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RESEARCH ARTICLE

Determination of Kinetic and Thermodynamic Parameters of Partially Purified *Borassus flabellifer* L.Peroxidase.

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ABSTRACT

Peroxidases are widely believed to catalyze the last enzymatic step in the biosynthesis of lignin, the dehydrogenation of the *p*-coumaryl alcohols. The present study was under taken to develop a system for production of peroxidase enzyme in large scale production from *Borassus flabellifer*L.(Arecaceae). *Borassus flabellifer* peroxidase was partially purified. It had single isoform. The quantity of enzyme activity and assay were determined by substrates guaiacol, O-Dianisidine, Diphenyl amine, Benzidine and Tetra methyl Benzidine. It had greater affinity towards Guaiacol compare to Benzidine and o-Dianisidine as organic substrates. The partially purified enzyme has optimum pH around 4 to 5 in presence of substrates guaiacol, o-Dianisidine, Diphenyl amine, Benzidine and Tetra methyl Benzidine. The temperature optimum for the above substrates lies in the region around 60°C. The kinetic parameters, Vmax and Km were calculated from the Michaelis-Menten plot and LB plot. A haemoprotein as it was brownish in colour. Its relative molecular weight was 100KDa and had single isoenzyme.

Key words: *Borassus flabellifer*, Plant derived peroxidases,Isoenzyme ,Enzyme activity

INTRODUCTION

The Palmae plant, *Borassus flabellifer* L. (palmyra palm in English), is widely distributed and cultivated in tropical Asian countries such as Thailand, Bangladesh, India, Myanmar, Sri Lanka, Malaysia, *etc*. The fruit pulp of *B. flabellifer* has been used in traditional dishes and the sap, which was trapped from the flower part, has been used as a sweetener for diabetic patients. In the previous studies, several steroidal saponins, a polysaccharide, and a triterpene constituents were isolated from the fruit pulp, seeds, and young shoot of *B. flabellifer*. However, the chemical and pharmacologic studies for the flower parts of this medicinal food were left uncharacterized[5].

Peroxidases occur throughout the biosphere and participate in a large number of essential oxidative reactions. The homologous super family of heme peroxidases discussed here has been divided into three classes based on biological origin and sequence alignments. The first class which shows the Intracellular plant and bacterial peroxidases (Prokaryotic Cytochrome C peroxidase)[7]. The second class shows that the secretory fungal peroxidases (fungal peroxidase Lignin peroxidase [12]. The third class consists of the Plant peroxidases (HRP), Zapota, R.communis, tomato peroxidases.

Plant peroxidases participate in a wide variety of pathways, including the synthesis of the cell wall components lignin and suberin, metabolism of hormones such as indole-3-acetic acid (IAA), stress response mechanisms, and fatty acid metabolism[8].Of the various peroxidases, the class III enzymes are most readily available and have been more extensively studied and used than other peroxidases. Their abundance, together with the stability and distinguishing spectral properties of the various peroxidase intermediates, led plant peroxidases to serve an important role in the early development of modern enzymology[10]. The dramatic and measurable color changes accompanying the oxidation of certain peroxidase substrates such as tetramethylbenzidine is the basis for various commercial applications, including bioassays[11]. Most of the functions attributed to plant peroxidases occur in cell walls. These functions can be divided into two main categories. The first is the oxidative cross-linking or coupling of many aromatic molecules by using hydrogen peroxide as an electron acceptor. This leads to the formation of lignin or suberin and also to the establishment of covalent bonds between hydroxycinnamate ester moieties or flavonoids associated with pectins or hemicellulose. By catalyzing these reactions, peroxidases are involved in the construction of cell walls and in the control of cell wall plasticity [1]. It has 5- coordinated high spin ferric ion as the prosthetic group. In addition to one mole of ferric ion, two moles of calcium per mole of enzyme were present which are required to maintain the structural integrity of the protein at high temperatures [6].

In common with other disease situations, rust-resistant wheat leaves show a large increase in peroxidase activity during infection**.** Peroxidase isozymes from healthy or infected lines of wheat (*Triticum aestivum* L.) near isogenic for resistance and susceptibility to race 56 of *Puccinia graministritici* were separated by gel electrophoresis and the activity of each was estimated by photometric scanning. 14 isozymes were detected in both healthy and infected leaves, increases in only 1 (isozyme 9) were associated consistently with the development of resistant disease reaction at 20^0 C [13]. Nickel toxicity was evaluated in *Triticum aestivum* L. by its effects on root and shoot length, dry matter production and water content. The degree of toxicity increases as a function of the Ni^{2+} concentration in the medium. Ni^{2+}-treated roots show enhanced lipid peroxidation. In roots and shoots, Ni^{2+} enhances both guaiacol and syringaldazine extracellular peroxidase activity. The increase in extracellular peroxidase activity is also associated with an increase in the phenolic contents of roots and shoots. Intracellular soluble peroxidases are also stimulated by Ni^{2+}. Intracellular peroxidases might act as scavengers of peroxide radicals produced as a result of nickel toxicity.

Lignin is an integral cell wall component of all vascular plants. Peroxidases are widely believed to catalyze the last enzymatic step in the biosynthesis of lignin, the dehydrogenation of the *p*-coumaryl alcohols. Five different peroxidases of *Populus trichocarpa* (PXP 1, PXP 2, PXP 3-4, PXP 5, and PXP 6) were isolated from the xylem .Because these isoenzymes were specifically or preferentially expressed[3]. xylem, PXP 3-4 and PXP 5 are suggested to be involved in lignin polymerization [12]. Nicotiana tabacum and N. sylvestris transformed plants with peroxidase activity that is 10-fold higher than in wild-type plants by introducing a chimeric gene composed of the cauliflower mosaic virus 35S promoter and the tobacco anionic peroxidase cDNA. The elevated peroxidase activity was a result of increased levels of two anionic peroxidases in *N. tabacum*, which apparently differ in post-translational modification. Transformed plants of both species have the unique phenotype of chronic severe wilting through loss of turgor in leaves, which was initiated at the time of flowering [3].It was concluded that the over expression of the tobacco anionic peroxidase in transformed plants results in diminished root mass from fewer root branches, which contributes to the wilting phenomenon seen in these plants. Further, this developmental change in transformed plants may be a consequence of the metabolism of IAA by the anionic peroxidase [9].

Enzyme linked immunorbent assay (ELISA) tests on which peroxidase is probably the most common enzyme used for labeling an antibody, are a simple and reliable way of detecting toxins, pathogens, cancer risk in bladder and prostate, and many other analyte[4]. Peroxidase-modified amperometric electrodes have been widely studied and developed, not only because of hydrogen- and organic peroxides are important analytes but also because of the key role of hydrogen peroxide detection in coupled enzyme systems, in which hydrogen peroxide is formed as the product of the enzymatic reaction. Many important analytes, such as, aromatic amines, phenolic compounds, glucose, lactate, neurotransmitters, etc. could be monitored by using bi- or multi-enzyme electrodes [11]. Adsorption of the reaction products on the talc effectively protected the biocatalyst against contamination by oxidative products, thereby prolonging its catalytic action and leading to almost complete elimination of phenols in aqueous media [2].

MATERIALS AND METHODS

Chemicals like acetic acid, sodium acetate, sodium dihydrogen orthophosphate, Disodium hydrogen phosphate, (a) Benzidine , (b) o-Dianisidine Hydrochloride (c) Guaiacol (d),hydrogen peroxide [30%] (e)and Himedia dialysis membrane [mw.cut off 10KDa] were used. Quartz double distilled water (f) was used in preparing all solutions. B and C were dissolved in water whereas (a) was dissolved in ethanol. All other chemicals utilized in our experiments were of analytical grade and used as such.

UV-VIS Spectrophotometer was employed in inhibition studies and products were measured at 470nm respectively. The molar extinction coefficient was 8.3 $M^{-1}cm^{-1}$ whereas the molar coefficient of (g) was $2.66x10^4$ $M^{-1}cm^{-1}$). Sigma plot software was used in analyzing Vmax and Km. Proteins were quantified by using BSA as a standard in Lowry's method. Borassus flabellifer fruits were purchased from the local market and grinded mechanically. The homogenate was centrifuged at 10,000 rpm for 30 min at 4 ^{0}C. The uninterested protein precipitated by ammonium sulphate saturation.

Ammonium sulphate precipitation was used as a purification step for the crude soluble peroxidase fraction but not for the crude ionically bound peroxidases. To the supernatant added required amount of ammonium sulphate to reach 45% saturation and left overnight. The precipitates were collected by centrifugation and to the above supernatant added ammonium sulphate to attain 95% saturation. These precipitates were dissolved in 50mM acetate buffer pH 4.5 and dialyzed against 50 mM same buffers overnight [1:1000]. The dialysate was considered as partially purified peroxidase. Buffers like Acetic acid/Sodium acetate buffer pH 4.0 – 5.5 were used.

0.9 milliliter of reaction mixture contained 0.2 ml of acetate buffer pH 4.5, 2.30µg of enzyme, 8mM of Guaiacol and water. Incubated for fifteen minutes and transferred into cuvette containing 0.1 ml of 4mM hydrogen peroxide. Absorbance change was monitored at 470 nm. Molar extinction coefficient of tetra guaiacol was $2.26x10^4M^{-1}CM^{-1}$. Each experiment was performed in triplicates. Linear regression analysis was utilized in drawing all graphs. Amount of enzyme [mg] required to produce one micro moles of product per minute under standard assay conditions.The enzyme was mixed with inhibitors/organic solvents and vortexed, incubated for 30 minutes at 30ºC and dialysed exhaustively for 12 hours with three changes. The control doesn't contain inhibitor.

The kinetic parameters, Vmax and Km were calculated from the Michaelis-Menten plot and LB plot, plotted against the substrate concentration in the X-axis and the enzyme activity in the Y-axis in case of MM plot and 1/[v] V_s 1/[s] in the case of LB plot.The pH optimum of peroxidase was found out graphically by incubating the reaction mixture at different ph at saturating substrate concentrations. The enzyme was incubated in different buffer solutions (pH 3.0-8.0) for 15 minutes at 45°C in presence of substrate and assayed after addition of 0.05ml of hydrogen peroxide.

The reaction mixtures were incubated at different temperatures at saturating substrate concentrations and then plotted the graph. The enzyme was incubated at different temperature (35°C-65°C) for 15 minutes in presence of

substrate and brought to room temperature before assaying it. The assay was done after addition 0.05ml of hydrogen peroxide.The native gel electrophoresis was performed at 8°C with partially purified sample. The enzyme was stained by transferring the gel in to a plastic container containing assay solutions. The result is shown in the plate 2. The portion of the gel at a particular RF value were sliced and crushed in 50mM acetate buffer pH 4.5 at 4°C. The supernatant purity was checked by SDS-PAGE. The results are not shown. The protein obtained from native gel was dialysed against assay buffer were used in all experiments.

RESULTS AND DISCUSSION

Borassus flabellifer was partially purified by salting out process. Saturating the crude filtrate with 45 to 95% ammonium sulfate completely precipitated peroxidases. The specific activity of this peroxidase and its purification fold were 1 and 5 respectively. As shown in the figure 1-3 the Vmax of the enzyme with Guaiacol, Benzidine and O-Dianisidine as substrate were 24, 34 and 64μM respectively. Similarly Km was 66μM/min, 84mM/min and 13mM/min respectively. This clearly indicated that Guaiacol was the best substrate. Km of Peanut, Mango, Milk, HRP, Pyrus, Bringal peroxidases and lignin peroxidase with Veratryl alcohol as substrates were around 50μM .

Partially purified *Borassus flabellifer* peroxidase affinity towards Guaiacol was more or less same as reported. This peroxidase may also be involved in lignifications of cell walls. The delignifying enzyme – Lignin peroxidase of Phanerochaete chrysosporium peroxidase has pH optimum around 3.0 .where as plant peroxidase pH optima were around 7.0 [7].

These plant peroxidase pH optima with Guaiacol, Benzidine and o-Dianisidine substrates were around 4 to 5, which seems that possible amino acid involved in catalysis was Aspartic acid. The temperature optima of peroxidase with Benzidine and o-Dianisidine were around 60 ^{o}C. This clearly indicated the enzyme was highly active at higher temperature compare to so for known peroxidases [7].The enzyme was active at higher temperature for weeks without losing its activity [Not shown]. Thus this enzyme was thermo stable. The relative molecular weight of this peroxidase was around 100 KDa compare to so for known 44 KDa plant peroxidase. It was brownish in colour and probably a heamoprotein as already known plant peroxidases [12].The Native gel electrophoresis experiment clearly indicated it possessed singly enzyme compare to HRP which has nearly three isoenzymes.

CONCLUSION

Our future plan is to purify to apparent homogeneity. Its utility in degradation of recalcitrant organic pollutants, mutagens, carcinogens and dyes will be exploited in our further studies with adsorbed/covalently immobilized peroxidases. Finally we wish to couple this thermo stable enzyme with monoclonal IgY antibodies and utilize in ELISA experiments.

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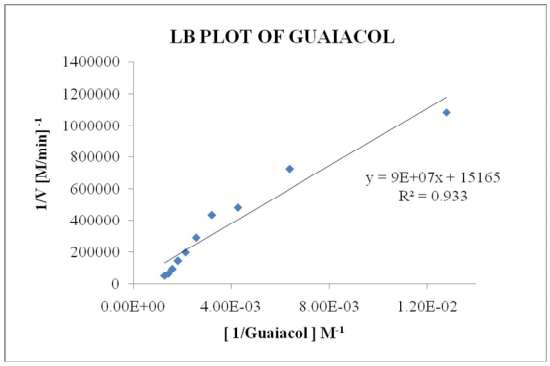
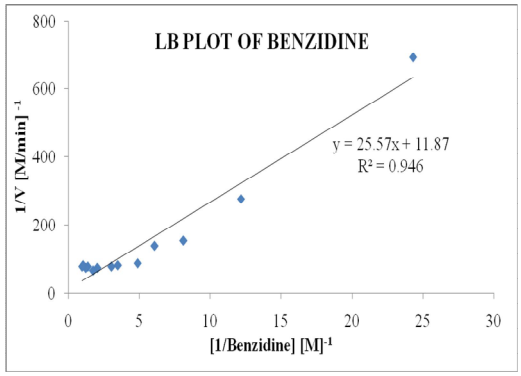


Fig 1: As in materials and methods except Guaiacol concentrations varied



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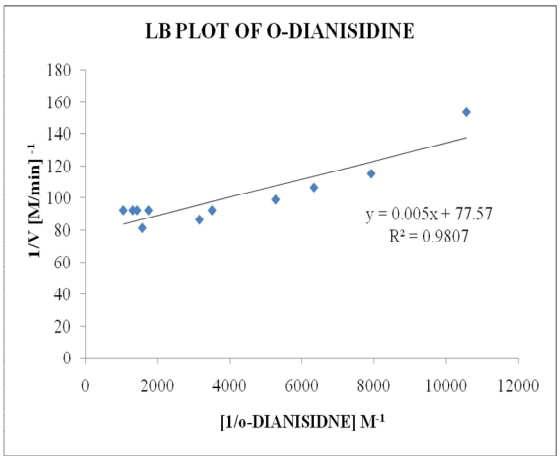

Fig 3: As in materials and methods except Dianosodine concentrations varied

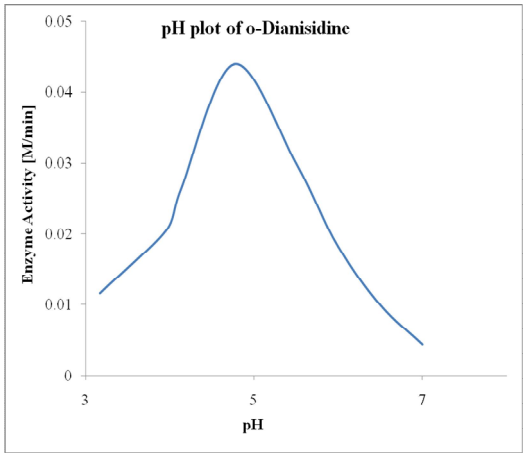

Fig 4: As in materials and methods except p O- has been varied

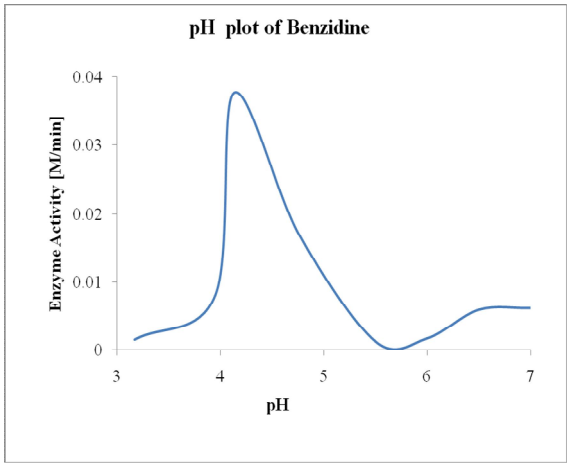

Fig 5: As in materials and methods except pH has been varied.

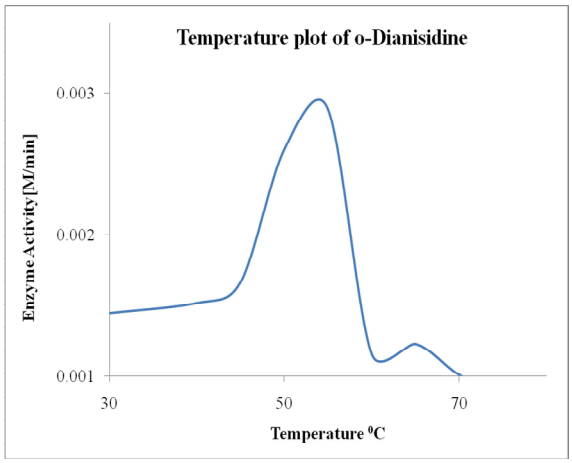

Fig 6: As in materials and methods except temperature varied

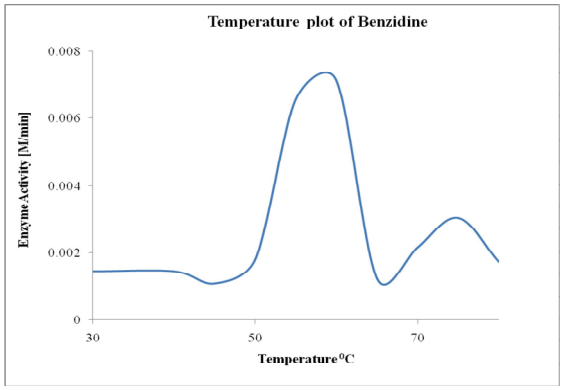

Fig 7: As in materials and methods except temperature varied.

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RESEARCH ARTICLE

Docking Studies and Phytochemical Analysis of *Trigonella foenum-graecum* L.

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ABSTRACT

This study provides the detailed analysis about phytochemical compounds extracted from *Trigonella foenum graecum* L. using GC-MS techniques. We also focussed on docking studies and calculate the score values for all ligands. The receptors FABP4, FASN, MTP, CSP which is responsible for diabetes, cancer, malaria and dyslipedimea respectively. The parameters and the secondary structure factors are to be calculated for these proteins. We are going to utilize Expasy servers for the calculation of protein parameters and secondary structure factors.

Key words: *Trigonella foenum graecum* L., Phytochemical Analysis, Receptors, Diabetes, cancer, malaria, dyslipedimea, Protein Parameters, Secondary structure factors.

INTRODUCTION

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. The trend of using natural products is increasing steadily. The use of traditional medicines and medicinal plants in most developing countries as normative bases for maintenance of good health has been widely observed further an increasing reliance on the use of medicinal plants in the industrialized societies has been related to the development of several drugs and chemotherapeutics from plants species as well as from traditionally used rural herbal preparations. Herbal remedies have attained much more popularity in the treatment of minor ailments, due to increasing awareness of personal health maintenance through natural products[1].

