from about 3 to 3.08ppm in pre and post-monsoon seasons respectively. As results the groundwater is not suitable for the drinking water purposes as per the IS: 10500 specifications.

CONCLUSION

The study area, drinking water quality aspects where 20 water samples were collected during pre and post monsoon seasons and analyzed to understand their quality as APHA standard. As a result are in both seasons fluoride, hardness, calcium, magnesium and salinity, contents are more than permissible limits as for the Indian standards specification: IS:10500(Reaffirmed 1993), however the chloride sulfates and nitrates are within the permissible limits, accordingly as a result the groundwater is not suitable for drinking purpose.

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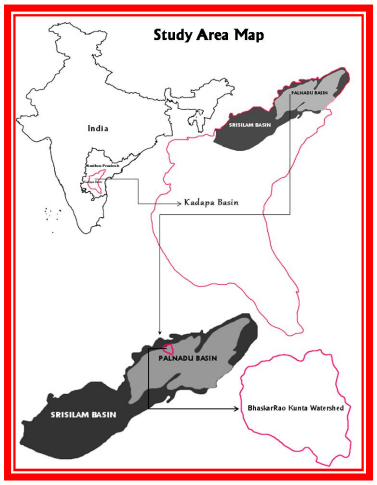

Fig.1: Location of the Study Area Map

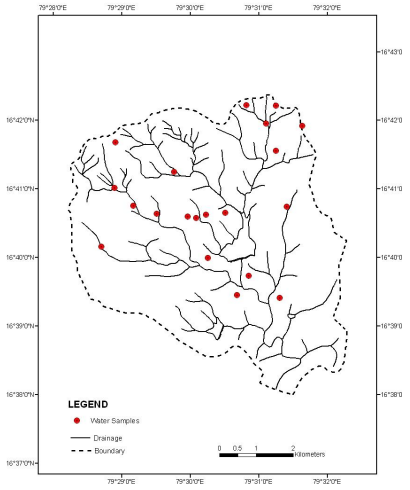

Fig.2: Groundwater Samples Locations Map

Table-1: Groundwater Quality Parameters and Results (Pre-monsoon)

Longitude	Latitude	PH	EC	TH	Ca	Mg	Na	K	HCO_3	Cl	SO4	NO_3	TDS	F	Well Type
79.48184	16.69462	8.3	859	407	96	72	72	0.82	199	57	23	25	425	2.5	BW
79.52091	16.69261	8.45	795	446	79	83	120	1.02	231	37	39	26	395	3.35	OW
79.51853	16.69924	8.1	840	495	88	87	80	0.98	219.2	95	33	25	425	3.8	BW
79.50391	16.67703	7.8	1300	423	127	88	110	0.97	191.7	115	28	26	650	4	BW
79.50153	16.67625	8.1	751	422	97	65	103	0.83	183.5	50	31	23	377	2	BW
79.49611	16.68746	7.67	1349	523	140	94	81	0.99	237	110	23	40	670	3.3	BW
79.49947	16.67665	8.01	873	396	116	100	70	0.83	217.4	60	19	29	435	2.5	BW
79.49188	16.67732	8.09	965	446	118	72	81	0.79	241	95	31	30	480	3.3	BW
79.52731	16.69863	7.9	689	323	108	53	89	0.67	165.4	93	23	29	348	2.03	BW
79.51375	16.70373	8.5	781	397	86	91	81	0.57	195.5	47	27	21	390	2.2	BW
79.52091	16.70356	8.05	829	370	91	67	75	0.98	221.4	38	43	35	414	3.35	BW
79.47841	16.66934	8.07	661	573	95	102	102	0.92	179.3	30	25	40	328	3.62	BW
79.48162	16.68361	8.4	650	349	73	56	63	0.73	177.4	35	19	38	321	2.55	BW
79.48616	16.67927	8.8	852	447	130	76	71	0.78	196.3	73	39	24	420	3.95	BW
79.52356	16.67901	8.49	1001	347	85	69	106	0.98	260.1	46	36	17	500	1.75	OW
79.51143	16.65753	7.9	1421	396	114	70	80	0.99	244.3	102	37	49	711	2	BW
79.51427	16.66217	8.5	1453	520	151	91	77	1	236.3	54	35	20	729	3.2	BW
79.50864	16.67751	8.3	1027	542	124	95	80	0.87	191.5	97	24	31	573	2.97	BW
79.50442	16.66661	8.7	901	497	97	97	91	0.97	211.5	98	27	41	452	2.9	BW
79.52186	16.65682	8.97	1497	536	119	88	91	1.53	237.2	83	38	34	651	2.9	BW
Mean		**8.25**	**981**	**445**	**107**	**81**	**87**	**0.92**	**212**	**71**	**30**	**30**	**488**	**3**	
Min		**7.67**	**650**	**323**	**73**	**53**	**63**	**0.57**	**165**	**30**	**19**	**17**	**321**	**1.75**	
Max		**8.98**	**1497**	**573**	**151**	**102**	**120**	**1.53**	**260**	**115**	**43**	**49**	**729**	**4.00**	