Trigonella foenum-graecum L. is an annual crop from the family Leguminosa. The major constituents of this plant seeds contain only minute quantities of an essential oil, which contain 40 different compounds. Furthermore, n-alkanes, sesquiterpenes, alkanoles and lactones were also reported. The dominant aroma component in fenugreek seeds is a hemiterpenoid, lactone, and sotolone (3-hydroxy-4, 5-dimethyl-2(5H)-furanone). Phytochemical investigations on *Trigonella foenum graecum* resulted in the isolation of diosgenin, an important substance in the synthesis of oral contraceptives and sex hormones and are popular where meatless diets are customary. It is otherwise used as a tonic, for mouth ulcers, chapped lips, stomach complaints and also in veterinary practices as a source of diosgenin. Cosmetically, fenugreek is said to improve the complexion and the condition of the hair. It contains phyto-estrogen which is a term applied to non-steroidal plant materials displaying estrogenic activity[1]. Phyto-estrogens encompass several classes of compounds including flavonoids, isoflavonoids and coumestans [2].

Diabetes mellitus (DM) is a metabolic disorder caused by insufficient or inefficient insulin secretary response and it is characterized by increased blood glucose levels (hyperglycemia). DM is a heterogonous group of syndromes. Glucose is the main energy source for the body, and in the case of DM, management of glucose becomes irregular. There are three key defects in the onset of hyperglycemia in DM, namely increased hepatic glucose production, diminished insulin secretion, and impaired insulin action. Conventional drugs treat diabetes by improving insulin sensitivity, increasing insulin production and/or decreasing the amount of glucose in blood[3] Ayurvedic formulations are used to treat a wide variety of diseases including diabetes mellitus Standardization of herbal formulation is essential in order to assess the quality of drugs[4]. Bone is a frequent target of lung cancer metastasis and is associated with significant morbidity and a dismal prognosis. Interaction between cancer cells and the bone microenvironment causes a vicious cycle of tumor progression and bone destruction [5].

Musashi1 is an evolutionarily conserved RNA-binding protein that has been implicated in processes like stem cell fate, nervous system development, and tumorigenesis via its activities as a specific regulator of translation. While Msi1 is barely detected in normal adult tissue, it has been observed to be highly expressed in numerous tumour types [6]Prevalent bacterial pathogens isolated were Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis, Proteus vulgaris and the fungal pathogens. Among variety of medicinal plants Trigonella foenum graecum showed significant antimicrobial activity against most of the isolates [7].Developing countries, where malaria is one of the most prevalent diseases, still rely on traditional medicine as a source for the treatment of this disease. For the present study, Trigonella foenum-graecum L. was collected. The test plant has been used in India by traditional healers for the treatment of fever as well as other diseases. The active principle was extracted out in different solvent systems to assess the anti-plasmodial potential, with an aim that they can further be utilized to formulate drugs. In vitro anti-plasmodial assay of the extracted fractions of Trigonella foenum-graecum L. leaves was carried out using laboratory adapted chloroquine sensitive and resistant[8].

The soluble dietary fibre (SDF) fraction of Trigonella foenum graecum has been shown to reduce postprandial elevation in blood glucose level of Type 2 model diabetic rats by delaying the digestion of sucrose. The Trigonella foenum graecum has now been investigated for its chronic effect on serum fructosamine, insulin and lipid levels, and on platelet aggregation in Type 2 diabetic rats [9]. Oral supplements of Trigonella foenum graecum seeds have been shown to treat glucose and lipid homeostasis in several metabolic disorders [10].

A combination of protein-ligand docking and ligand-based QSAR approaches has been elaborated, aiming to speed-up the process of virtual screening. In particular, this approach utilizes docking scores generated for already processed compounds to build predictive QSAR models that in turn assess hypothetical target binding affinities for yet undocked entries. This progressive-docking procedure therefore substantially accelerates high throughput screening, especially when using high accuracy (slower) docking approaches and large-sized datasets, and has allowed us to identify several novel potent nonsteroidal SHBG ligands [11].

MATERIALS AND METHODS

Trigonella foenum-graecum L. was collected at various locations around V.Kalathur and Trichy. The leaves were washed thoroughly under running tap water and dried under shade. They were then finely ground to a powder in an electric blender. Then we retrieved the protein sequences from NCBI database. The Protein sequences which includes Diabetes Mellitus, Cancer, Malaria and Ameliorate dyslipidemia. The sequences were subjected to PROSITE tool and calculate the binding sites present in it. The Protein structure can be downloaded from PDB database and converted in to .pdb format. The structure of the Phyto chemicals was retrieved from Pub chem. Compound database. Then the retrieved structures of both these compounds for each disease were subjected to docking studies using Hex tool. We can also dock the structure for various proteins with these phytochemical compounds. The docking scores can be calculated from Hex tool. The lists of Phyto chemicals were extracted from *Trigonella goenum graecum* and subjected to ALOGPS tool to calculate the hydrophic nature of the proteins. The Parameters of the Protein and secondary structure factors were calculated by Expasy Server and Proteomics tools. Finally, the results were compared and discussed.

RESULTS

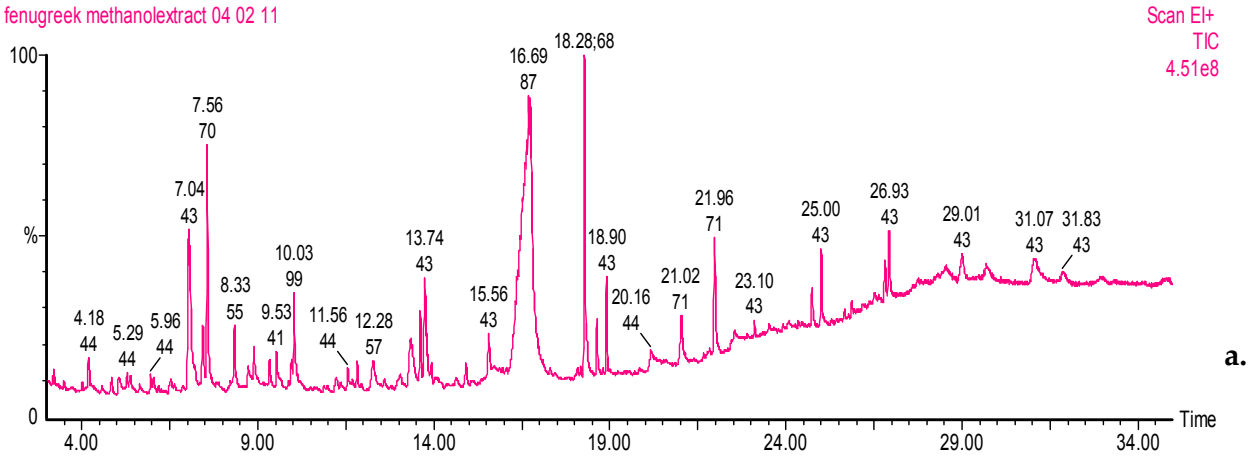
Table 1: *Trigonellafoenum graecum* L.was subjected to Gas Chromatography and Mass Spectrometry technique to identify the variety of phytochemical compounds. *Trigonella foenum-graecum* which contains 37 phytochemicals identified by GCMS.

S.No.	Peak Name	Retention time	Peak area	%Peak area
1.	Pyridine, 1,2,3,6-tetrahydro-1,2-dimethyl- Formula: $C_7H_{13}N$ MW: 111	3.49	280059	0.1184
2.	Piperazine, 2,6-dimethyl-, cis- Formula: $C_6H_{14}N_2$ MW: 114	4.01	300863	0.1272
3.	Piperazine, 1-nitroso- Formula: $C_4H_9N_3O$ MW: 115	4.18	2960516	1.2517
4.	2-Methoxymethyl-2-methylpyrrolidine-1-carboxaldehyde Formula: $C_8H_{15}NO_2$ MW: 157	4.58	538246	0.2276
5.	Cyclohexanol,3,5-dimethoxy-, cis-1,3,trans-1,5- Formula: $C_8H_{16}O_3$ MW: 160	4.85	1197499	0.5063
6.	4-Piperidinemethanamine Formula: $C_6H_{14}N_2$ MW: 114	5.29	839429	0.3549
7.	Pyrrolidin-1-acetic acid Formula: $C_6H_{11}NO_2$ MW: 129	5.65	780391	0.3300
8.	Piperazine, 3-butyl-2,5-dimethyl- Formula: $C_{10}H_{22}N_2$ MW: 170	5.96	985176	0.4165
9.	3,6-Pyridazinedione, 1,2-dihydro-4-methyl- Formula: $C_5H_6N_2O_2$ MW: 126	6.53	1415646	0.5985

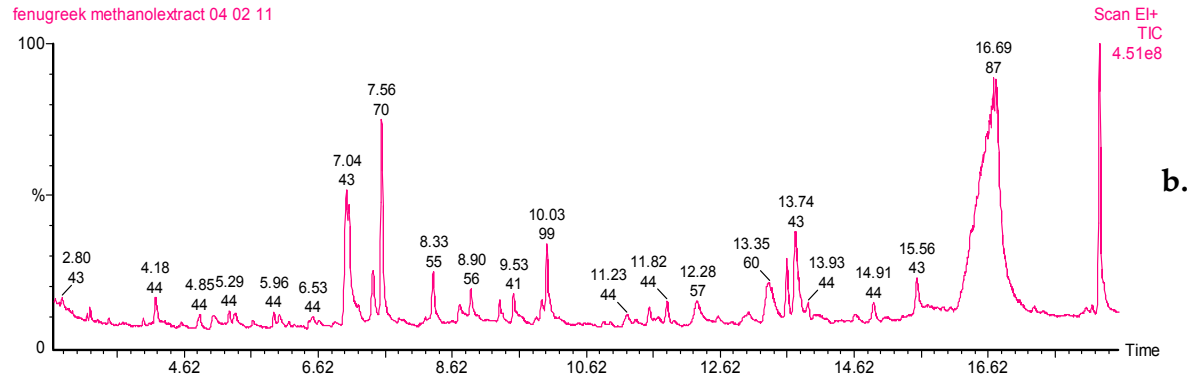
10.	2-Methyl-4-pentenoic acid Formula: $C_6H_{10}O_2$ MW: 114	7.44	3301882	1.3960
11.	2-Propen-1-amine, N-ethyl- Formula: $C_5H_{11}N$ MW: 85	7.56	11885554	5.0253
12.	Pyrrolidine, 1-nitro- Formula: $C_4H_8N_2O_2$ MW: 116	8.22	547373	0.2314
13.	2-Oxonanone Formula: $C_8H_{14}O_2$, ü-Caprylolactone MW: 142	8.33	4580591	1.9367
14.	Benzofuran, 2,3-dihydro- Formula: C_8H_8O MW: 120 Coumaran	8.73	2197469	0.9291
15.	3-Amino-4,5-dimethyl-2(5H)-furanone Formula: $C_6H_9NO_2$ MW: 127	8.90	2858412	1.2085
16.	2H-Furo[2,3-b]pyrrole-2,5(3H)-dione, tetrahydro-3a,6a-dimethyl-, cis- Formula: $C_8H_{11}NO_3$ MW: 169	9.33	1400786	0.5923
17.	2-Methyl-8-nitroisoxazolizidine Formula: $C_6H_{10}N_2O_4$ MW: 174	9.53	1582873	0.6692
18.	2-Piperidineacetic acid, à-phenyl-, methyl ester Formula: $C_{14}H_{19}NO_2$ MW: 233	9.87	362177	0.1531
19.	2-Methoxy-4-vinylphenol Formula: C9H10O2 MW: 150	9.95	1478397	0.6251
20.	2-Oxonanone Formula: $C_8H_{14}O_2$ MW: 142	10.03	5229534	2.2111
21.	Hydrocinnamic acid, o-[(1,2,3,4-tetrahydro-2-naphthyl)methyl]- Formula: $C_{20}H_{22}O_2$ MW: 294	10.50	202980	0.0858
22.	1H-Azepin-1-amine, hexahydro- Formula: $C_6H_{14}N_2$ MW: 114	10.87	500711	0.2117
23.	2-(2-Vinyloxy-ethoxy)-cyclohexanol Formula: $C_{10}H_{18}O_3$ MW: 186	11.23	1478880	0.6253
24.	2H-Pyran-2-one, tetrahydro-6-nonyl- Formula: $C_{14}H_{26}O_2$ MW: 226 ë-Nonyl-ë-valeralactone	11.56	1115492	0.4716
25.	9-Aza-1-methylbicyclo[3.3.1]nonan-3-ol Formula: $C_9H_{17}NO$ MW: 155	11.82	1374851	0.5813
26.	Sucrose Formula: $C_{12}H_{22}O_{11}$ MW: 342	12.28	5483238	2.3183
27.	Formamide,N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl]- Formula: $C_{10}H_{16}N_2O$ MW: 180	12.59	1248775	0.5280
28.	1,6-Anhydro-á-D-glucopyranose (levoglucosan) Formula: $C_6H_{10}O_5$ MW: 162	13.35	7606930	3.2162

29.	9-Azadispiro[3.1.3.0]nonane Formula: $C_8H_{13}N$ MW: 123	13.61	3337592	1.4111
30.	Benzen-1,2-diol, 5-fluoro-4-aminomethyl- Formula: $C_7H_8FNO_2$ MW: 157	14.63	1149900	0.4862
31.	3-O-Methyl-d-glucose Formula: $C_7H_{14}O_6$ MW: 194	16.69	129066744	54.5699
32.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	18.28	16288217	6.8867
33.	n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	20.16	5278493	2.2318
34.	Phytol Formula: $C_{20}H_{40}O$ MW: 296	21.96	7901946	3.3410
35.	2-Propenoic acid, 2-(dimethylamino)ethyl ester Formula: $C_7H_{13}NO_2$ MW: 143	25.86	802008	0.3391
36.	Methyl (Z)-5,11,14,17-eicosatetraenoate Formula: $C_{21}H_{34}O_2$ MW: 318	28.54	7808738	3.3016
37.	Spirost-5-en-3-ol, acetate, (3á,25R)- Formula: $C_{29}H_{44}O_4$ MW: 456 (Diosgenin acetate)	34.83	1148198	0.4855

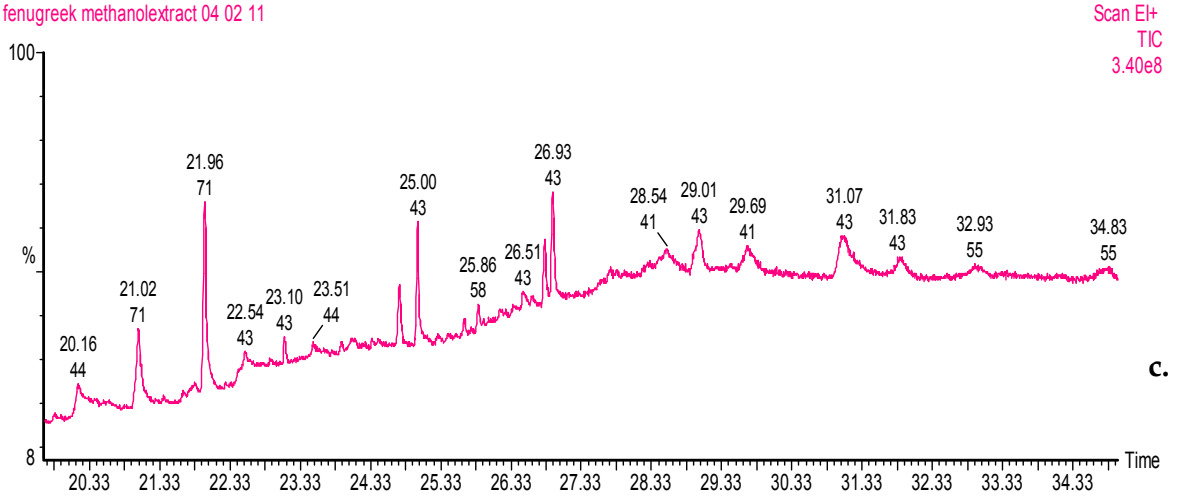
Chromatogram



a.

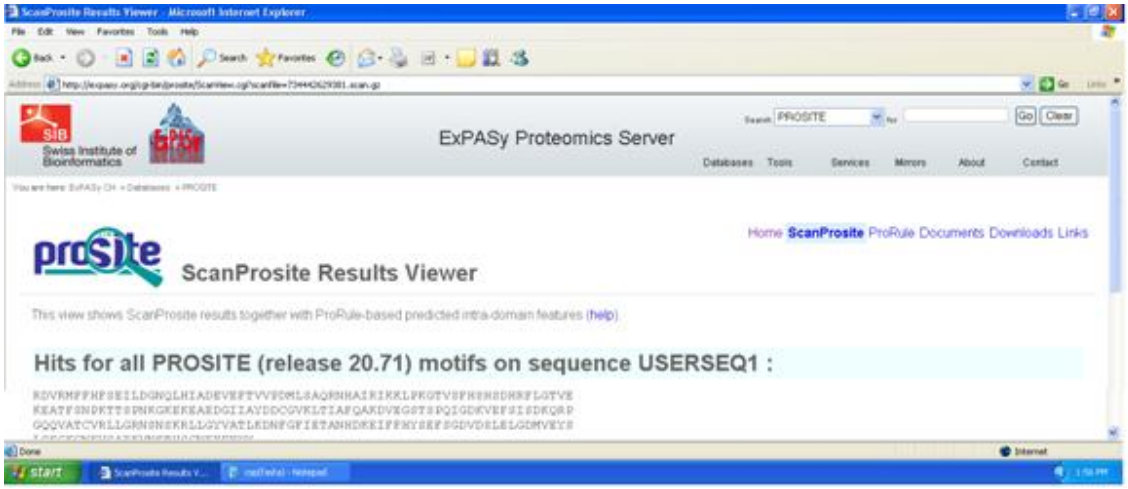


b.



c.

Fig 1 (a,b,c) : Chromatogram of phytochemical compounds.This graphs shows that the geometric representation of phytochemical compounds



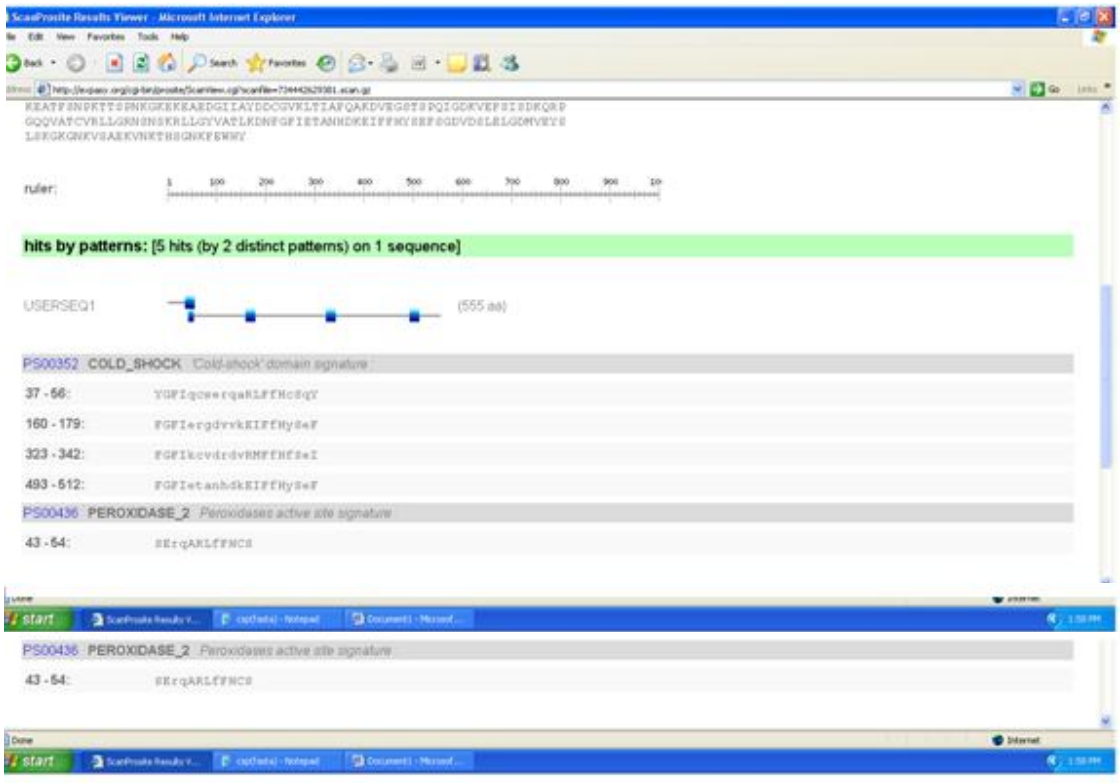


Fig 2: Screen shot of FABP4 Receptor and their hits, the pictures shows that FABP4 Receptor has 5 Active sites and these sites may ready to bind with the ligand.This Receptor which contains 565 residues.

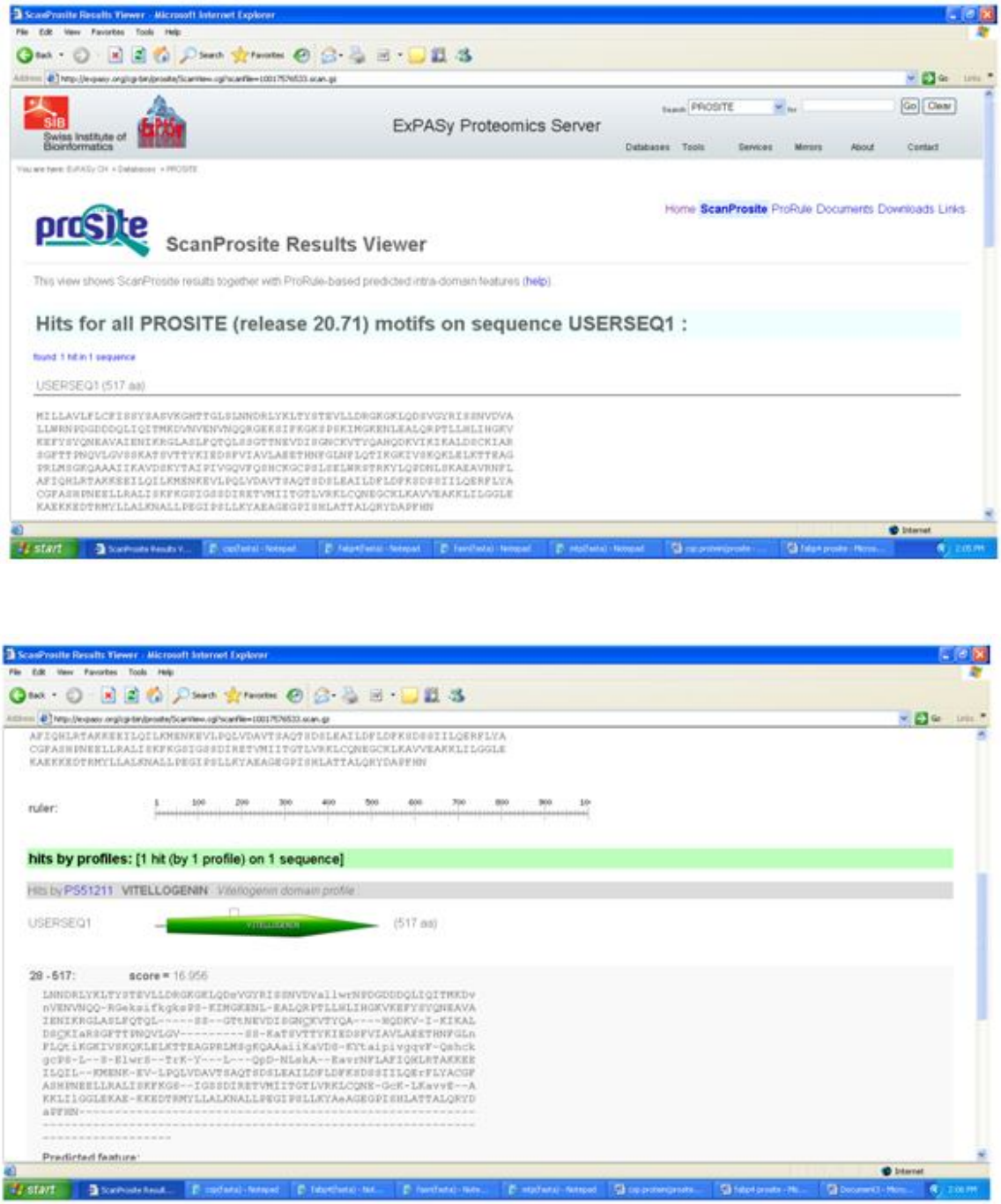


Fig 3: Screen shot of FASN Receptor and their active sites, It shows that the FASN receptor has 517 residues and it has only one hit. The ligand molecules are ready to interact with this receptor through this hit.

Table 2: Results for Hydrophobic Nature from ALOGPS tool. This table shows that the hydrophobic activity of the receptors.

mol_NlogPlogSSMILES
mol_1 -2.49 0.65 COC1C(C(C(C(O1)CO)O)O)O
mol_NlogPlogSSMILES
mol_1 6.82 -6.88 CCC=CCC=CCC=CCCCCC=CCCCC(=O)OC
mol_NlogPlogSSMILES
mol_1 7.89 -6.43 CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C
mol_NlogPlogSSMILES
mol_1 7.89 -6.43 CC(C)CCCC(C) CCCC(C)CCCC(=CCO)C

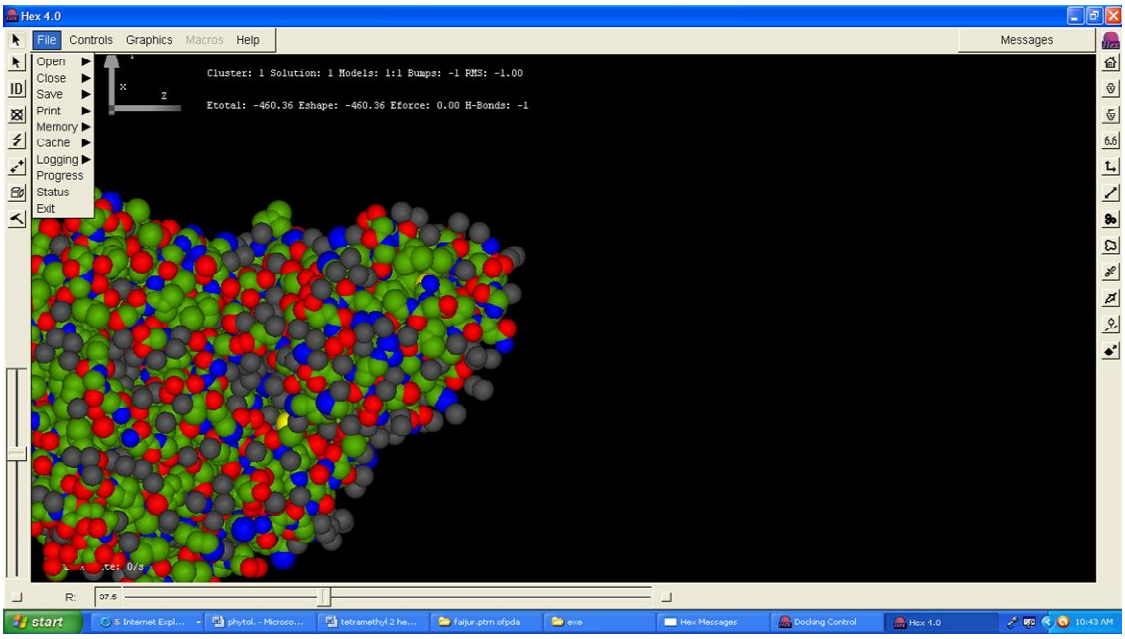


Fig 4: Docking result for FABP4 receptor with phytol. It shows that the interaction of FABP4 receptor with the ligand Phytol. These interactions can be identified by Hex tool.

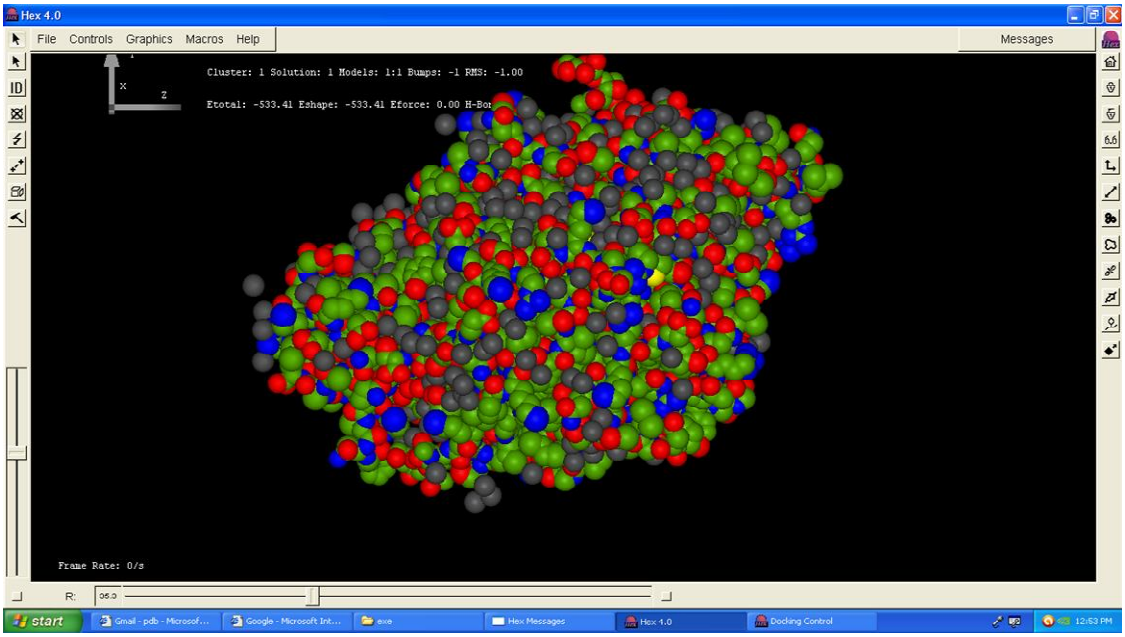


Fig 5: Docking result for FASN receptor with phytol. It represents the biding of FASN receptor with phytol.

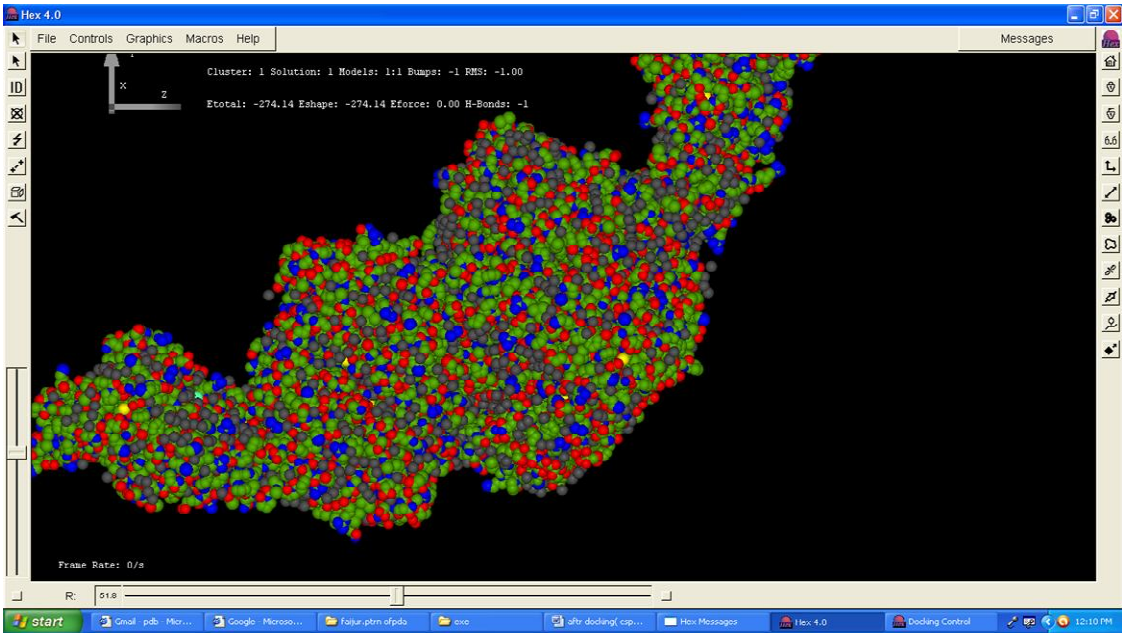


Fig 6: CSP receptor docked with Phytol. This fig shows that the docking of CSOP receptor with phytol and score values are also calculated.

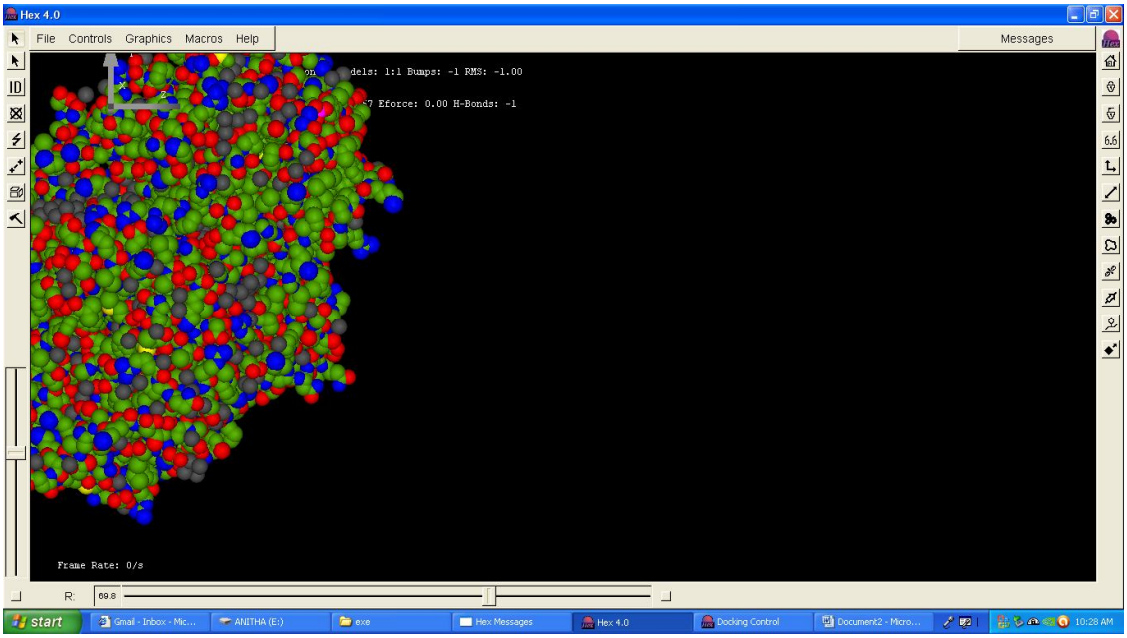


Fig 7: MTP receptor docked with Phytol. This fig which represents the interaction between MTP receptor and phytol.

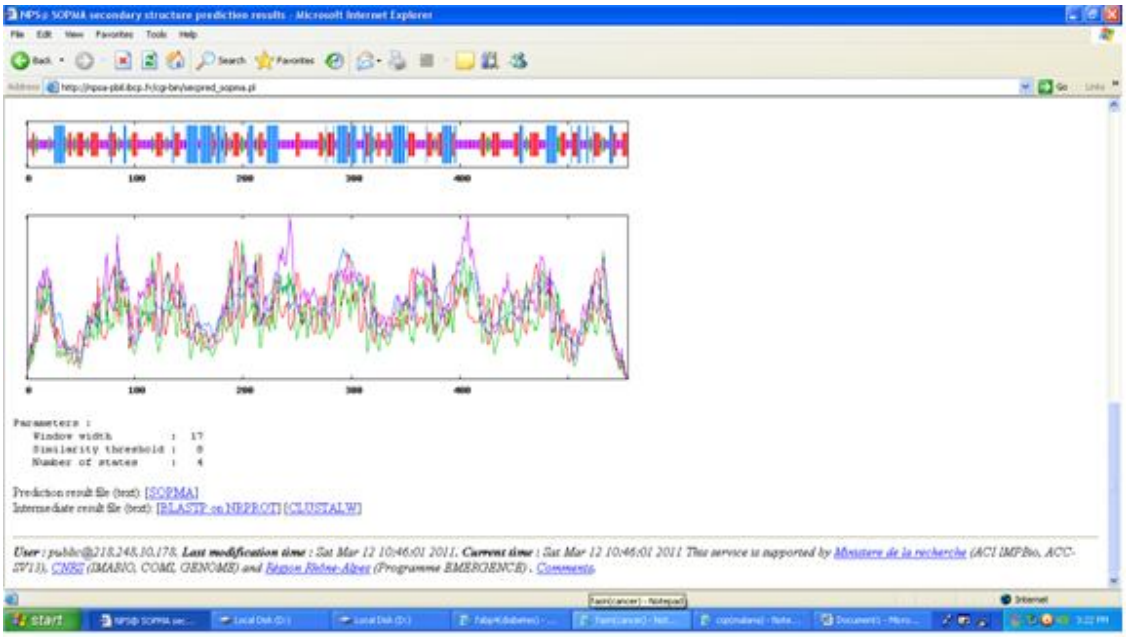


Fig 8: which indicates that the calculation of secondary structural factors by SOPMA tool. We can able to understand the composition of the factors from SOPMA tool.

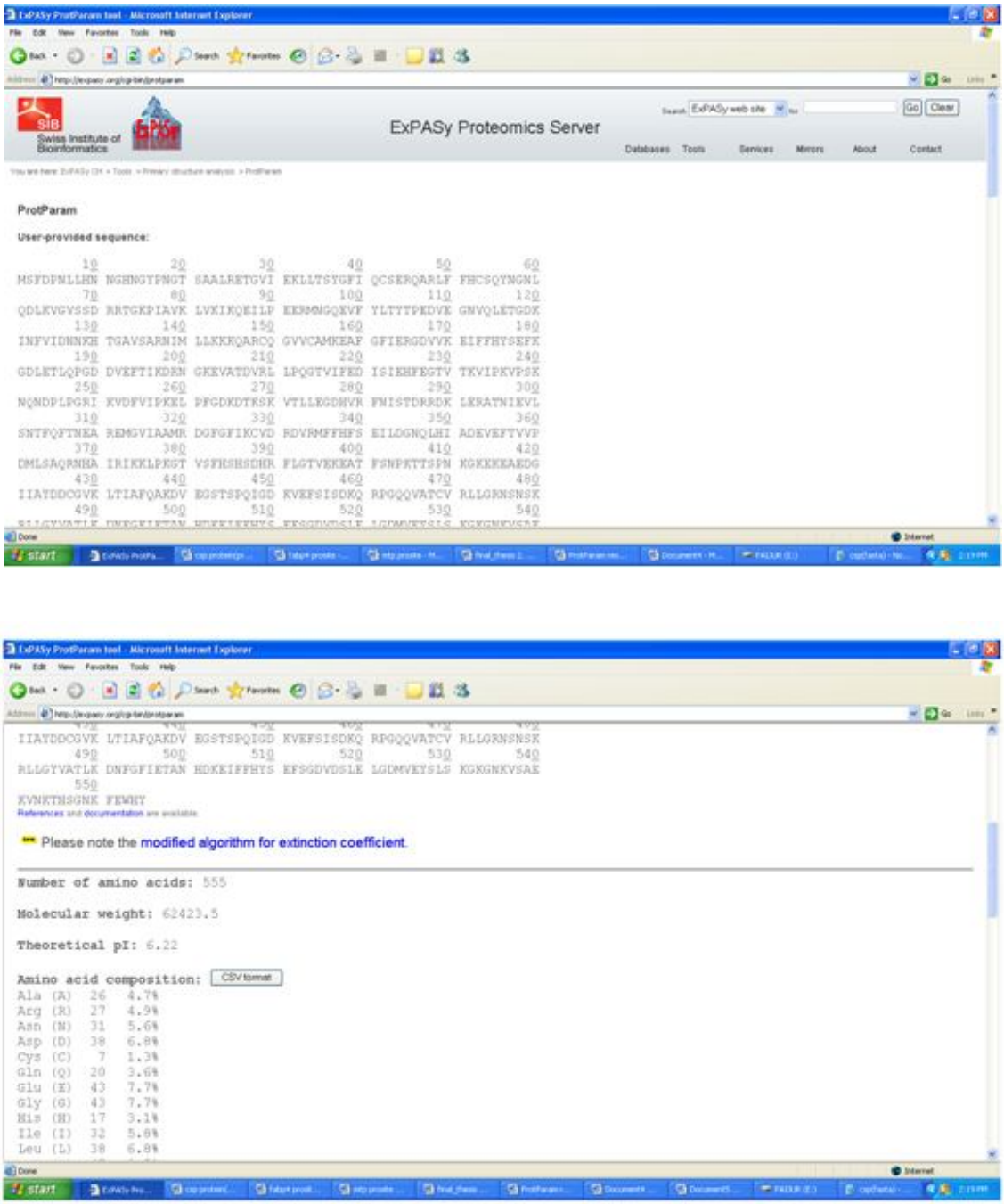


Fig 9: Calculation of protein parameters and composition of residues present in the sequence.