Post –monsoon

Longitude	Latitude	PH	EC	TH	Ca	Mg	Na	K	HCO_3	Cl	SO_4	NO3	TDS	F	Type well
79.48184	16.69462	7.9	862	418	103	78	75	0.76	206.4	61	27	29	431	2.98	BW
79.52091	16.69261	8.35	798	450	82	89	122	0.98	241	39	41	28	399	3.45	OW
79.51853	16.69924	8.05	855	500	92	89	81	0.88	225.7	97	34	29	427	3.91	BW
79.50391	16.67703	7.58	1304	425	132	89	109	0.91	195.2	120	29	26	652	4.02	BW
79.50153	16.67625	8.01	758	425	101	68	106	0.65	189	51	32	24	379	2.01	BW
79.49611	16.68746	7.35	1355	525	144	97	86	1.05	244	113	25	41	677	3.38	BW
79.49947	16.67665	7.85	876	400	118	103	74	0.79	219.6	61	19	30	438	2.67	BW
79.49188	16.67732	7.94	967	450	122	73	82	0.72	247	96	32	32	483	3.67	BW
79.52731	16.69863	7.83	698	325	112	53	91	0.63	167.8	96	26	31	349	2.02	BW
79.51375	16.70373	8.07	782	400	89	92	82	0.46	198.3	48	29	22	391	2.36	BW
79.52091	16.70356	7.9	830	375	92	69	74	1.01	228.8	39	46	39	415	3.38	BW
79.47841	16.66934	7.93	662	575	96	107	106	0.88	183	31	26	41	331	3.72	BW
79.48162	16.68361	8.19	650	350	72	58	66	0.68	183	36	19	39	325	2.67	BW
79.48616	16.67927	8.07	849	450	131	76	72	0.69	198.3	76	41	26	425	4.01	BW
79.52356	16.67901	8.33	1008	350	88	71	110	0.92	262.3	47	38	18	504	1.98	OW
79.51143	16.65753	7.87	1428	400	116	73	83	0.91	250.1	109	39	51	714	2.06	BW
79.51427	16.66217	7.79	1460	525	152	93	78	1.12	241	59	37	21	730	3.98	BW
79.50864	16.67751	7.92	1031	550	128	96	81	0.71	189.1	99	25	32	576	3.02	BW
79.50442	16.66661	8.15	910	500	101	98	93	1.04	213.5	106	29	43	455	2.98	BW
79.52186	16.65682	8.04	1526	550	127	94	97	0.96	242.7	87	42	39.7	663	3.3	BW
Mean		**7.96**	**987**	**449**	**110**	**84**	**89**	**1**	**217**	**74**	**32**	**32**	**491**	**3.08**	
Min		**7.35**	**650**	**325**	**72**	**53**	**66**	**0.46**	**168**	**31**	**19**	**18**	**325**	**1.98**	
Max		**8.35**	**1526**	**575**	**152**	**107**	**122**	**1.12**	**262**	**120**	**46**	**51**	**730**	**4.02**	

Note: SD-Standard Deviation; All units are expressed in mg/L except pH, EC (μS/cm); TH-Total Hardness as CaCO3; TA-Total Alkalinity; TDS-Total Dissolved Solids; BW-Bore Well; OW; Open Well.

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RESEARCH ARTICLE

Ambient Copper Induced Histopathological Changes in the Gill and Liver of the Major carp, *Labeo rohita* (hamilton).

Meenakumari.S[1] , T.S. Saravanan[2]* and M. Sundaramoorthy[3]

[1]P.G. Department of Zoology, National College, Tiruchirappalli-620001.TamilNadu,India.