Table 3: Comparison of Docking Score values for various receptors with Phytol. The score values can be calculated from the Hex tool.

S.No	Receptor	Ligand	Docking Score value
1	FABP4	Phytol	86.21
2	FASN	Phytol	84.02
3	CSP	Phytol	83.91
4	MTP	Phytol	78.06

DISCUSSION

Trigonella foenumgraecum L. sample were collected and subjected to phytochemical study and identify the phytochemicals involved in it. Then the composition and percentage of each phytochemical compound identified from Gas Chromatography Mass Spectrometry results (Table 1). The diseased sequences were retrieved from NCBI database and subjected to PROSITE tool for the identification of active sites. Fig 2 and Fig 3 shows that the identification of binding sites for FABP4 and FASN protein by PROSITE tool. FABP4 protein has 5 hits and FASN protein has only one hit. (Fig 2 and 3). The hydrophobic nature of the protein was calculated by ALOGPS tool. (Table 3). The proteins which are subjected to hydrophobic analysis and calculate the stability. Fig 4 shows that the interaction of FABP4 receptor with the compound phytol. This interaction may be possible using Hex tool. FASN receptor docked with the compound phytol and calculates the docking scores by Hex tool. (Fig 5). Fig 6 and 7 shows that the docking of CSP and MTP receptor with the ligand. Table 4 shows that the comparison of docking scores among the receptors. The docking score was calculated using Hex tool. The secondary structure factors of the protein were calculated by SOPMA tool. (Fig 8) The parameters of the proteins were also calculated by PROTPARAM tool. (Fig 9)

CONCLUSION

This study provides the detailed analysis about phytochemical compounds extracted from *Trigonella foenum graecum* using GC-MS techniques and the process of drug designing. The phytochemical analysis shows that the compounds present in the plant *Trigonella foenum graecum.* We also focussed on docking studies and calculate the score values for all ligands. The receptors FABP4, FASN, MTP, CSP which is responsible for diabetes, cancer, malaria and dyslipedimea respectively. The parameters and the secondary structure factors are also calculated for these proteins. From these observations, we concluded that *Trigonella foenum graecum* is the ligand which is most responsible for diabetes mellitus. *Trigonella foenum graecum* is a best anti diabetic agent when compared with others.

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RESEARCH ARTICLE

Ethanol Production from Cheese Whey by Baker's Yeast

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ABSTRACT

The commercial baker's yeast was used for ethanol production from cheese whey. The initial lactose (42 g l -1) was metabolized by yeast within 24 hrs resulting in the formation of 0.5 g l -1 ethanol and biomass of about 1 g l -1. The same experiment was continued up to 72 hrs and the results were observed in 48 hrs and 72 hrs respectively. No variation was found in the ethanol production. Sugar utilization rate was observed within the respective fermentation time of 24 hrs, 48 hrs and 72 hrs. The yield co-efficient was decreased from 0.227g EtOH g-1S to 0.147 g EtOH g-1S when the time of fermentation was increased from 24 hrs to 72 hrs. From this study it is concluded that baker's yeast used for ethanol production from whey is not economically feasible but this raises a new perspective for alcoholic fermentation of whey by commercial baker's yeast.

Key words: Fermentation, Whey, Ethanol; Baker's yeast; Lactose; Anaerobic processes, EtOH.

INTRODUCTION

The world production of cheese whey is estimated to be over 108 tons and approximately10 liter of cheese whey is produced from every kg of cheese [1]. In India, 4.84 million tons of cheese whey is produced annually [2]. Cheese whey is a high strength wastewater with the BOD and COD contents of around 50 and 80g l -1 respectively and hence it is considered as an important source of environmental pollution [3]. Biological treatment of cheese whey by conventional activated sludge process is reported to be very expensive [4]. Typical cheese whey contains lactose (5-6 %), protein (0.8 – 1 %) and 0.06 % of fat [5] and has been used as starter material for production of organic acids, single cell protein, methane and cheese whey powder. The bioconversion of lactose in cheese whey to ethanol is an

attractive alternative to manage the huge volume of cheese whey generated, besides reducing the environmental impact caused by cheese whey discharge [6].

In Ireland some distillers are producing alcohol from cheese whey on commercial scale [7] while in New Zealand one fifth of cheese whey is being converted to ethanol [8]. Several studies had been carried out on the production of ethanol from cheese whey [9-10], cheese whey powder [11] and cheese whey permeate. *Kluyveromyces marxianus* [12],*Kluyveromyces fragilis, Saccharamyces cereivisiae*, and *Candida pseudotropils* [5] are being used as fermentative organisms in ethanol fermentation studies. Cost of raw material, mass culturing of fermentative microorganisms and ethanol separation from the fermentation broth are the major expenses involved in any ethanol fermentation process [4].

Cheese whey is a very inexpensive raw material for ethanol production having lactose content in the range of 5- 6 % [9]. Majority of the studies on ethanol production reported hitherto have employed axenic culture of fermentative microorganisms. Commercially available baker yeast is an inexpensive source of fermentative organism and can be employed for ethanol production in order to reduce the overall cost of ethanol production. To our knowledge no report exists on the use of commercially available baker's yeast in the bioconversion of lactose. Therefore, the present study was undertaken to assess the usefulness of commercially available baker's yeast in the bioconversion cheese whey to ethanol.

MATERIALS AND METHODS

Preparation of Inoculums culture

First grade Bakers yeast was obtained from the local supermarket. The inoculum culture was prepared in Yeast peptone lactose medium (YPL) [12]. One hundred milligram of baker yeast was inoculated in a 250 ml capacity Erlenmeyer flask containing 100ml of YPL medium aseptically. The culture flasks in duplicate were kept over a rotatory shaker set at 150 rpm for 72 hrs at room temperature.

Analytical methods

Biomass was measured in terms of dry weight. Yeast cells were harvested by centrifugation for 10 min at 5000 rpm and then washed twice with distilled water and weighed after 24 hrs at 100 0C. Lactose in cheese whey was estimated following the method of Lane- Eynon [13]. The total reducing sugar was estimated according to the method of phenol- acid method [14]. Ethanol concentrations were measured using a Gas Chromatography with an FID detector and WCOT used silica capillary column (15m x 0.25 mm i.d., 0.25µm film thickness). The column temperature was set at 75 ^{0}C for 1 min and raised to 130 ^{0} C with a rate of 20 ^{0} C/min yielding a total holding time of 4.75 min. Temperatures of injector and detector were 150 and 200 ^{0} C, respectively. Nitrogen was used as the carrier gas with a velocity of 25ml min -1.

Fermentation

Fermentation was performed in a 250 ml capacity Erlenmeyer conical flask containing 100 ml of deprotenized cheese whey. To this 10 ml of inoculum culture was introduced and kept on rotatory shaker at an agitation of 150 rpm for 72 hrs at room temperature. Then, the fermented broth was filtered through whatman No.4 and the supernatant was used for estimating ethanol yield.

RESULTS AND DISCUSSION

Ethanol production from cheese whey was studied in detail. Fig.1 shows that the bio conversion of whey to ethanol by commercial baker's yeast. Most of the initial lactose 42 g l -1 was metabolized by the yeast within 24 hrs resulting in the formation 0.5 g l -1. Ethanol and biomass produced was about 1gl-1. The same experiment was continued up to 72 hrs and the results were observed respectively in 48 hrs and 72 hrs. No variation had been found in the ethanol production. The sugar utilization rate was observed within the respective fermentation time of 24hrs, 48 hrs and 72 hrs. The peak utilization was found at the fermentation time of 72 hrs as shown in Fig .2. The ethanol concentration reached maximum at 24 hrs. But it decreased considerably after the fermentation time of 24 hrs. This may be attributed to the accumulation of ethanol in the broth leading to product inhibition. There was no ethanol formation or sugar utilization in the control flask. The results of duplicate experiments were almost the same with (0.2) deviation.The variation of the product yield co-efficient within different fermentation time was as shown in Fig.3. The yield co-efficient was decreased from 0.227 g EtOH g-1S to 0.147 g EtOH g-1S when the time of fermentation was increased from 24 hrs to 72 hrs.

CONCLUSION

The fermentation of whey by using commercial baker's yeast without any supplements resulted in low ethanol production. Maximum ethanol production 0.5 g l -1 was observed at 24 hrs. The lactose of whey is 4.2 % and protein 0.6 %. From this study it is concluded that baker's yeast used for ethanol production from whey is not economically feasible but this raises a new perspective for alcoholic fermentation of whey by commercial baker's yeast.

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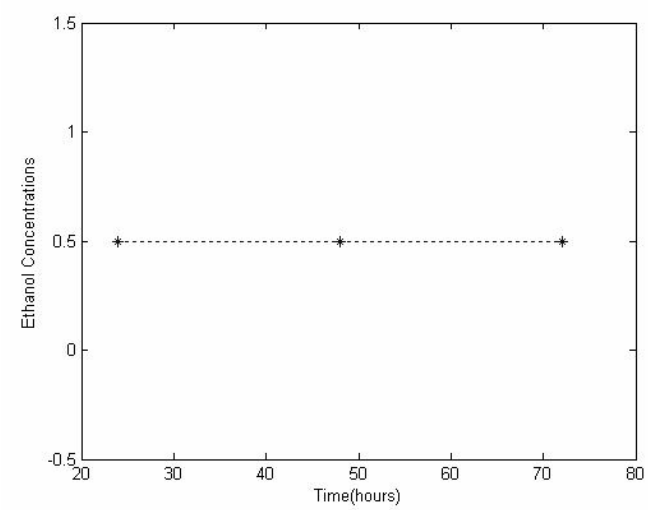

Fig.1 Ethanol production ratio in different hours

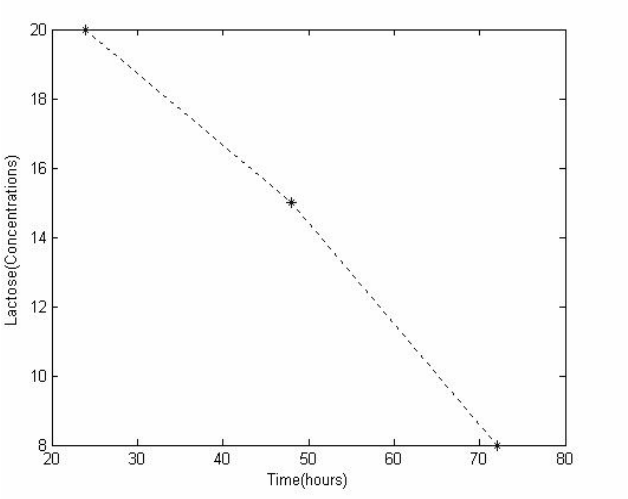

Fig.2 Sugar consumption ratio in different hours.

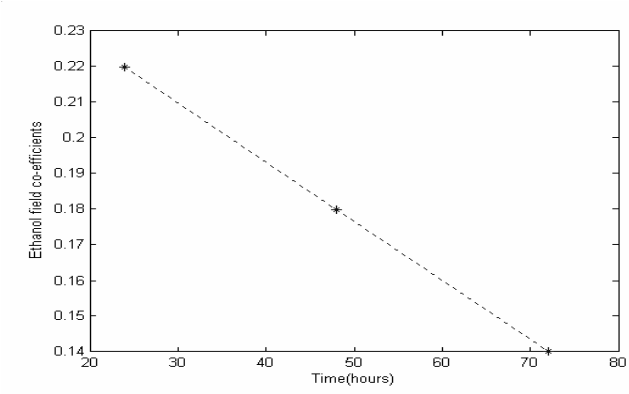

Fig.3 Ethanol yield coefficient in different hours.

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RESEARCH ARTICLE

Insilico Studies on *Borassus flabellifer*L. Peroxidase using Bioinformatics Tools.

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ABSTRACT

Peroxidases are widely believed to catalyze the last enzymatic step in the biosynthesis of lignin, the dehydrogenation of the *p*-coumaryl alcohols. This study shows that the insilico analysis of *Borassus flabellifer* L. peroxidase. The sequence can be retrieved from Protein Data Bank and subjected to Protparam and SOPMA tool. It shows the parameters and composition of the protein. The amino acid such as Alanine and Serine has the highest composition 46 and 40 respectively in *Borassus flabellifer* L. peroxidase. The composition of hydrogen atom and Carbon atom is 2257 and 1405 respectively. The secondary structure Parameter alpha helix has present in highest contribution (42.90%) when compared with other parameters. The SOPMA tool shows the other secondary structure parameters such as Random Coil, Beta Bridge, Extended Strand etc. From these observations we concluded that due to the larger number of the residues Alanine and Serine, it has various medicinal applications.

Keywords: *Borassus flabellifer* L., Plant derived peroxidases ,Isoenzyme , *Insilico* analysis

INTRODUCTION

Peroxidases occur throughout the biosphere and participate in a large number of essential oxidative reactions. The homologous super family of heme peroxidases discussed here has been divided into three classes based on biological origin and sequence alignments. The first class which shows the Intracellular plant and bacterial peroxidases (Prokaryotic Cytochrome C peroxidase) [6].The second class shows that the secretory fungal peroxidases (fungal peroxidase Lignin peroxidase [11]. The third class consists of the Plant peroxidases (HRP), Zapota, R.communis, tomato peroxidases.Plant peroxidases participate in a wide variety of pathways, including the synthesis of the cell wall component lignin and suberin, metabolism of hormones such as indole-3-acetic acid (IAA), stress response mechanisms, and fatty acid metabolism [7].

Of the various peroxidases, the class III enzymes are most readily available and have been more extensively studied and used than other peroxidases. Their abundance, together with the stability and distinguishing spectral properties of the various peroxidase intermediates, led plant peroxidases to serve an important role in the early development of modern enzymology[9]. The dramatic and measurable color changes accompanying the oxidation of certain peroxidase substrates such as tetramethylbenzidine is the basis for various commercial applications, including bioassays [10].

Most of the functions attributed to plant peroxidases occur in cell walls. These functions can be divided into two main categories. The first is the oxidative cross-linking or coupling of many aromatic molecules by using hydrogen peroxide as an electron acceptor. This leads to the formation of lignin or suberin and also to the establishment of covalent bonds between hydroxycinnamate ester moieties or flavonoids associated with pectins or hemicellulose . By catalyzing these reactions, peroxidases are involved in the construction of cell walls and in the control of cell wall plasticity [1].

It has 5- coordinated high spin ferric ion as the prosthetic group. In addition to one mole of ferric ion, two moles of calcium per mole of enzyme were present which are required to maintain the structural integrity of the protein at high temperatures [56].In common with other disease situations, rust-resistant wheat leaves show a large increase in peroxidase activity during infection**.** Peroxidase isozymes from healthy or infected lines of wheat (*Triticum aestivum* L.) near isogenic for resistance and susceptibility to race 56 of *Puccinia graministritici* were separated by gel electrophoresis and the activity of each was estimated by photometric scanning. 14 isozymes were detected in both healthy and infected leaves, increases in only 1 (isozyme 9) were associated consistently with the development of resistant disease reaction at 20^0 C[12].

Nickel toxicity was evaluated in *Triticum aestivum* L. by its effects on root and shoot length, dry matter production and water content. The degree of toxicity increases as a function of the Ni^{2+} concentration in the medium. Ni^{2+}-treated roots show enhanced lipid peroxidation. In roots and shoots, Ni^{2+} enhances both guaiacol and syringaldazine extracellular peroxidase activity. The increase in extracellular peroxidase activity is also associated with an increase in the phenolic contents of roots and shoots. Intracellular soluble peroxidases are also stimulated by Ni^{2+}. Intracellular peroxidases might act as scavengers of peroxide radicals produced as a result of nickel toxicity.Lignin is an integral cell wall component of all vascular plants. Peroxidases are widely believed to catalyze the last enzymatic step in the biosynthesis of lignin, the dehydrogenation of the *p*-coumaryl alcohols. Five different peroxidases of *Populus trichocarpa* (PXP 1, PXP 2, PXP 3-4, PXP 5, and PXP 6) were isolated from the xylem .Because these isoenzymes were specifically or preferentially expressed[3]. xylem, PXP 3-4 and PXP 5 are suggested to be involved in lignin polymerization [11].

Nicotiana tabacum and N. sylvestris transformed plants with peroxidase activity that is 10-fold higher than in wild-type plants by introducing a chimeric gene composed of the cauliflower mosaic virus 35S promoter and the tobacco anionic peroxidase cDNA. The elevated peroxidase activity was a result of increased levels of two anionic peroxidases in *N. tabacum*, which apparently differ in post-translational modification. Transformed plants of both species have the unique phenotype of chronic severe wilting through loss of turgor in leaves, which was initiated at the time of flowering [3].

It was concluded that the over expression of the tobacco anionic peroxidase in transformed plants results in diminished root mass from fewer root branches, which contributes to the wilting phenomenon seen in these plants. Further, this developmental change in transformed plants may be a consequence of the metabolism of IAA by the anionic peroxidase [8]. Enzyme linked immunorbent assay (ELISA) tests on which peroxidase is probably the most common enzyme used for labeling an antibody, are a simple and reliable way of detecting toxins, pathogens, cancer risk in bladder and prostate, and many other analytes[4]. Peroxidase-modified amperometric electrodes have been widely studied and developed, not only because of hydrogen- and organic peroxides are important analytes but also

because of the key role of hydrogen peroxide detection in coupled enzyme systems, in which hydrogen peroxide is formed as the product of the enzymatic reaction. Many important analytes, such as, aromatic amines, phenolic compounds, glucose, lactate, neurotransmitters, etc. could be monitored by using bi- or multi-enzyme electrodes [10]. Adsorption of the reaction products on the talc effectively protected the biocatalyst against contamination by oxidative products, thereby prolonging its catalytic action and leading to almost complete elimination of phenols in aqueous media [2].

METHODOLOGY

The sequence for *Borassus flabellifer*L.Peroxidase was retrieved from Data Bank and subjected to Protein Parameter Analysis. The sequence was submitted in to Prot Param tool for calculating the Protein Parameters. This tool provides the composition of Amino Acids and Atoms which is present in the sequence. The Sequence was also subjected to SOPMA tool for the prediction of secondary structure parameters. It shows the structural parameters such as alpha helix, beta sheet, Random Coils, Extended Strand, etc. These parameters are very essential for structure prediction.

RESULTS AND DISCUSSION

PROTPARAM TOOL

ProtParam computes various physico-chemical properties that can be deduced from a protein sequence. If you provide the accession number of a Swiss-Prot/TrEMBL entry, you will be prompted with an intermediary page that allows you to select the portion of the sequence on which you would like to perform the analysis. The choice includes a selection of mature chains or peptides and domains from the Swiss-Prot feature table, as well as the possibility to enter start and end position in two boxes.

The parameters computed by ProtParam include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY). Molecular weight and theoretical pI are calculated http://expasy.org/tools/pi_tool.htmland the amino acid and atomic compositions are self-explanatory. The extinction coefficient indicates how much light a protein absorbs at a certain wavelength. It is useful to have an estimation of this coefficient for following a protein which spectrophotometer when purifying it.

SOPMA

Recently a new method called the self-optimized prediction method (SOPM) has been described to improve the success rate in the prediction of the secondary structure of proteins. In this paper we report improvements brought about by predicting all the sequences of a set of aligned proteins belonging to the same family. This improved SOPM method (SOPMA) correctly predicts 69.5% of amino acids for a three-state description of the secondary structure (alpha-helix, beta-sheet and coil) in a whole database containing 126 chains of non-homologous (less than 25% identity) proteins. Joint prediction with SOPMA and a neural networks method (PHD) correctly predicts 82.2% of residues for 74% of co-predicted amino acids.