[2]P.G. Department of Zoology, Jamal Mohamed College, Tiruchirappalli-620020,TamilNadu,India.

[3]P.G. Department of Zoology, AVC College, Mannampandal, TamilNadu- 609305,India.

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***Address for correspondence**
Dr. T.S. Saravanan
P.G. Department of Zoology, Jamal Mohamed College,
Tiruchirappalli-620020,TamilNadu,India.
E.Mail: drtssaravanan50 @ yahoo.com, thiruver@gmail.com
Mobile: 919894421487

ABSTRACT

The present investigation was carried out to find the hazardous effects of sublethal concentration of copper on *Labeo rohita*. After 30 days of exposure to the sub lethal concentration of copper, the mortality, behavioral, histological and cytological changes were observed on fish. There was no mortality during the entire period of study whereas the normal functions were found to be affected such as increased opercular movements and fast swimming within an hour of exposure. In the gill of treated fish, the histopathological changes like shrinkage, disintegration and vaculation were noticed. The light microspcopic view of treated fish liver showed abnormal changes like enlargement, vacuolization, congestion of central vein, histolysis and necrosis. The ultra structural changes such as enlargement and shrinkage of nucleus with irregular nuclear membrane, clumping of chromatin in the nucleus, dilation of the endoplasmic reticulum and damaged organelles resulting in profuse cytoplasmic vacuolization were also noticed in the hepatocytes of fishes exposed to copper sulphate.

Key words : *Labeo rohita*, gill, liver, ultrastructural, histolysis, necrosis and hepatocytes

INTRODUCTION

It is well known fact that the water bodies are looked upon as a source of exploitation for urban, agriculture and industrial wastes. This leads to the contamination of water with heavy load of pollutants and which has become a serious global problem. Heavy metal contamination may have the devastating effect of the ecological balance of the recipient environment and a diversity of aquatic organism [1-3]. Although copper is a trace element essential to life it is one of the most toxic heavy metals**.** It is continuously concentrated in water and adversely affect aquatic organism including fish. The direct contact of gills in water favors the bioaccumulation of copper in fish. Aquatic organisms such as fish and shell fish accumulate many times more concentration of metals than the concentration present in water or sediment [4,5].

The toxicity and bioaccumulation of copper were studied in fingerlings and sub adults of the silver sea bream by Wong *et al*.[6] The impact of pollutants on an organism is expressed as perturbations at different levels of functional complexity. The detection of responses to toxicant at the cellular or tissue level is of great value. The consequences of pollutants on aquatic animals are valuable. For example Saravanan et al.[7] have reported the significant changes in respiration and excretion of *Sarotherodon mossambicus* on exposure to copper in the medium. At cellular level they cause biochemical alterations, size variations in organelles like mitochondria, cytoplasmic vaculation and apoptosis, nuclear shrinkage, fragmentation of nucleus, chromosomal aberrations, gene mutation etc.,[8-10]. Das and Nanda [11]demonstrated the genotypic effect of paper mill effluents on *Heteropneustes fossilis*. The effect of copper toxicity on growth and reproductive potential in an ornamental fish, *Xiphophorus helleri* was reported by James *et al*.[12]The histopathological and ultra structural changes of fishes exposed to different types of stresses were analyzed by many workers[13-15].

The severity of a toxicant can be measured at the level of the molecular, cellular, tissue, organ, individual or population[16]. The histopathological study of an organ reflects the impact of toxicant on metabolic process at the cellular level[17,18]. In this view, the present study was undertaken to find out the ultra structural alteration caused by a heavy metal pollutant (copper) in vital tissues such as gill and liver of the major carp *Labeo rohita*.

MATERIALS AND METHODS

Sample collection - Healthy carps, *Labeo rohita* weighing around 60 gms were colleted from a fish farm located at Tiruchirappalli city, Tamilnadu, India. They were subjected for acclimatization to the laboratory condition for a month providing tap water and pellets of groundnut oil cake.

Experimental design - After acclimatization the fishes were divided into two groups, each containing five individuals as control and experimental groups. Each group was introduced into separate rectangular plastic tanks filled with 40 liters of tap water. In the experimental tank 4 ml of stock copper sulphate solution was added to obtain the sub lethal concentration of $1mgL^{-1}$. This stock solution was prepared by dissolving 3.929g of copper sulphate in 100 ml of distilled water according to Saravanan *et al*.[19]. This solution would yield 1 gm of copper in 100 ml. The experiment was carried out for the period of 30 days. During the entire course of this study, the medium of both tanks was changed daily and aerated. The fishes of both the groups were fed with pellets of groundnut oil cake. The physical parameters like temperature, pH, salinity and dissolved oxygen of the water were also measured periodically and kept in the normal range of 26˚C±1˚C, 7.45± 0.2, 0.76± 0.08% and 7.10±0.13ml/L respectively.

Light Microscopy - On the 30th day exposure, the fishes of both control and experimental groups were sacrificed and the tissues of gills and liver were removed and fixed in bouins fluid for 24 hrs. Following this, the tissue fragments were dehydrated in ascending grades of alcohol and cleared in xlylene adapting the usual process. Then the tissues were embedded in paraffin wax (50-60˚) and sectioned into 8 micron thickens. After deparafinisation the sections

were stained in Delafields hematoxyline and alcoholic eosin stains. Finally, the stained sections were mounted in DPX and studied under light microscope and photographed using Meiji (Japan) M.15 microscope with cannon T-70 model camera.

Transmission electron microscopy - For the ultra structural analysis, the liver fragments were fixed in 2.5% glutaraldehyde in 0.2 M Phosphate buffer (pH 7.2). The ultra fine sections of 50 nm were cut by ultramicrotome and stained with uranyl acetate and lead citrate. Then the ultra structure of the hepatocytes of control and experimental fishes were analyzed and the pictures were captured with the help of Philips CM-10 transmission electron microscopy attached with video-equipped Hitachi VK-C150DE and Image® Imagen software.

RESULTS AND DISCUSSION

After 30 day exposure of fishes to the sub lethal concentration of copper, the mortality, behavioral, histological and cytological changes were observed. The results showed that there was no mortality during the entire period of study. But the normal functions were found to be affected. There was an increased Opercular movements and fast swimming in some of the treated fishes within an hour of exposure.

The gill of control fish showed normal gill filaments (GF) with Primary and Secondary lamellae (PGL & SGL) as seen in Fig.1. Whereas in treated fish the following histopathological changes were noticed in the gill; shrinkage, disintegration and vaculation (Fig,2). The light microspcopic view of control fish liver showed compact hexagonal cells (Fig.3). On the other hand the treated fish liver showed abnormal changes like enlargement, vacuolization, congestion of central vein, histolysis and necrosis (Fig.4). This is in agreement with a recent report of Patel and Bahadur[20]. They have observed many histopathological changes such as cytolysis, haemorrhage withinin sinusoids and their dilation, focal necrosis, fibrosis, cytoplasmic vacuolization etc., in the liver of *Catla catla* exposed to sublethal concentration of copper ions.

According to Triebskorn and Kohler[17], the cellular damages occur in an organism exposed to even trace metal pollutants. Saravanan *et al*.[21] have evidenced the chronic effects of endosulfan on the histology of liver of *Anabus testudineus*. They have noticed the enlargement of liver cells with vacuolation, histolysis and necrosis on 30 days of exposure and more degenerative changes after 60 days of exposure.

Electron microscopic images were also analyzed in the liver of control and experimental groups. The ultra structure of control fish liver reveals a normal hepatocyte with a spherical nucleus and a prominent nucleolus. The cytoplasm is filled with compact Mitochondria, Endoplasmic reticulum and Golgi complex around the nucleus and also dominated by vast accumulation of glycogen (Fig.5 & 7). Due to the exposure to sublethal concentration of copper, in the treated fish a lot of drastic cellular changes have occurred in the hepatocytes. The ultra structural changes like enlargement and shrinkage of nucleus with irregular nuclear membrane, clumping of chromatin in the nucleus, dilation of the endoplasmic reticulum and damaged organelles resulting in profuse cytoplasmic vacuolization were noticed (Fig 6 & 8). The similar abnormal changes in the hepatocytes were also reported in copper induced fishes. The increased size of nucleus and nucleolus and large lysed cytoplasmic areas were observed in the liver cells of the fish, *Brachydanio rerio* exposed to sublethal concentrations of coppers sulphate[22]. Copper induced ultrastructural alterations in the snake-headed fish, *Channa punctatus* such as extensive proliferation of smooth endoplasmic reticulum, dilation of rough endoplasmic reticulum, degradative changes of mitochondria and clumping of chromatin materials at the center of the nucleus were studied by Khangarot[23]. He has also suggested that proliferation and dilation of endoplasmic reticulum might be due to active detoxification attempted by liver. It is notable that the present investigation also reveals the dilation of endoplasmic reticulum in the liver cells of treated fish. The abnormal alterations like increase of rough endoplasmic reticulam, necrosis and apotopsis have also been documented by Scaff and Scussel [15] in the hepatocytes subjected to Fumonisin B1 stress at different dose levels and