Prot Param – Primary

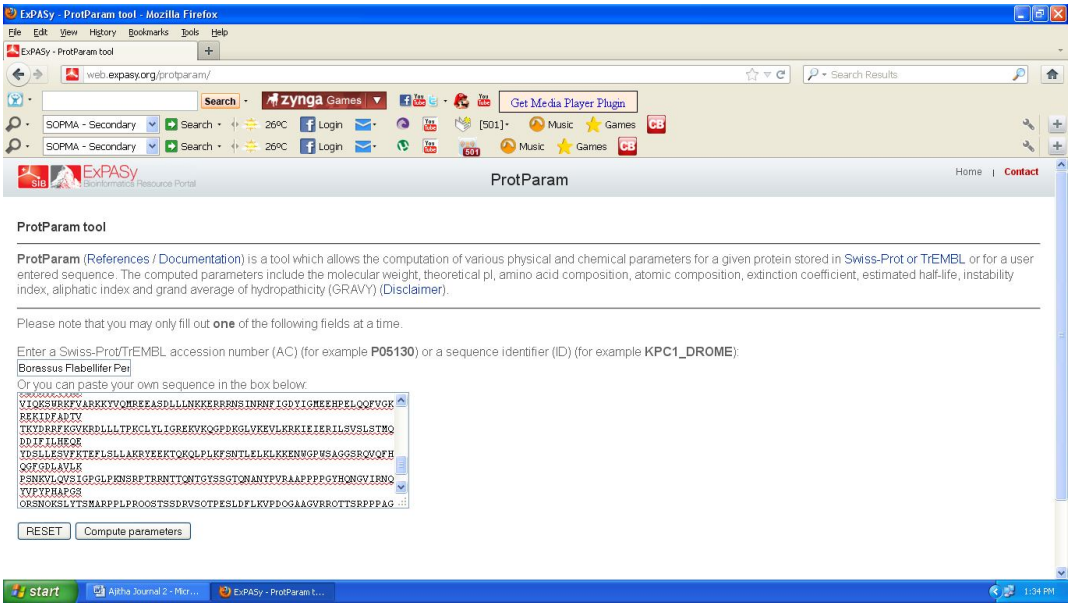

Fig 1: *Borassus flabellifer* L. Peroxidase submitted to Prot Param tool Protein Parameter Calculation

Number of amino acids: 317, Molecular weight: 32875.7, Theoretical pI: 5.77

Amino acid composition:

Ala (A) 46	14.5%	Arg (R) 14	4.4%	Asn (N) 24	7.6%
Asp (D) 17	5.4%	Cys (C) 8	2.5%	Gln (Q) 13	4.1%
Glu (E) 6	1.9%	Gly (G) 21	6.6%	His (H) 4	1.3%
Ile (I) 11	3.5%	Leu (L) 30	9.5%	Lys (K) 6	1.9%
Met (M) 7	2.2%	Phe (F) 12	3.8%	Pro (P) 10	3.2%
Ser (S) 40	12.6%	Thr (T) 23	7.3%	Trp (W) 1	0.3%
Tyr (Y) 4	1.3%	Val (V) 20	6.3%	Pyl (O) 0	0.0%
Pyl (O) 0	0.0%	Sec (U) 0	0.0%		

Total number of negatively charged residues (Asp + Glu): 23 ,Total number of positively charged residues (Arg + Lys): 20

Atomic composition

Carbon C	1405
Hydrogen H	2257
Nitrogen N	411
Oxygen O	468
Sulphur S	15

Formula: $C_{1405}H_{2257}N_{411}O_{468}S_{15}$
Total number of atoms: 4556
Extinction coefficients:
Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.
Ext. coefficient 11960
Abs 0.1% (=1 g/l) 0.364, assuming all pairs of Cys residues form cystines
Ext. coefficient 11460
Abs 0.1% (=1 g/l) 0.349, assuming all Cys residues are reduced

SOPMA - Secondary

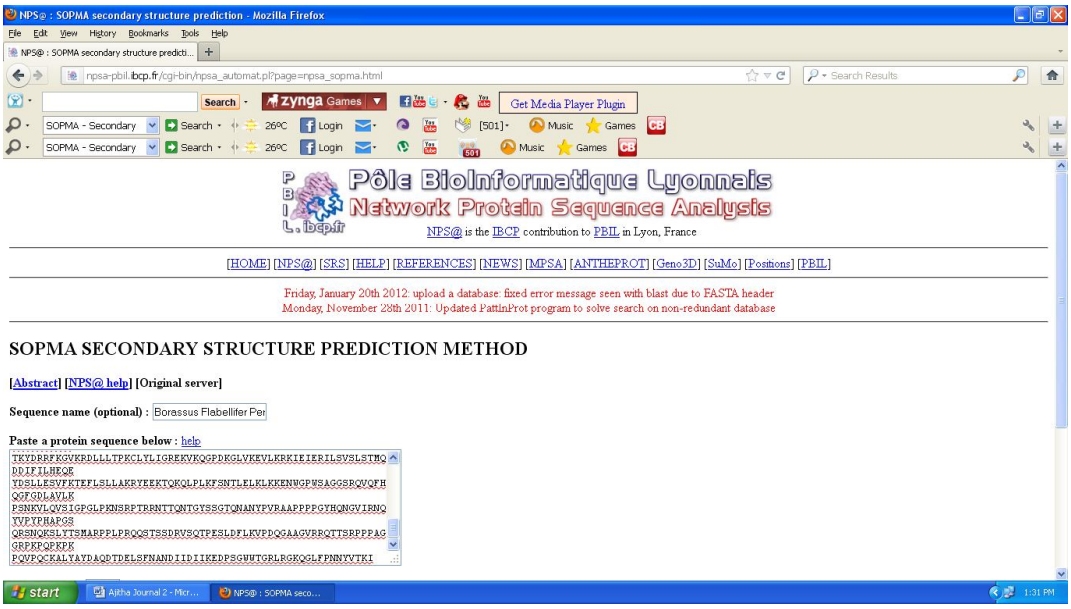

Fig 2: Secondary Structural Parameters by SOPMA tool

```
        10        20        30        40        50        60        70
         |         |         |         |         |         |         |
MASATNSSLSLMLLVAAAMASVASAQLSATFYDTSCPNALSTIKSVITAAVNSEARMGASLLRLHFHDCF
hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh
VQGCDASVLLSGQEQNAGPNVGSLRGFSVIDNAKARVEAICNQTVSCADILAVAARDSVVALGGPSWTVL
ettccceeeeeccccccccccccccchhhhhhhhhhhhhhccccccchhhhhhhhhtchheeeetccceeee
LGRRDSTTASEALANTDLPAPSSSLAELIGNFSRKGLDATDMVALSGAHTIGQAQCQNFRDRIYNETNID
ecccccchhhhhhccccccccccchhhhhhhhhhhttcchhheeeettcccccccchhhhhheecccccc
SAFATQRQANCPRPTGSGDSNLAPVDTTTPNAFDNAYYSNLLSNKGLLHSDQVLFNGGSADNTVRNFASN
chhhhhhhhccccccccccccccccccccccchhhhhhhhhttceeccheeecccccchhhhhhtc
AAAFSSAFTTAMVKMGNISPLTGTQGQIRLSCSKVNS
chhhhhhhhhhhhhhtcccccccccccceeeccheect

Sequence length :   317

SOPMA :
   Alpha helix      (Hh) :   136 is  42.90%
   3(10) helix      (Gg) :     0 is   0.00%
   Pi helix         (Ii) :     0 is   0.00%
   Beta bridge      (Bb) :     0 is   0.00%
   Extended strand  (Ee) :    40 is  12.62%
   Beta turn        (Tt) :    17 is   5.36%
   Bend region      (Ss) :     0 is   0.00%
   Random coil      (Cc) :   124 is  39.12%
   Ambigous states  (?)  :     0 is   0.00%
   Other states          :     0 is   0.00%
```

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RESEARCH ARTICLE

Isolation and Identification of Polyhydroxyalkanoates (PHAs) Producing Bacteria *Pseudomonas aeruginosa* EB4 from Petroleum Oil Contaminated Soil.

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ABSTRACT

To study the production of Poly(3-hydroxybutrate-co-3hydroxyvalerate) ,P (3HB-co-3HV) copolymer by strain *P.aeruginosa* EB4 isolated from petroleum oil contaminated soil. The Nile blue A staining was done to screen for PHAs producing bacteria followed by nucleotide sequencing which analysis further confirmed the bacterial strain, and finally these isolates were then subjected to identification and quantification of P(3HB-co-3HV) co polymer using GC-MS. These results demonstrate that the environmental bacterial stains were able to accumulate the PHA which was confirmed by Nile blue A staining method, and also GC-MS was used to quantify by the PHA produced by the stain. The result obtained confirms that the PHA produced by *P.auroginosa* EB4 was P(3HB-co-3HV)copolymer.An important part of this study is that the isolated and identified bacterial strain was able to produce significant amount of P(3HB-co-3HV) copolymer from the commercial medium. The condition for achieving higher yield and productivity will be optimized in the next phase using fermentation studies. This approach can be adapted for potential industrial applications. Its also opens up new possibilities for various industrial applications owing to the superior properties of this co polymer using biological systems.

Key words: Microbial polyester, *Pseudomonas* sp., Nile blue A, GC-MS, P (3HB-co-3HV)

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are a group of polyesters synthesized by bacteria, archaea, as well as some fungi [10].This polyester are biodegradable in nature and the properties are similar to commercial plastics[1]. Pure Poly(3-hydroxybutyrate) P(3HB) is brittle and has a low extension to break, and thus limits its range of applications. In the recent time, copolymer such as P(3HB-co-3HV)have capture much interest as it is highly crystalline, more ductile, easier to mold and tougher, compared to pure P(3HB)[6]. In addition, it has been known to posses to have potential applications in medicine, material science and agriculture, and etc.

A few bacterial species, such as *Aeromonas caviae*, *Bacillus megaterium* and some species of Pseudomonas (*P. fluroresens, P. sp. A33, P. marginalis, P. mendocina and P. sp 61-3)* were found to accumulate the copolymer of SCL- MCL -PHAs with a maximum carbon skeleton up to C_{14}.[7]. The major drawback encountered is due to the high cost involved in large scale production[16]. These troubles are various important factors influencing the production cost which include the product yield, polymer recovery and the capital equipment costs. These troubles are being mitigated by using an efficient recombinant PHA production system to advance the product yield [17]. The copolymer of the P(3HB-co-HV) are far less permeable to oxygen than polyethylene and polypropylene. This character makes copolymers a superior material for food –packing as there is a reduced necessity for addition pf antioxidents [16,18]. In order to satisfy the minimal industrial standards for screening commercially useful organisms for PHA production, we have adopted a polyphasic approach to screen for environmental microbes that are not only able to produce MCL-PHAs, but can also accumulate substantial amount of PHAs inside the cells. More then 140 different hydroxyalkanoic acids have been acknowledged as constituents of microbial PHAs when bacteria were cultivated under nitrogen limited conditions [15].

Pseudomonas classified under RNA homology group I were capable of synthesizing MCL–PHAs when they are cultivated on various aliphatic alkanes or aliphatic fatty [8], but only few exceptions are able to synthesize MCL-PHAs from glucose alone and other structurally unrelated carbon source[7,14]. New strains that are capable of utilization cheap carbon substrate should be identified. Interest has been centered on Gram-negative bacteria due to the because of selective pressure which was caused by the imbalance of the carbon/nitrogen ration. This imbalance was caused by decomposition of residues in the soil leading to an excess of carbon and nitrogen limitation. Our major purposed in this study is screen for and identify bacteria which would produce significant amounts of PHAs from oil contaminated site.

MATERIALS AND METHODS

Isolation of PHA – producing microorganisms from petroleum contaminated soil

Soil samples were collected from oil contaminated soil from Indian petroleum Chennai, Tamilnadu. Soil samples were serially diluted, whereby each dilution was spread on nutrient agar plates, and the plates were incubated at 37°C for 24 hours. Bacterial strains isolated from these environments were screened for PHA production. Mineral salt medium (MSM) was used for the production of PHA. pH of the media was neutralized and was sterilized before use.

Cell cultivation

Identification bacterial strains was first grown in nutrient broth for 24 h in 250 ml Erlenmeyer flask and it was incubated at 37 °C with shaking. After 24 h, cells were then harvested by centrifugation at 8000 rpm for 12 min, and were washed aseptically with sterile distilled water and were resuspeneded into 1L of MSM medium. The carbon source used is glucose (10g/ml) and were incubated at 37 °C for 48 h. Upon r incubation, cells were harvested by

centrifugation at 8000 rpm for 12 min, Cells was washed with sterile distilled water and recentrifuged similarly. Cell pellets was used for further studies.

Nile blue A Staining

Heat fixed bacterial smear was stained with 1% aqueous solution of Nile blue A at 55 °C for 10 min. The slide was washed with tap water to remove excess stain and then washed with 8% aqueous acetic acid for 1 min .The staining smear was again washed with water and blot dried. Prior to observation, the slide was remoistened with a drop of water and coverslip was placed on the smear [19]. The slides were viewed in fluorescence microscopy at wavelength of 480 nm.

Extraction of DNA for sequence analysis

Bacterial cells were harvested and washed twice with 1ml of phosphate buffered saline (PBS). Extraction of DNA was carried out .For amplification of 16s rRNA gene by polymer chain reaction (PCR). The 16s rRNA was amplified using with bacterial universal primers f24 and f25 as previous. Purified DNA obtained from PCR was then sequenced by using a LICOR IR2 4200 DNA sequencer (LI-COR, Lincoln, NE, USA) according to manufacturer's instructions. The sequence of 16S rRNA gene was compared with the 16S rRNA gene sequences available in the NCBI public data-bases.

Extraction and purification of PHA

PHA was extracted from dried cells material with chloroform at 100 °C for 3 h. The chloroform solution was filtered to remove any cellular debris and it was concentrated by rotary evaporator. PHA polymer was precipitated from the chloroform solution by adding chilled methanol (1:10) drop wise. The methanolchloroform mixture was then centrifuged at 5000 rpm.Then the polymer was dissolved in chloroform and was again precipitated in methanol to obtained highly purified polymer[14]. Finally, it was dried in (Bucchi, Switzerland) room temperature and weight of the polymer was measured. The polymer content was calculated as the percentage of PHAs in the cell upon drying. Residual cell mass (RCM) was defined as cell dry weight (CDW) minus the PHAs concentration.

Preparation and analysis of PHAs

To determine the polymer content of the cells and its composition, the precipitated PHAs was subjected to methanolysis.Approximately 5mg of precipitate was subjected to methanolysis (3h at 100 °C) with a solution consisting of 1.7 ml of methanol, 0.3 ml of 98% sulfuric acid and 2ml of chloroform [9]. After phase separation and two washes with water, the organic phase (bottom) was dried with anhydrous sodium sulphate. Separation of methylesters was performed using a Shimadzu GC-MS (QP-2010) with splitless injection. The GC-MS was operated with an interface temperature of 270ºC and an ion source temperature of 230ºC. The mass spectrometer was used with silica capailary column (30m x 0.32 mm, thinness 0.25µm, J & W scientific, folsom, CA, USA). Helium with a maximum purity 99.999 % was used as the carrier gas at a flow rate of 2.25 ml/min.The gas chromatography was equipped with split/splitless injector port operated mode at a colum temperature of 70ºC using an auto sampler AOC-20; containing 10µl syringe the GC oven temperature was programmed as follows ;initial temperature of 50 ºC for 1 min, from 50 ºC to 220 ºC at a rate of 30 ºC/min and finally to 320 ºC at a rate of 20 ºC/min.The mass spectrometer was operated in the positive ion electron impact EI mode using ionization energy of 70 eV and an emission current of 60µA. Full scan data were obtained with a mass range of m/z 35-500. Scanning interval and SIM sampling rate were 0.5 and 0.2 sec, respectively. The mass selective detector was operated in selected ion monitoring (SIM) mode. The initial structural assignment of the methylesters analyzed based on their retention times compared to those of authentic standards.

RESULTS

PHA producing bacterial strains that was isolated from oil contaminated soil were investigated for its PHA producing ability by staining and then further confirmed through GC-MS. The strains were stained based on the production capacity is tested by using Nile blue A staining method its observed in florescence microscopy at wavelength of 480 nm followed by stain identification and it was evident that the belongs to the genus *Pseudomonas* and closely related to *P.aeruginosa* stain. We can further confirm that *Pseudomonas aeruginosa* EB4 strain is producing PHA (Genbank accession Number is GU 131270). The carbon source was always supplied in excess to allow maximum accumulation of PHB. However under nonoptimized condition, the total accumulation of PHB in *P.aeruginosa* EB4 does not exceed 20% (wt/wt) of the cell dry weight when compared to other organism, such as *A .eutrophus* which has been reported to accumulated up to 90% (wt/wt) of the biomass production [5].

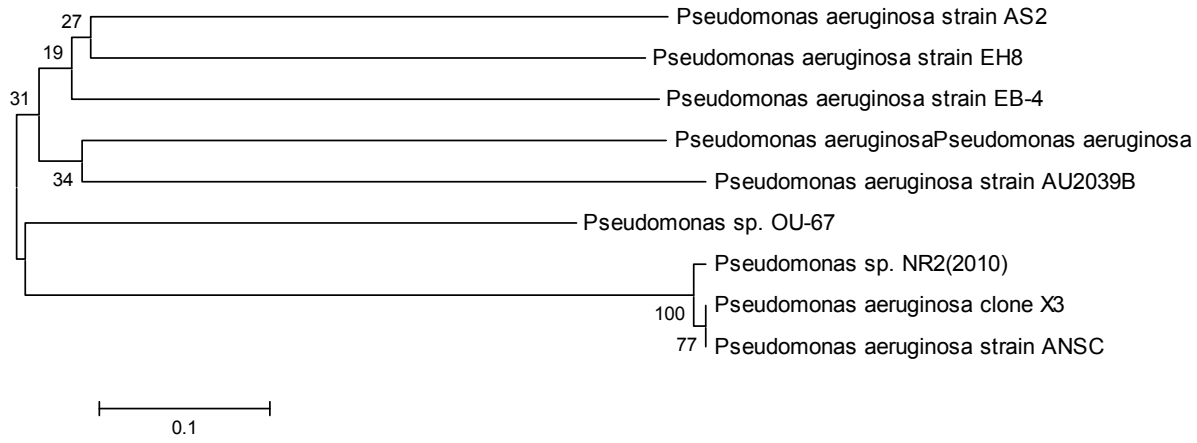

Fig: 1. Phylogentic tree based on 16s rRNA gene sequence for *Pseudomonas aeruginosa* EB 4

Further confirmation was done using GC-MS to identify and quantify PHA production. From shank-flask cultivation, up to 20% of P(3HB-co-3HV) copolymer was accumulated at 48 h under controlled culture condition. This polyhydroxyalkanoates accumulation was studied under the interactive condition of N- deficiency in presence of glucose as the substrate. The cassation of P(3HB) accumulation in *A. eutrophus* was a result from a physical space limitation [2]. This is probably not the case for *P. pseudoflava* and studies should be done to optimize the growth and PHB accumulation as well as to identify and modify the control mechanism that limits accumulation in organism that produced little PHB.