periods. They have also suggested that the increasing amount of rough endoplasmic reticulum might be due to over synthesis of proteins in order to reduce the stress.

Thus, the copper sulphate, contaminate the aquatic ecosystem and adversely affect the fish. This will in turn bioaccumulate in the human body through consuming fish. Hence, we would like to make the public and concern governmental organizations to understand about the seriousness of the heavy metal pollution in aquatic ecosystem and to take necessary steps in minimizing this type of pollutions considerably through the present communication. Further, the ultra structural changes recorded in liver of *Labeo rohita* exposed to copper sulphate can also be used as hisopathological tools for screening the copper pollution in aquatic environments.

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Lightmicroscopic studies of Gill

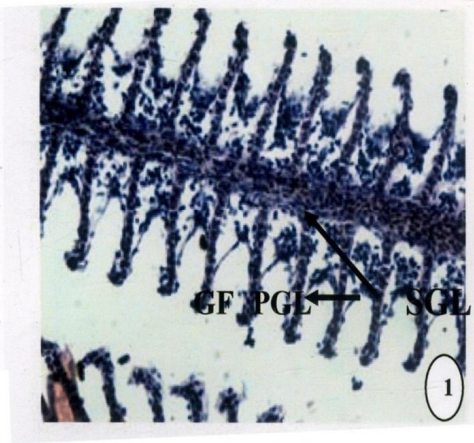

Fig. 1. Control gill of Labeo rohita showing gill filaments (GF) with primary and secondary lamellae (PGL & SGL). X 100 (H&E).

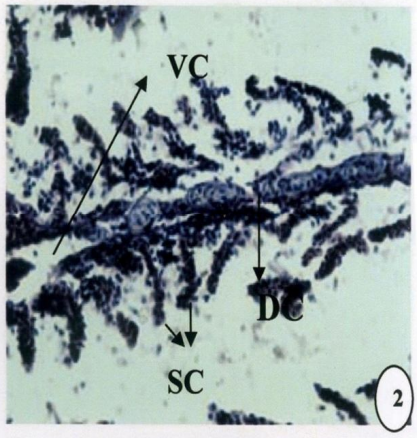

Fig. 2. The gill of Labeo rohita exposed to copper showing the following histopathological changes; a) Shrinkage (SC) b) Disintegaration of cells (DC) c) Vacuolation (VC). X100 (H&E).

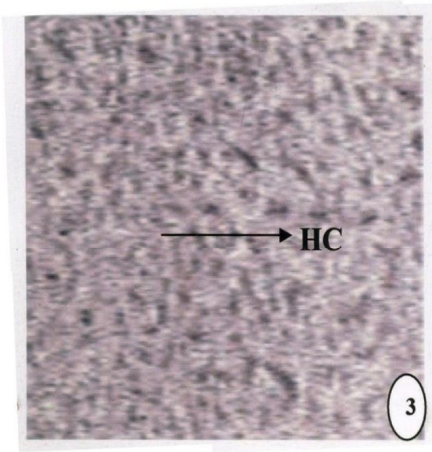

Fig. 3. Control liver of Labeo rohita showing compact hexagonal cells (HC). X 100 (H&E).

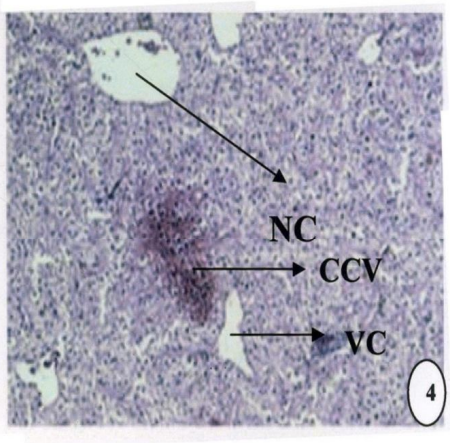

Fig. 4. The liver of Labeo rohita exposed to copper showing the following histopathological changes; a) Vacuolation (VC), b) Necrosis (NC), c) Congestion of central vein(CCV). X100 (H&E).