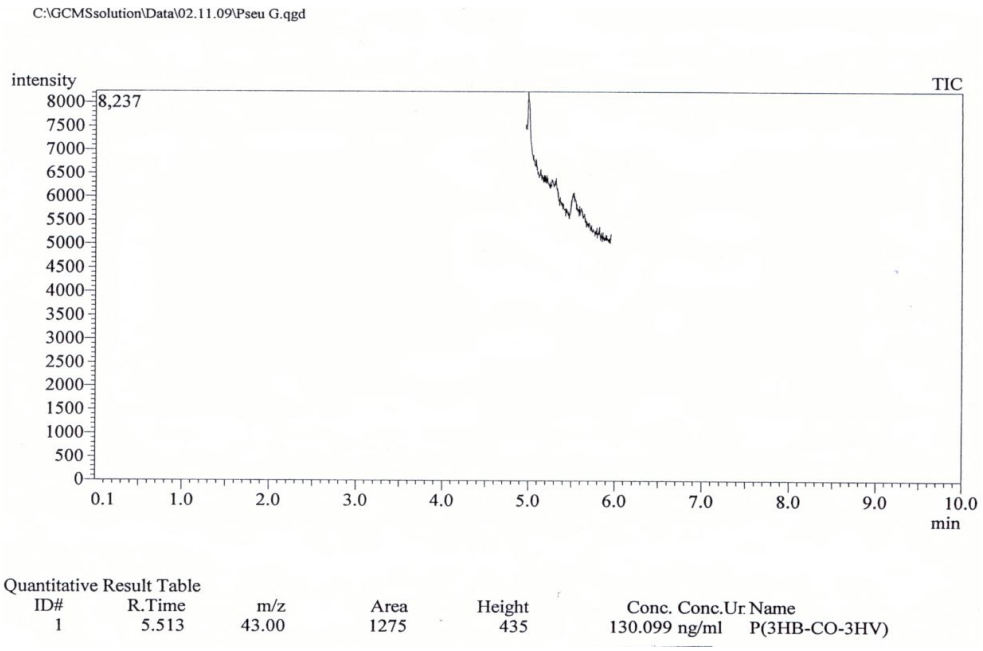

Fig :2.GC-MS chromatogram of P(3HB-co-3HV) from *P. aeruginosa* EB 4

DISCUSSION

Many *Pseudomonas* belonging to the rRNA homology group I have the ability to produce PHA polymer from a number of different substrates. In most cases, the building blocks of PHAs are incorporated in to the polymer only if the cells are cultivated on carbon skeleton that is related to the structure of the constituent monomer unit [8]. In some cases, of course, the PHA monomers do not have similarity with carbon sources; this was described as the capacity of the organism to synthesize PHA from unrevealed substrates[1,5,14]. This is very important as these organisms allow synthesis of PHA from simple substrate rather than from expensive substrate, these increasing the possibility of cost - effective production in large scale [4]. *Pseudomonas aeruginosa*, *P. stutzeri* and other fluorescent pesudomonads including *P.putida* and *P.mendocina* were reported to PHA through accumulate glucose and gluconate. The isolated organism, *P. aeruginosa* EB4, accumulated (3HB-co-3HV) copolymer which indicates PHA accumulation through fluorescence, Pseudomonas belonging to r RNA homology group I, where fatty acid de novo biosynthesis is the main route that supplies (R)-3–hydroxyacyl–coA intermediate as metabolic precursor for PHAs biosynthesis[9]. This is advantageous in the sense that under limited condition the oil contaminated soil isolated *P. aeruginosa* EB4 accumulate co polymer (wt/wt)20 % P(3HB-co-3HV) copolymer.

However, under nitrogen deficient condition, NADPH consumption was decreased due to unavailability of nitrogen pool, which blocks the aminoacid synthesis pathway, especially the reaction from α –ketoglutamate, thus resulting in an accumulation of excess NADPH in the cells. This residual NADPH might be responsible for the enhanced PHA synthesis in nitrogen deficient cells [2,3]. In this study, the polymer yield in *P. aerugienosa* EB4 not only boosted up to 20% but also the production of copolymer P(3HB-co-3BHV), which exhibits novel capacity to accumulate PHA utilizing glucose as a sole carbon source. Apart from this , it is also evident that the test organism is capable of synthesizing the co-polymer which is desirable for commercial application as the polymer properties are greatly influenced by their polymer composition [11,12,13].`However, this warrant future research on using statistical

optimization tools, such as response surface mythology,which is a better economical approach that could located that real optimum levels of most significant parameters for maximum yield of the copolymer by *P. aeruginosa* EB4 with minimum effort and time. Thus the isolated strain *P. aeruginosa* EB4 produced the polymers and P(3HB-co-3HV), using single carbon source, with novel properties, which suggests that it is an ideal and a potent stain that could effectively replace the commercial nondegradable plastic production.

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RESEARCH ARTICLE

Assessment of Water Quality and Chemical Parameters of Bhima River, Near Saradagi Barrage, Gulbarga, Karnataka,India.

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ABSTRACT

The water quality and some chemical parameters were measured at three sampling stations namely Before Sewage entry (S1), After Sewage entry (S2) and Water Lifting Station near Barrage (S3) for a period of three month March to May 2012 in the River Bhima Near Saradagi Barrage, Gulbarga of Karnataka State (India). The pH ranged from 8.3 to 8.61, the level of total hardness in S1 station is lower as compare other stations. Station S1 is 250 mgl^{-1}, S1 station registered a minimum level of magnesium and calcium is 22.84 mgl^{-1} and 22.4 mgl^{-1} respectively. The amount of chloride content ranges from 92 to 96 mgl^{-1}. In the present study S1 station (before sewage entry) getting values in minimum concentration as compare to other stations. In this current investigation river water is slightly polluted due to sewage entry to the river. According to World Health Organization the concentration of few chemical parameters are getting within permissible limit, so the water is used for drinking and agricultural usage.

Keywords: Sewage entry, chemical parameters, World Health Organization.

INTRODUCTION

Water is most essential factor for all living organisms. Availability of fresh drinking water is negligible amount in the planet of Earth. Water is polluting from several man-made activity. An increase of population growth and migration of rural to urban areas is one of the major causes to pollute surface water as well as ground water. Water quality is a very important consideration for all water development projects as it effects of water use-for humans, for animals, for crops and even for industry. Increasing urban population and changing their life style may increases chemical concentration of sewage water and quantity of sewage water.

Human health threatened by improper management of drainage pattern and city sewage. Waterborne diseases occur mainly due to lack of safe drinking water and sanitation facilities (EICK, 2009). According to World Health Organization (WHO), about 80% of all the diseases in human beings are caused by water. The improper management of local municipal authority, point source of sewage water entering into River Bhima near Saradagi Barrage. Agricultural and human activity may also cause water pollution. The negligence of sewage water entire to the river may cause water pollution; once water gets contaminated it is very expensive to remove impurities in the water. After entering sewage water to Bhima river, water is lifting for drinking purpose to Gulbarga city. In the summer season most of the health related problems causes due to drinking water usage. In this study were assessing effects on public health.

Ganapati and Chacko made an investigation the river Godavari and examined the effects of paper mill pollution at Rajamundry [1]. Chakrabarty et.al, the study of physico-chemical condition and reported that EC value fall below 350 µmohs/cm, salinity of those of Chambal, Yamuna, Tapti, Godavari and Krishna ranged from 450 to 1400 µmohs/cm [2]. Rafiullah et.al, has studies physico-chemical analysis of Triveni lake water of Amaravati district and reported that most of the parameters are in normal range [3]. Mahesha et.al, studies Hydrogeochemical of Dalvoy lake ecosystem of Mysore City, India were reported that physic-chemical parameters well within the limits except Calcium, Magnesium, Copper, Nickel and Manganese [4]. Mahatre et.al, studied the effect of industrial pollution on the aquatic ecosystem of Kali river [5]. R.C. Dixit et.al, reported that there is a continuous increase in the demand of water supply on cities due to the industrialization and growing population [6].

MATERIALS AND METHODS

For the study of water quality monthly samples were collected from all three established stations in early morning (7.00am to 9.00am) of the day during first week of every month for a period of three month. The water sample should collected integrated sampling methods and standard sampling methods. The surface water has been collected by using two liter capacity of white coloured plastic containers and was transferred to the laboratory.

Study Area

The study area (fig.1) is situated at latitude 17^0 15' to 17^0 25' longitude of 76^0 45' to 76^0 55', at mean sea level of 454 m. The study area is located in the south direction at 29 km distance from Gulbarga District. In this river Bhima has been chosen for sampling, namely Before sewage entry (S1), After Sewage entry (S2) and Water lifting station (S3) were selected for collecting sample. The rive flows from upstream of west to downstream east direction. The city sewage water enters to Bhima river at the upstream from 100 to 200 meter distance from the barrage. The barrage constructed in the downstream for storage of water for drinking and agricultural purpose.

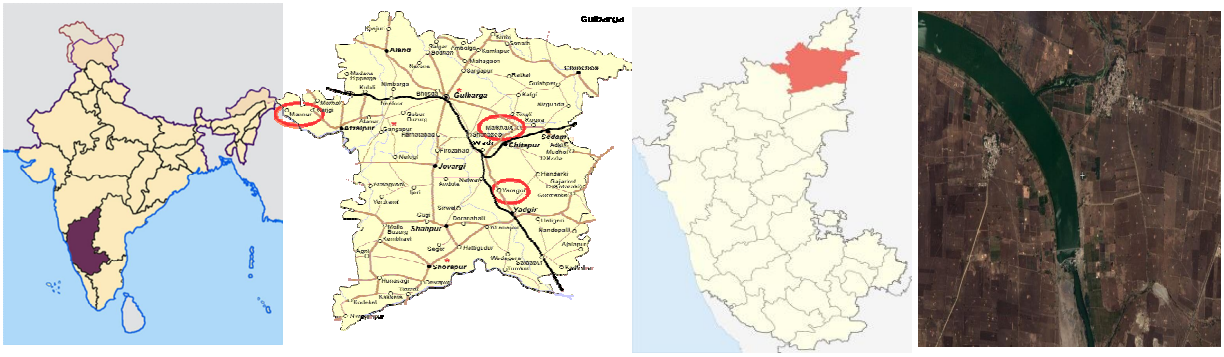

Fig. 1: Study Area Map - River Bhima, Gulbarga District,Karnataka state,India.

RESULTS AND DISCUSSION

Water quality problems cannot be solved by isolated means, or for only a particular or region. Water quality is nationwide problem that needs examining from all points of view. There are some essential common problems such as drinking water supplies and sanitation treatment. The chemical parameter includes in this study, namely total hardness, magnesium, calcium, chloride, dissolved oxygen etc.

Total Hardness (TH)

Waters which contain a significant concentration of dissolved minerals like calcium, magnesium, iron, and manganese, are called 'hard' because it takes a large amount of soap produce a lather or foam with these waters. When hard waters are heated in water heaters, hot water pipes and boilers, for example, they leave a mineral deposit called 'scale'. Total hardness is expressed as mg/L of calcium carbonate because calcium and carbonate are the dominant ions in most hard waters.

Hardness in natural water comes mainly from the leaching of igneous rock and carbonate rocks (dolomite, calcite and limestone). Water containing the soluble salts of calcium and magnesium such as chlorides, sulphates and bicarbonates is called hard water [9]. Generally hard water originates in the areas where thick topsoil and lime stone formations are present. Soft water originates in the areas where the topsoil is thin and limestone formats are absent. The hardness in water is derived largely from contact with the soil and rock formation. The ability to dissolve the ions is gained in the soil where CO_2 exists in equilibrium with carbonic acid.

In the present study, Total hardness value ranged from an average of minimum is 250 mg/l to maximum of 380 mg/l (Table 2). The three month average value of TH is getting 289.22 mg/l. According to recommendation (BIS 1998) standard permissible limit is 300 mg/l to excessive limit 600 mg/l. So the present study of three month average value getting below the permissible limit.

Calcium (Ca^+)

Calcium is an element that is found naturally in water due to its abundance in the earth's crust. Large bodies of surface water, such as rivers, typically contain 1-2 mg/L of calcium. High levels of calcium in surface water mean that the water is hard, which helps aquatic life by buffering the pH of the water and protecting those organisms with gills from direct metal uptake. However, if calcium and hardness are too high, hardening of pipes and staining may occur. In the present investigation, Calcium value is recorded for the month of March to May the minimum value is 21 mg/l and the maximum value is 40 mg/l. The average value of calcium is found 26.76 mg/l (Table 2). According to recommendation (BIS 1998) and (WHO 1993) [12] standards, the permissible limit is 75 and excessive limit is 200 mg/l. The average of three month value is 26.76 mg/l. The obtaining result is within the permissible limit.

Magnesium (Mg^+)

Magnesium is found in large concentrations in both the Earth's crust and the human body. It is highly soluble in water, and is the third most abundant element in sea water. Concentrations of magnesium in fresh water vary according to surrounding geological conditions. Along with calcium, magnesium concentrations are used to determine water hardness. High concentration of magnesium cause similar problems to high concentrations of calcium, including staining and hardening of pipes and fixtures.In the present investigation, Magnesium value is found in the month of March to May of minimum value is 8.37 mg/l and maximum value is 24.3 mg/l. The average mean value of the three month is 18.20 mg/l (Table 2). According to BIS standard permissible limit is 30 mg/l and excessive limit 100 mg/l. In my investigation the average result 18.20 mg/l.

Dissolved oxygen (DO)

Dissolved oxygen refers to the amount of oxygen dissolved in water. Because fish and other aquatic organisms cannot survive without oxygen, dissolved oxygen is one of the most important water quality parameters. Dissolved oxygen is usually expressed as a concentration of oxygen in a volume of water (mg/l). Dissolved oxygen is an important factor for the dissolution of inorganic substances in water (NEERI, 1998). In nature, oxygen gets into water in two ways. Oxygen from the atmosphere is mixed into the water from the atmosphere. Where water is rough (for example, where water is tumbling over rocks or where there are waves), the oxygen and the water mix more oxygen from the atmosphere being dissolved in the water. Oxygen is also introduced into water by green aquatic plants and algae during photosynthesis.

The DO level in natural water depends on physical and biological activities prevailing in the water quality. However, in drinking water, DO values should lie between 3 ppm to 8 ppm at 25ºC. Therefore, DO is one of the important parameters for assessing the quality of water and plays a key role in water pollution control activities and to evaluate the potential waste. In the present finding, the value of DO ranged from a minimum of 6.3 mg/l and maximum is 7.14 mg/l. The average value of three month of the DO concentration is 6.74 mg/l (Table 2). The value of DO decreases due to precipitation and surface water run-off that raises the water table through percolation[8]. In the present study shows DO values are well within the permissible limits for drinking and domestic purpose.

Chloride (Cl^-)

Chlorides occur in natural water in varying concentrations. The chloride content increases as the mineral contents increases. It is commonly found in soils and rocks. The primary source of chloride is sedimentary rocks and saline water intrusion and the minor sources are igneous rocks. High concentration of chloride makes water unpalatable and unfit for drinking and other purposes. Chloride ion is generally present in natural water and its presence can be attributed to the dissolution of salt discharge from chemical industries, oil wells, sewage discharges, and contamination from leachates. The salty taste produced by chloride ion depends on chemical composition of the water.

Chlorides in excess, imparts the salty taste to water and people are not accustomed to high chloride are subjected to laxative effect [10,11]. Chlorides in reasonable concentration are not harmful to humans. Concentration greatly in excess of 100 mg/l may cause physiological damage. At concentration above 250 mg/l the water becomes salty taste. Hence, the chlorides are generally limited to 250 mg/l in supplies intended for public use. In the present study, chloride values ranged from a minimum of 92 mg/l to a maximum of 130 mg/l in the average of three months. The average mean value of three month of chloride concentration is 107.78 mg/l. According to references (BIS 1998) standards the chloride value is ranges from 250 mg/l to 1000 mg/l. In the present study the average value of Chloride is in the range of minimum limit.

Table: 1. Chemical parameters during the month of March- May 2012.

Sl. No.	Parameters	March			April			May		
		S1	S2	S3	S1	S2	S3	S1	S2	S3
01	Total Hardness	250	260	266	315	250	280	380	312	290
02	Calcium as Ca	22.4	24	26.4	31.6	25.2	23.8	40	26.4	21
03	Magnesium as Ca	22.84	24.3	24.3	21.2	17.8	14.4	19.4	11.18	8.37
04	Dissolved Oxygen (DO)	6.99	6.92	7.14	6.75	6.80	6.90	6.5	6.3	6.4
05	Chloride	92	96	94	102	118	104	110	130	124

Table: 2. Statistical Analysis of March to May months.

Sl. No.	Parameters	Mean	Standard Deviation	Minimum	Maximum
01	Total Hardness	255.89	80.86	150	380
02	Calcium as Ca	26.76	5.81	21	40
03	Magnesium as Ca	18.20	5.78	8.37	24.3
04	Dissolved Oxygen (DO)	6.74	0.28	6.3	7.14
05	Chloride	107.78	13.65	92	130

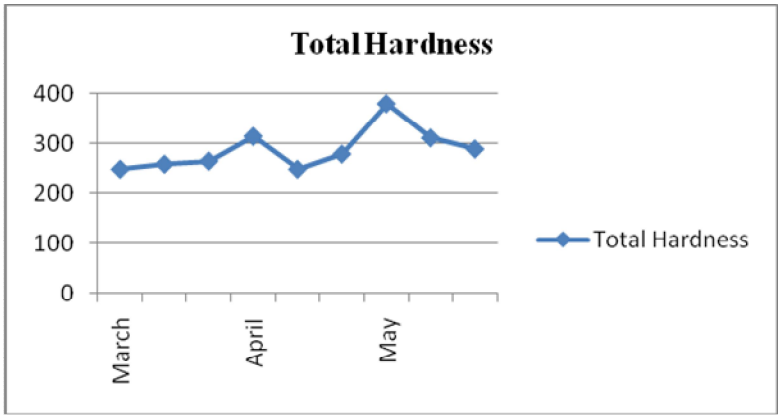

Fig.:2. Total Hardness concentration in the month of March to May 2012.

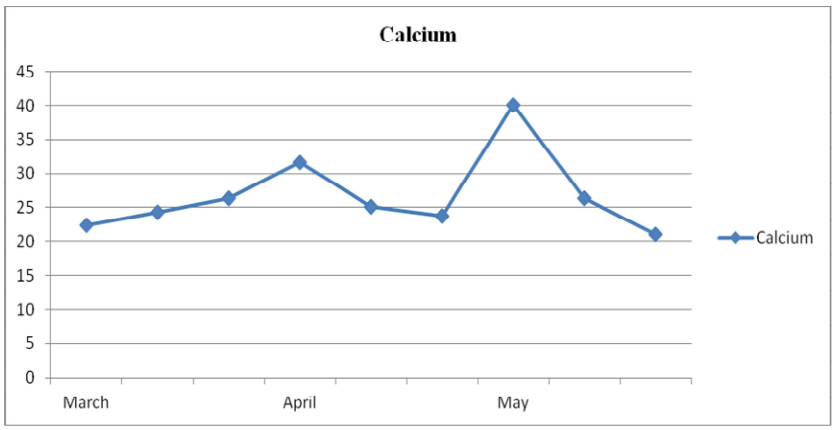

Fig.3: Calcium concentration in the month of March to May 2012.

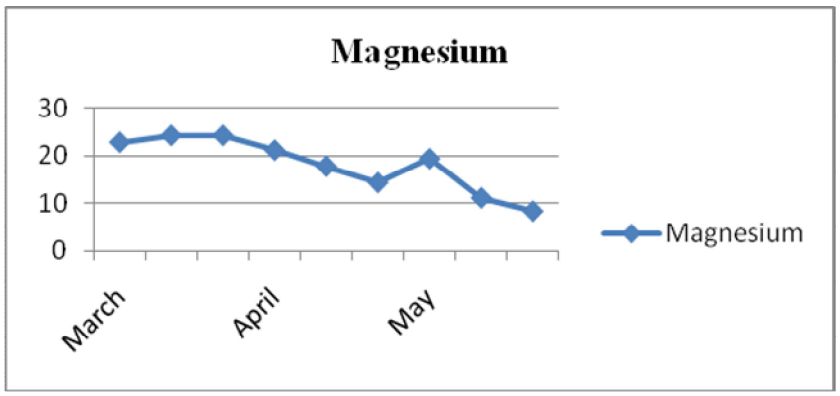

Fig.4: Magnesium concentration in the month of March to May 2012.

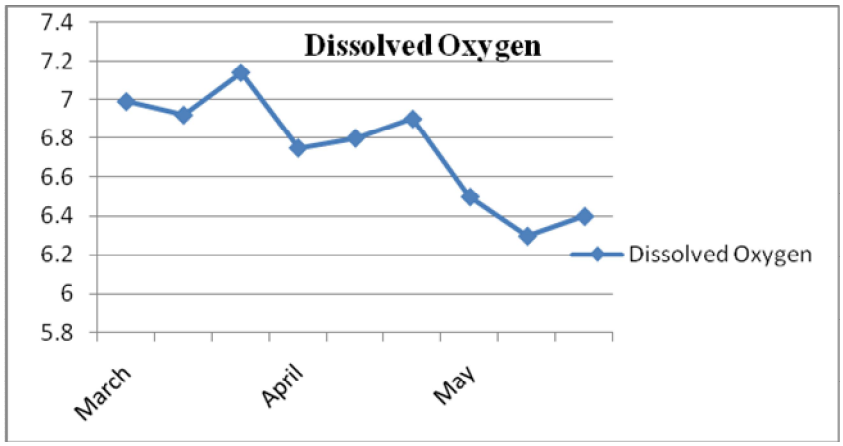

Fig.5: Dissolved Oxygen concentration in the month of March to May 2012.

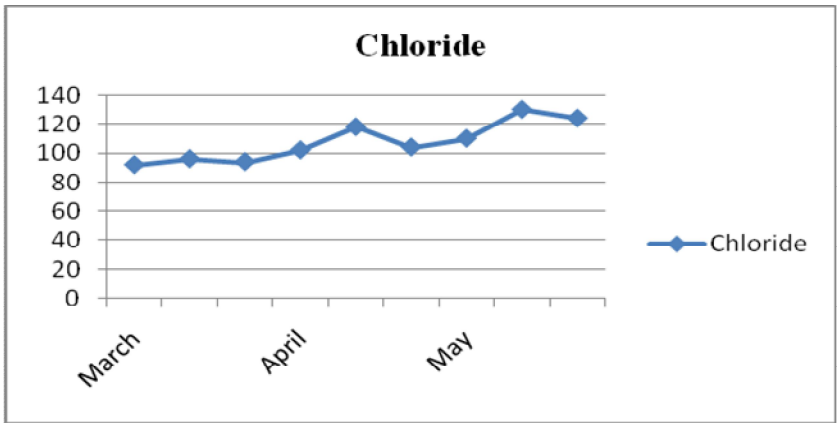

Fig.6:Chloride concentration in the month of March to May 2012.

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RESEARCH ARTICLE

Spatio-Temporal Evaluation of Land Use / Land Cover Changes in Noyyal River Basin, Tamil Nadu, India: A Case Study using Geospatial Techniques.

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ABSTRACT

The present study is conducted to find out the land use /land cover changes in Noyyal River basin situated in Coimbatore, Erode, Tiruppur and Karur Districts of Tamil Nadu State. The total area of the study area is 3,510 sq.km. For the spatio-temporal study, the following years are considered 1973, 1991 and 2006. Satellite images of LANDSAT MSS (1973), LANDSAT TM (1991) and LANDSAT ETM+ (2006) are used to demarcate the land use / land cover categories. ERDAS IMAGINE 8.5 image processing and ArcGIS 9.3 software are used to classify the land use / land cover categories and to detect the changes occurred during the above mentioned periods. Overlay technique is applied to find out the difference in land utilization pattern in the years 1973, 1991, 2006.The analyses conclude that the barren, built-up, forest, gullied ravenous, irrigated crop, sand and water body categories have increased in the year 1991 when compared to the year 1973 whereas the other categories like forest, marshy, scrub and unirrigated crop land have decreased in their spatial extent compared to 2006. It is clearly understood that urbanization is gradually grabbing all natural land uses which results in depletion of various other categories.

Keywords: Land use / Land cover, Change detection, Remote Sensing, GIS.

INTRODUCTION

Land use describes various ways in which human beings make use of the land and manage its resources. Land cover is the physical and biological materials (vegetation or the built-ups) found on the surface of the land, which is a human created structures. In the early years humans used the land for the basic needs like shelter, food and defense. But today this has changed appreciably and humans have developed the earth's surface environment and due to this land use has undergone extensive changes in the landscape. Analysing the spatial and temporal changes in land use / land cover is one of the effective ways to understand the current environmental status of an area and ongoing changes [1].

As far as a river basin is concerned, the spatio-temporal changes of land use in the basin have a direct influence on its hydrological realm.Currently fresh water resources in several parts of the globe is facing severe crisis in its availability due to unsustainable uses aggravating the unpredictable and unforeseen changes in the global and local climate. The climate change coupled with urbanization and rampant alterations in land use of the basins have made most of the world's fresh water flow regimes under severe pressure and change [2]. Deforestation and conversion of water logged wetlands in to built-up areas have directly affected the groundwater recharging capacity and natural water flow regimes.Remote Sensing and GIS have been widely applied to understand the land use / land cover changes and are considered to be a powerful tool to identify the spatio-temporal changes of an area [3].

Study Area

The Noyyal is a sacred river in Tamil Nadu. Its original name was Kanchinadi but changed later to the name of the place where it drains into the Cauvery River. The Noyyal River originates from the hills of Velliangiri and it confluences with river Cauvery at Kodumudi. It is a tributary of the Cauvery, a large inter-state river which cuts through the States of Karnataka and Tamil Nadu and enters the Bay of Bengal. The Noyyal flows through the districts of Coimbatore, Erode, Tiruppur and Karur and the urban centres of Coimbatore and Tiruppur, in the western Tamil Nadu.

The Noyyal river basin covers a total area of 3,510 sq.km and the boundary of the river basin is between North latitudes 10°54′00″ - 11°19′03″ and East longitudes 76°39′30″ - 77°05′25″. The river flows for a distance of 180 kms from west to east. The average width of the basin is 25 km. The basin is widest in the central part with a width of 35 km. The entire area of the basin is situated within the state of Tamil Nadu, in parts of Coimbatore, Erode, Tiruppur and Karur Districts (Fig. 1).

METHODOLOGY

The LANDSAT MSS (Multi Spectral Scanner); LANDSAT TM (Thematic Mapper); LANDSAT ETM+ (Enhanced Thematic Mapper) images taken in the years 1973, 1991 and 2006 respectively are utilized for land use / land cover analysis. However, various primary and secondary data are also used as collateral data for the study. The basin boundary is delineated using the Survey of India (SOI) toposheet series of 1:50,000 scale. Geometric correction is carried out by field survey using Global Positioning System (GPS). The ERDAS IMAGINE 8.5 software is used to classify the image digitally by applying NRSA land use / land cover classification techniques in the study area and ArcGIS 9.3 software is used to process and analyse the land use / land cover changes.

RESULTS AND DISCUSSION

The land use / land cover classification of the study area consist of built-up land, irrigated cropland, unirrigated cropland, fallow land, forest, marshy land, gullied ravenous land, scrub land, sand, barren land and water bodies[4]. The study assesses the spatio-temporal changes of land use / land cover of the Noyyal basin during three different periods in 1973, 1991 and 2006 (Fig. 2, 3 and 4, Table 1 and 2).

It is obvious that the land use / land cover are undergoing a shift from predominantly rural based society to urban. The land use / land cover changes, transformations and conversions, are the results of various pressures on ecosystems and have been progressed largely around human settlements. The areas around the settlements/industries/institutions often undergo diverse kinds of transformation and changes in varying degrees. Similar conversion analysis of Noyyal basin is studied using overlaying method and the results are given in the matrix Table 1.3 and Table 1.4 for the years 1973-1991 and 1991-2006 [5].

Built-Up Land

The built-up land comprised areas of intensive use with much of the land covered by physical structures. Comparison of the temporal data shows that built-up area has considerably increased in Noyyal basin showing urban sprawl in and around the urban centres.It was as low as 1.63% in 1973 and it has increased to 4.98% in 1991. This is due to the conversion of 33.84 sq.km of irrigated crop land, 37.89 sq.km of scrub land, 26.71 sq.km of unirrigated crop land and 20.3 sq.km of fallow land into built-up category. Similarly in 2006 it has shown a gradual improvement as 5.06% where 5.89% of fallow land 21.7% of irrigated crop land and 16.98% of unirrigated cropland have changed as built-up lands. The encroachment of urban in the water body is 3.06sq.km in 1991 and it has increased to 3.97 sq.km in 2006.

Irrigated Crop Land

Irrigated crop land is used for growing agricultural crops with the artificial application of water to the soil. Such land is seen in the entire basin because Noyyal is a seasonal river, which has good flow only for a short period during the North-East and South-West monsoons[6,7]. The irrigated crop area shows a change of 876.92 sq.km (24.98%) in 1973 to 1,085.07 sq.km (30.91%) in 1991 where the difference is 208.15 sq.km and in 2006 it has shown appreciable improvement to 1,383.27sq.km (39.41%) where the difference is 298.2 sq.km as the unirrigated cropland lands, fallow lands and scrub lands has changed in to irrigated cropland.

Unirrigated Cropland

The unirrigated crop land is the area where irrigation is mainly by means of rainfall. Occasionally flash floods occur when there is heavy rain in the catchment areas[8]. Out of the total area of about 1,289.21 sq.km (1973) under the unirrigated crop land, an area of 1215.75 sq.km has retained the same vegetation cover over the fifteen-year period till 1991 and 73.46 sq.km has transformed into other categories in the next fifteen-year period up to 2006 which shows the decrement of 1,081.93 sq.km against the same category in 1991.

Fallow Land

The agricultural land which is taken up for cultivation but temporarily allowed to rest un-cropped for one or more seasons, but not less than one year, is a fallow land. Here in the Noyyal basin 471.2 sq.km of fallow land has

decreased to 377.86 sq.km in 1991 and again it has shown a depletion of 328.76 sq.km in 2006. This decrease is probably due to the extension of cultivation lands as a result of the increased demand for housing, industries, agriculture, development of irrigation etc.

Forest Land

The western part of the basin covered by forest lands of the Western Ghats has shown an increment of 12.05 sq.km in 1991 when compared to the statistics of 1973 and this is a good sign for the improved ecosystem but when it comes to 2006 it has decreased by 5.01 sq.km. Thus the changes in the forest and land cover in the southern region of the Western Ghats exhibit great spatial variations, a decrease in forest area and increase in fallow and other unirrigated areas where as in northern region it has increased the irrigated crop land and scrub land[9].

Marshy Land

Noyyal basin possesses very little area of marshy land which is permanently or periodically inundated by water mostly polluted ones and is characterized by vegetation, which includes grasses and weeds. The marshy land with 12.05 sq.km of area has decreased to 1.32 sq.km in 1991 due to increased awareness to afforestation whereas in 2006, it has increased to 1.54 sq.km due to increase in water bodies, mostly near industrial centres.

Gullied Ravenous Land

The gullies are formed as a result of localised surface runoff affecting the friable unconsolidated material in the formation of perceptible channels resulting in undulating terrain. The gullies are the first stage of excessive land dissection followed by their networking which leads to the development of ravenous land[4]. Since the study area is a basin, the gullied ravenous covered with 26.39 sq.km in 1973 has increased to 33.76 sq.km in 1991 and it has spread to 55.76 sq.km in 2006 due to changes in fallow, scrub, irrigated and unirrigated crop lands.

Scrub Land

The land that is uncultivated and covered with sparse stunted vegetation is defined as scrub land. Compared to 1991, the scrub lands are identified as scattered patches in the entire basin during the year 1973. This shows that these lands have been converted to agriculture lands for the purpose of cultivation. As per the study, 563.18 sq.km of scrub land in 1973 has reduced to 389.46 sq.km in 1991 and further decreased to 265.17 sq.km in 2006 where the conversion occurred in the categories of irrigated, unirrigated, fallow, built-up and gullied ravenous lands.

Sand

As the study area is a river basin, the sand cover dominates in and around the river and its tributaries of the entire basin. In 1973 the sand cover seems to be less (7.23 sq.km) whereas in 1991 it has increased to 10.1 sq.km but it has decreased to 5.47 sq.km in 2006 which is due to the increase in water body, irrigated and unirrigated crop lands.

Barren Land

In general, the barren area consist of thin soil, sand, or rocks and has the limited ability to support life and in which less than one-third of the area has vegetation or other cover. In the basin, the barren land constitution is 12 sq.km in 1973 and it has increased to 14.33 sq.km in 1991 but in 2006 it has reduced to 13.51 sq.km. It has been observed that there is a conversion of other land categories such as irrigated cropland (0.24 sq.km), scrub land (0.34 sq.km) and

unirrigated cropland (0.69 sq.km) into barren land. These lands may appear barren because of human activities and sometimes seasonal impact could also pose for the changes.

Water Bodies

Water is one of the major determining factors in the study as it is the cause for the civilization. In 1973 the 55.98 sq.km of the area is covered by water bodies and in 1991 it has marginally increased to 56.7 sq.km but in 2006 it has reduced appreciably to 51.48 sq.km, which shows a fluctuating tendency of areas under water. The encroachment of the water body is mainly for the purpose of built-up and industries. Apart from this, the natural changes like gullied ravenous, floods, drought etc also plays their role in the decrease of water bodies in the basin.

Table 1 – Land Use / Land Cover Changes in Noyyal River Basin during 1973 - 1991

Sl. No.	Land Use / Land Cover Categories	Area in sq.km		Difference in area (sq.km)	Percentage	
		1973	1991		1973	1991
1	Built-up Land	57.21	174.93	117.72	1.63	4.98
2	Irrigated Crop Land	876.92	1,085.07	208.15	24.98	30.91
3	Unirrigated Crop Land	1,289.21	1,215.75	-73.46	36.73	34.64
4	Fallow Land	471.2	377.86	-93.34	13.42	10.77
5	Forest	138.63	150.68	12.05	3.95	4.29
6	Marshy Land	12.05	1.32	-10.73	0.34	0.04
7	Gullied Ravenous Land	26.39	33.76	7.37	0.75	0.96
8	Scrub Land	563.18	389.46	-173.72	16.05	11.10
9	Sand	7.23	10.14	2.91	0.21	0.29
10	Barren Land	12	14.33	2.33	0.34	0.41
11	Water bodies	55.98	56.7	0.72	1.59	1.62
	Total	3,510.00	3,510.00		100.00	100.00

Table 2 – Land Use / Land Cover Changes in Noyyal River Basin during 1991 - 2006

Sl. No.	Land Use / Land Cover Categories	Area in sq.km		Difference in area (sq.km)	Percentage	
		1991	2006		1991	2006
1	Built-up Land	174.93	177.44	2.51	4.98	5.06
2	Irrigated Crop Land	1,085.07	1,383.27	298.2	30.91	39.41
3	Unirrigated Crop Land	1,215.75	1,081.93	-133.82	34.64	30.82
4	Fallow Land	377.86	328.76	-49.1	10.77	9.37
5	Forest	150.68	145.67	-5.01	4.29	4.15
6	Marshy Land	1.32	1.54	0.22	0.04	0.04
7	Gullied Ravenous Land	33.76	55.76	22	0.96	1.59
8	Scrub Land	389.46	265.17	-124.29	11.10	7.55
9	Sand	10.14	5.47	-4.67	0.29	0.16
10	Barren Land	14.33	13.51	-0.82	0.41	0.38
11	Water bodies	56.7	51.48	-5.22	1.62	1.47
	Total	3,510.00	3,510.00		100.00	100.00

Table 3 - Matrix Results of Land Use / Land Cover Changes of Noyyal River Basin during 1973 -1991

1973-1991	Built-up land	Irrigated Cropland	Unirrigated Cropland	Fallow land	Forest	Marshy land	Gullied Ravinous Land	Scrub land	Sand	Barren Land	Water body	Total Area in sq.km (1973)
Built-up land	52.35	1.47	0.59	2.4	0	0	0	0	0	0	0.4	57.21
Irrigated Cropland	33.84	621.02	14.53	99.45	0	0.76	9.89	90.64	2.09	0.95	3.75	876.92
Unirrigated Cropland	26.71	91.7	1109.3	29.71	0	0	0.03	22.09	4.15	2.51	3.01	1289.2
Fallow land	20.3	236.73	31.75	155.55	0	0	6.1	17.47		0.5	2.8	471.2
Forest	0	0	0	0	138.63	0	0	0	0	0	0	138.63
Marshy land	0	0	0	0	12.05	0	0	0	0	0	0	12.05
Gullied Ravinous Land	0	9.75	0.6	0.04	0	0	15.95	0.05	0	0	0	26.39
Scrub land	37.89	116.81	56.63	90.31	0	0	1.75	259	0	0.09	0.75	563.18
Sand	0.73	0.74	1.4	0	0	0	0	0	3.9	0	0.46	7.23
Barren Land	0.05	0.51	0.95	0	0	0	0	0.21	0	10.28	0	12
Water body	3.06	6.34	0	0.4	0	0.56	0.04	0.05	0	0	45.53	55.98
Total Area in sq.km (1991)	174.93	1085.07	1215.75	377.86	150.68	1.32	33.76	389.5	10.1	14.33	56.7	3510

Table 4 - Matrix Results of Land Use / Land Cover Changes of Noyyal River Basin during 1991 -2006

1991-2006	Built-up land	Irrigated Cropland	Unirrigated Cropland	Fallow land	Forest	Marshy land	Gullied Ravinous Land	Scrub land	Sand	Barren Land	Water body	Total Area in sq.km (1991)
Built-up land	126.64	25.35	18.79	2.99	0	0	0	0	0	0.19	0.97	174.93
Irrigated Cropland	21.7	998.2	22.84	10.5	0	0	11.91	12.67	0.06	0.24	6.95	1085.1
Unirrigated Cropland	16.98	175.27	995.79	7.99	0	0	5.9	8.36	2.79	0.69	1.98	1215.8
Fallow land	5.89	38.59	33.98	293.86	0	0	0.58	4.6	0	0	0.36	377.86
Forest	0	0	0	0.33	145.67	0	1.89	0.59	0	2.2	0	150.68
Marshy land	0	0.75	0	0	0	0.57	0	0	0	0	0	1.32
Gullied Ravinous Land	0.29	1.38	0.16	0.03	0	0	31.03	0	0	0	0.87	33.76
Scrub land	1.97	132.84	2.86	8.99	0	0	2.76	239	0	0.34	0.75	389.46
Sand	0	4.46	1.46		0	0	0	0	2.59	0	1.63	10.14
Barren Land	0	1.43	2.92	0	0	0	0.13	0	0	9.85	0	14.33
Water body	3.97	5	3.13	4.07	0	0.97	1.56	0	0.03	0	37.97	56.7
Total Area in sq.km (2006)	177.44	1383.27	1081.93	328.76	145.67	1.54	55.76	265.2	5.47	13.51	51.48	3510

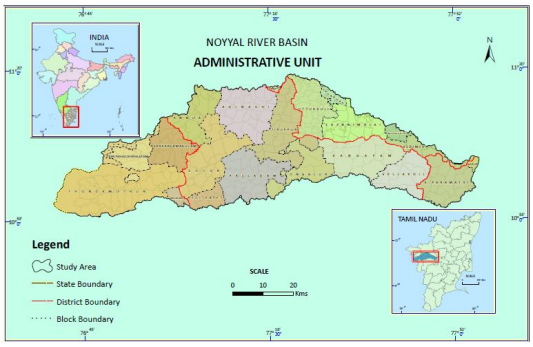

Fig. 1 - Study Area

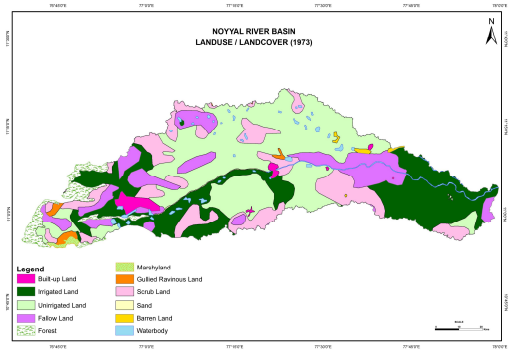

Fig. 2 – Land Use / Land Cover 1973

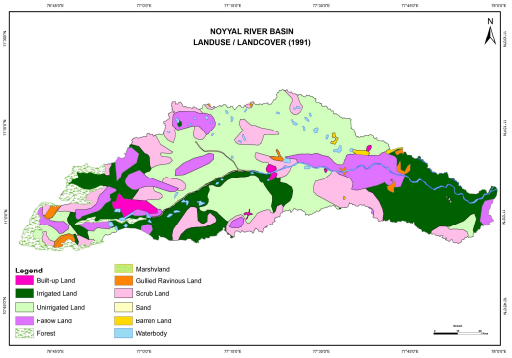

Fig. 3 – Land Use / Land Cover 1991

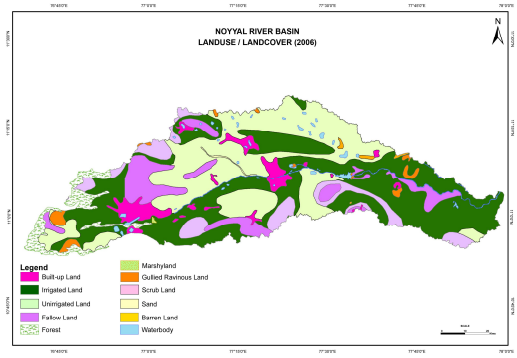

Fig. 4 – Land Use / Land Cover 2006

CONCLUSION

From this paper, it is understood that the spatio-temporal changes happened in land use / land cover categories of Noyyal basin is almost due to urbanization/industrialization processes that has been witnessed in the last two decades. Since the Noyyal flows through the Manchester of South India or cotton city, Coimbatore-Tirupur region the major impact of industrialization where the humans with their ever expanding needs, often resolve to take on mother nature in order to meet their own requirements. The most rapid change has occurred in the built-up land from 1.63% in 1973 to 5.06% in 2006. As like the many other hazardous changes, due to the lifestyle changes and change in culture, the lands have been converted or reformed in to various other categories where the agriculture lands including irrigated, unirrigated and fallow lands show improvement from 1973 to 2006, but the forest land has show a decrease in 2006 where the urban activities has developed in those areas[10]. Due to this change we have lost our natural ecosystem and biodiversity. Comparison of spatial data shows a considerable decrease in barren land and scrub land which has reformed in to agriculture or built-up land. The decrease in water bodies may be due to insufficient rainfall, non perennial water flow in the river and encroachments.