Ultramicroscopic studies (SEM) of Liver cells

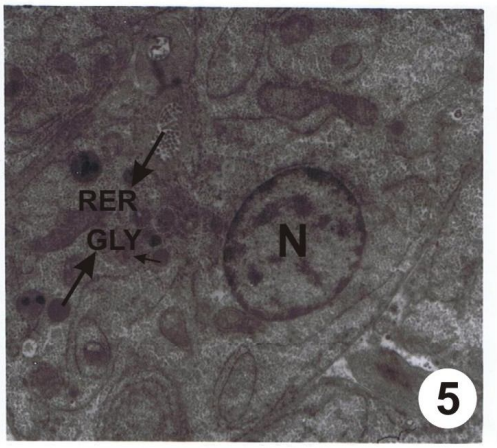

Fig. 5. A typical control liver cell of the carp showing a normal hepatocyte (X 7000) with more of glycogen (Gly) bodies, rough endoplasmic reticulum (RER) and a spherical Nucleus (N).

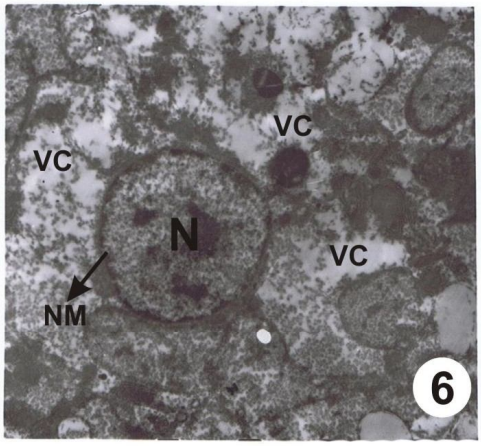

Fig. 6. Hepatocyte of carp exposed to copper showing enlarged nucleus (N) with an irregularnuclear membrane (NM). The cell organelles are completely damaged with more of vacuolation (VC). X7000.

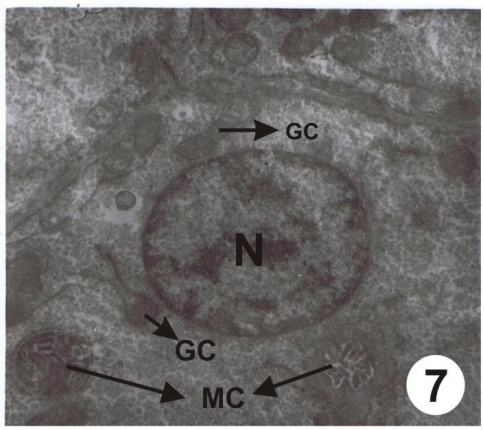

Fig. 7. A typical control liver cell of the carp showing a normal hepatocyte (X10000) showing a spherical nucleus with nucleolus (N), a compact mitochondria (MC) and Golgi complex (GC) are seen on either side of the Nucleus.

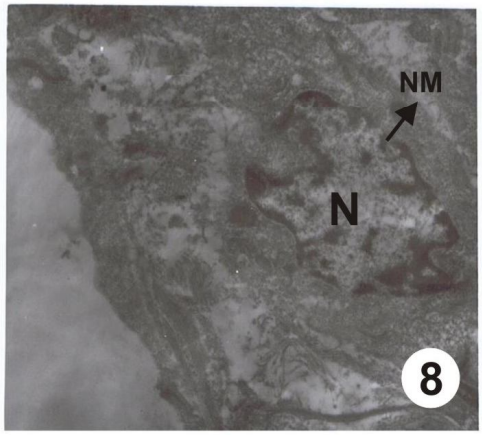

Fig. 8. Heaptocyte of carp exposed to copper (X10000) showing the distorted, amoeboid like nucleus (N) with irregular nuclear membrane (NM). The organelles like mitochondria (MC) and Golgi complex(GC) have lost their shape.

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3. Issue with supplement: Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1, Suppl 2):89-97.

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6. Chapter in a book: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

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