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RESEARCH ARTICLE

Assessment of Water Quality Parameters of Samudram Lake in Thanjavur District, Tamil Nadu, India.

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ABSTRACT

A physico-chemical characteristics study conducted in Samudram lake of Thanjavur district, Tamil Nadu. The present study deals with the analysis of quality of Samudram lake water samples were collected monthly for a period of June 2010 to December 2011. Showed that the concentration of parameters like pH, conductivity, Total Alkalinity, Nitrate, Phosphate, Ca, Mg, are within the permissible levels of surface water quality. However, Total hardness BOD and COD content was higher in most of the stations. The study shows that lake can be a very good source of water for irrigation. So, it should be conserved from the anthropogenic activities at any cost.

Keywords: physico-chemical, Total Alkalinity, anthropogenic activities.

INTRODUCTION

Thanjavur being the foremost district of the cauvery delta occupies an important position in the agricultural map of Tamil Nadu state. Since its formation, the district is called as the rice bowl of Tamil Nadu. Thanjavur District lies in the East Coast of Tamil Nadu. It is located between $9^0$50′ and $11^0$25′ of the Northern latitude and $78^0$45′ and $70^0$25′ of the Eastern longitude. Samudram Lake is located about 7 Km to the East of Thanjavur on the Thanjavur Nagapattinam, Velangkanni Road. The total area of Lake is 257.75 Acres (Fig.1). The Lake is one of the favourite fly away spots for migratory birds. The lake is basically an irrigation tank that receives water from Mettur and Northeast monsoon from July to February. It remains dry from April to June. The lake is one of the water reservoirs from where water is planned to be supplied to agricultural activities. The physico-chemical characteristics study was carried out during the period of June 2010 to December 2011.

Name of the lake	:	Samudram
Location of lake	:	Thanjavur,Tamil Nadu, South India
Length of Bund	:	3430 meters
Water spread Area	:	257.75 Acres
Capacity of Lake	:	13.40 M.Cft
Catchment Area	:	6.76 Sq.Miles
Ayact	:	1116 Acres
Highest depth of water	:	2.260 M
Village benefitted	:	5 Villages

The quality of water should be assessed on the basis of physico-chemical parameters in order to provide information for the purpose of water management [13].Water analysis is carried out to assess the optimum and harmful limits of various parameters for survival and general wealth of aquatic organisms. Physico-chemical properties of a water body play a major role in its productivity process and growth of aquatic organisms under culture. Several studies have been conducted to understand the physical and chemical properties of lakes, pond and reservoirs [14].

MATERIALS AND METHODS

The study was carried out in Samudram lake, Thanjavur district, Tamil Nadu, India.Lake water is used for irrigation, totally 5 villages benefited are by this lake. The physico- chemical parameters of water quality was analysed using standard methods given in APHA[15].

RESULTS AND DISCUSSION

The temperature varies from 24^{o}C to 34^{o}C .The temperature affects the metabolic rate of living organisms [6]. Aquatic organisms are affected by pH because most of their metabolic activities are pH dependent [10]. Scuthorpe (1967)[7] has reportd that pH, free CO2 and ammonia are more critical factors in the survival of aquatic plants and fishes than the oxygen supply. In the present study, the lake water was found to be slightly alkaline where pH varied from 6.9 to 8.2.Electrical conductivity (EC) values of lake water samples ranged between0.5 mmho/cm to 3.9 mmho/cm (Table 1). Conductivity of water depends upon the concentration of ions and its various dissolved solid content (Dilution of water during the rains causes a decrease in electrical conductance. EC is highly depends on the amount of dissolved solids in waters [22].

Calcium (Ca.H) is linked with the carbon dioxide and is an important constituent of the skeletal structure of organisms. Calcium forms the most abundant ions in fresh water [12]. The higher values may be due to accumulation of ions owing to evaporation, biological turnover and interaction with sediments [11]. Chloride (Cl^{-}) is found widely distributed in nature in the form of salts of sodium, potassium and calcium. The chloride status in water is indicative of pollution, especially of animal origin. In the present study chloride concentration in Samudram lake was ranged between 62 mg/L to 368 mg/L. The highest value of chloride has resulted due to large amount of organic matter, mass bathing activities, urination and waste of animals. These results are in conformity with the study of Zutshi and Khan (1988)[8]. They attributed high chloride values due to bathing activities and urination in the Dal Lake. The total hardness (TH) value ranged from 138 mg/L to 380 mg/L. Kannan (1991)[4] has classified water on the basis of hardness values in the following manner; 0-60 mg/L, soft 61-120 mg/L, moderately hard 121-160 mg/L, hard and greater than 180 mg/L very hard. Normal water hardness does not pose any direct health problems. Mohan and Patra (2001)[5] stated that addition of sewage, detergents and large scale human use might cause elevation of hardness of water. The BOD concentration (organic load in the present study remained very high ranging between42 mg/L to 72 mg/L indicating the fact that the lake is slightly eutrophic. Seasonally, it was high during summer, thus being in conformity with the observation of Chatterjee (1992)[9].

Total Alkalinity (TA) of water refers to the quality and kinds of components present such as bicarbonate, carbonate and hydroxide. According to Durrani (1993)[2] withdrawal of CO_2 from the bicarbonates for photosynthesis by algae may increase total alkalinity. Spence (1967)[1] classified the lakes into three categories based on alkalinity1. 1-15 mg/L is nutrient poor. Ii. 16-60 mg/L is moderately nutrient rich and iii. >60 mg/L is nutrient rich.The present investigation total alkalinity ranged between 129 mg/L to 410 mg/L, which may be due to higher input of nutrients in water through human activities.In water, total dissolved solids (TDS) are composed mainly of carbonates, bicarbonates, chlorides, phosphates and nitrates of calcium, magnesium, sodium, potassium and manganese, organic matter, salt and other particles. Their minimum values were recorded as 320 mg/L and maximum value of TDS was recorded as 950 mg/Lwhich reflects the pollution of Lake.

Magnesium (Mg.H) is and essential micronutrient for both autotrophs and heterotrophs. The magnesium content found from 36 mg/l to 162 mg/l. The amount of Mg in water samples was low as compared to the amount of Ca in water. The amount of Mg was low in rainy season indicating possible increase in water level [16]. The higher values of Mg may be due to its release from the bottom sediment and due to decomposition of biota.High Levels of both phosphate and nitrate can lead to eutrophication, which increases algae growth and ultimately reduces dissolved oxygen levels in the water [17,18]. It is widely assumed that nitrite concentrations in freshwaters are negligible [19], and the worldwide average concentration has beenestimated to be 1 mg of nitrite/liter[20,21].In the present study the value of Phosphate found from 0.02 mg/L to 0.14 mg/L and the value of nitrate ranged from 0.02 mg/L to 0.15 mg/L.

Chemical oxygen demand determines the oxygen required for chemical oxidation of organic matter. COD values convey the amount of dissolved oxidisable organic matter including the non-biodegradable matters present in it. Chemical oxygen demand (COD) value ranged from 188 mg/L to 88 mg/L. respectively. The sources of COD in Lake may be due to input of domestic drains and the use of soap and detergents for washing and bathing. Though the lake is regarded sacred, human activities in the form of washing and sewage disposal are strictly prohibited but it is very difficult to control these activities [3].

CONCLUSION

It is proved from the research findings that the quality of the water samples studied varies from season to season. However, in all stations of water collection, there is no proper drainage. There is a regular addition of large quantities of sewage and detergents from the local communities, and chemical fertilizers, pesticides from agricultural runoff. The present study shows that water quality of the lake in all the 5 stations suitable for domestic and irrigation purpose. Further they have good potential for fishery. Thus, this lake can be a very good source of water for domestic use and also for generating income from fishery which can be augmented with scientific management as small reservoirs are more manageable and high yielding than larger ones. Hence, it is important to protect and conserve these water bodies.

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Table:1. Physico-chemical characteristics of water samples from Samudram lake during the period from June 2010 to December 2011, mg/L.

	T°C	pH	EC*	TDS	TH	Ca.H	Mg.H	TA	Chloride	BOD	COD	Phosphate	Nitrate
June'10	33	7.9	2.4	720	234	145	89	156	159	43	90	0.06	0.12
July'10	32	7.6	3.9	690	264	152	112	189	236	58	97	0.06	0.06
August'10	28	7.8	3.2	520	249	146	103	172	189	63	96	0.04	0.12
September'10	29	7.6	1.2	704	258	153	105	149	169	55	92	0.06	0.04
October'10	26	8.2	1.8	770	228	149	79	153	173	46	93	0.07	0.08
November'10	27	7.5	1.4	652	258	169	89	226	62	53	98	0.05	0.15
December'10	26	7.8	1.1	380	244	146	88	235	95	57	107	0.06	0.06
January'11	28	7.8	0.6	360	364	162	202	169	134	43	98	0.06	0.12
February'11	29	7.7	0.8	370	378	196	182	152	220	68	120	0.04	0.03
March'11	30	7.9	0.5	620	244	146	98	320	368	65	140	0.09	0.18
April'11	31	8.2	2.3	700	140	86	54	410	270	72	182	0.09	0.02
May'11	34	7.2	2.2	760	138	102	36	398	261	64	101	0.12	0.02
June'11	31	6.9	1.9	700	190	101	89	252	236	48	92	0.14	0.03
July'11	31	7.6	1.5	770	220	144	76	192	134	64	102	0.06	0.03
August'11	27	7.5	2.4	890	248	156	92	139	360	53	98	0.06	0.12
September'11	26	7.4	2	950	380	162	118	156	278	46	88	0.04	0.06
October'11	25	7.1	1.8	710	234	154	80	152	173	62	104	0.06	0.04
November'11	25	7.2	1.5	320	276	184	92	161	126	53	94	0.02	0.06
December'11	24	6.9	2.6	380	265	153	112	129	134	42	96	0.05	0.04

*EC-mmho/cm.

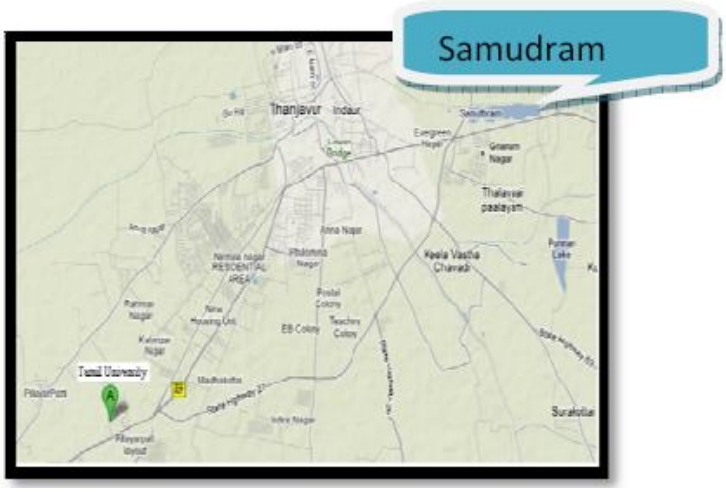

Fig:1 Location of Samudram lake in Thanjavur district,TamilNadu,India.

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RESEARCH ARTICLE

Conservation of Traditional Varieties of Rice using Geospatial Tool.

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ABSTRACT

Green revolution has added to the rosy picture of increase in productivity and production. But the actual situation is still grim struggling to feed the billion mouths of Indian population. Umpteen numbers of worthy traditional varieties of rice are falling into oblivion as they are sidelined by high yielding varieties. In this scenario, an attempt has been taken to restore some of these potential traditional varieties of rice using the geospatial tool-GIS. The success of application of this tool in conserving these varieties could be further used as a model for planning out conservation programmes for any species of plant varieties in mega scale.

Keywords: Green revolution, traditional varieties, geospatial tool, habitat suitability.

INTRODUCTION

Rice is a major food crop in tropical world.Nearly 400 varieties of traditional varieties of paddy had been in vogue during olden days in Tamil Nadu of South India. Pallu pattu - an ancient Tamil literary work on peasants quote about 150 varieties of paddy. These traditional varieties had been named after their shapes, duration, qualities of grain colour etc. Many varieties had been created in memory of many persons as a souvenir. Today many of these varieties can be seen only in the verses and hymns of literatures [1].

Green Revolution had been the single prime cause of the rapid vanishing of these traditional rice varieties. Rice cultivars exist that are adapted to a wide range of habitats. Different cultivars are grown widely throughout the world, from latitude 50°N in China to 35°S in New South Wales and Argentina [2], in tropical, temperate, lowland and highland regions and on a wide range of soil types. Although a wide range of environmental suitability prevails, however, each landrace –traditional variety has its own specificity for physical and chemical parameters of a location.

Keeping in mind this fact, a venture has been made to apply GIS in assessing and mapping other suitable locations still unexplored using GIS to conserve these varieties which are in the verge of extinction in Tamil nadu.

The recognition of geographic patterns in biodiversity is of considerable interest to biologists and natural resource managers. A key component is the mapping of geological models to predict biodiversity [3]. Remote sensing and GIS have proved to be potential in monitoring, mapping and detecting changes in natural resources and characterization of natural habitats and developing conservation strategies[4].They also help in monitoring areas of land for their suitability to endangered species (land suitability evaluation), through integration of various habitat variables of both spatial and non-spatial nature [5].

MATERIALS AND METHODS

The samples of 3 traditional varieties at the verge of extinction in Tamil Nadu namely Seeraga samba (Red), Seeraga samba (Black) and Seeraga samba (White) were procured from farmers of Tamil nadu and Non Governmental organizations located in Tamil Nadu and the places of collection were duly recorded for digitization using GIS.

Image processing and GIS techniques were adopted to generate a map, and the possible suitable places were projected for the conservation of three traditional varieties paddy varieties in Tamil Nadu, India.The analysis for habitat suitability was carried out using the version of Arc view 3.3 [ESRI Inc., 2002] to digitize, analyse and overlay the different themes ultimately figuring out the locations for propagation and conservation.

Two types of maps were used which included scanned maps and Topographic maps. The Tamil Nadu Political map of 'Info maps' with a scale of 1:960,000 cm and Lambert conical orthomorphic projection was scanned in nine parts and has been made into a single mosaic using ERDAS imagine image processing techniques. About seven maps relating soil and climatic parameters of Tamil Nadu were procured from NATMO, Calcutta. They include soil regions, water resources, crop regions, minerals, rocks, rainfall, drainage and annual temperature with a uniform scale of 1:2,000,000. All these maps were in conical equal area projection. Each of the maps was manually digitized using Arc view 3.3, into 8 different themes and the outline of Tamil nadu map was digitized into 9^{th} theme. Whereas the locations of each of the three Seeraga samba varieties were uploaded as 10^{th} theme. Care was taken to avoid errors and follow uniform projection during digitization of each thematic layer. To ensure the uniform projection during digitization, initial referencing with four reference points were registered as follows:

10^0 latitude	76^0 longitude
14^0 latitude	80^0 longitude (also)
8^0 latitude	76^0 longitude
16^0 latitude	80^0 longitude

After digitizing all the maps into various themes, then the themes were overlaid on each other. At the final stage, 9(n-1) unions of all 10 'n' themes were created and stored by using 'union' tool in the geo-processing window. Then, based upon the particulars of rainfall, temperature, water depth /availability, irrigation facilities, soil types, crop regions, rock types and minerals specific to each variety (Table 1), Boolean logic was applied as follows:

E.g. [[Temp _ cels] = 30] and [[name] = "Alluvium"] and
[[RF_c_m_m_m] = 900] and [[crop = "Rice"] and
[[Type = "young alluvial soil"] and [[Depth_m] = 100]]

Characteristics of Seeraga samba varieties

The samples of each landraces of Seeraga samba has been collected from Trichirappalli (Seeraga samba White) and Kancheepuram districts (Seeraga samba Red & Black) respectively. Each of the selected 3 land races had unique characters. Seeraga samba is a highly fine variety which is extremely aromatic. It is highly preferred for making Pulav or Briyani. It is thin like the Jeera(Cumin) and hence the name [6].In Kerala, it is known as Jeeraka Chamban - A mildly fragrant variety of long grain parboiled rice. Its grains are long and thin, almost resembling the shape of cumin seeds. When cooked, this rice has a fragrance similar to toasted cumin [7].

But this traditional variety exists as three types of landraces based on their external morphological characters of seed as well as grain. The three landraces employed in this study were:
1.Seeraga samba (White) - Straw coloured Lemma and Palea
2.Seeraga samba (Red) - Brown tawny coloured Lemma and Palea
3.Seeraga samba (Black) - Purple coloured Lemma and Palea

RESULTS AND DISCUSSION

From Table 1, it is evident that, Seeraga samba (White) and Seeraga samba (Red) differed in geology of rocks, water availability depth and rainfall. In case of location of Seeraga samba (White), the rainfall is comparatively lesser than Seeraga samba (Red)but, the water table availability depth is deeper than the case of 'Red' variety where the water table availability is less deep. The alluvium soil has less water holding capacity than charnokites. This may also serve as a cause for deeper water availability depth for Seeraga samba (White). These environmental parameters alone differ between the 'Red and White' varieties of Seeraga samba, which might have caused cumulative alteration in the gene level leading to genotype difference in grain characters.

The superimposition of all the 10 thematic map layers on the backdrop map and the application of query builder has generated the following land suitability map indicating the possible places of cultivation. The samples of each landraces of Seeraga samba has been collected from Trichy (Seeraga samba White) and Kancheepuram districts (Seeraga samba Red and Black) respectively. Whereas, the land suitability evaluation map has generated interesting results, highlighting the possibility of introduction of these landraces in new districts for Seeraga samba(White) in Tamil Nadu (Fig. 1 and 2).

Seeraga samba (White):-

Sample Location - Lalgudi - Trichy district.

Possible Locations:-

Trichy district - Gunaseelam, Samayapuram, Poovalur, Srirangam.

Perambalur district - Tirumanur.

Sivaganga district - Manamadurai, Tiruppachetti, Sivaganga, Tiruppuvanam.,

Seeraga samba (Red & Black): -

Sample Location:	- Chengelpet - Kanchipuram district.
Possible Locations:-	
Kanchipuram district.	- Walajabad, Maraimalainagar, Kanchipuram, Abdulapuram.

A note worthy point is that the land suitability map for the landraces such as Seeraga samba (Red) and Seeraga samba (Black) has resulted in identification of new villages and locations within the same district itself but not in new districts. Although rice has wide adaptability, the individual cultivars do not span this entire geographic and environmental range, being limited to specific ecological niches[2]. Hence, the Seeraga samba(Red) and Seeraga samba (Black) have inclined towards specific ecological niche, therefore the possible places of land suitability has been restricted to the same district but in new villages.

Moreover, the habitat preference between Seeraga samba (White) and Seeraga samba (Red and Black) is strikingly differing as tabulated in Table 1. The land suitability parameters determined for Seeraga samba (White) is prevalent in many districts of Tamil Nadu whereas, the set of land suitability parameters identified for Seeraga samba (Red & Black) can be located within only one district of Tamil nadu. Moreover by applying the Boolean logic query builder in developing the land suitability map, the resultant map indicates only the locations with very definite conditions [8].This may be attributed as the reason for the identification of few selected number of locations generated by GIS for possible cultivation of Seeraga samba landraces.

According to Robert [9], overlay maps may be inaccurate due to map class inconsistency, indistinct boundaries, scales or resolution inconsistency and silver error. Furthermore, the assumption that such maps are capable of capturing significant units of productivity and ecological response may be incorrect. He also suggests that to guard against these, efforts should be made to improve accuracy of map information. Where this is not possible, the map user should be made aware of the possibility of error. With regard to the use of overlay maps to identify productivity / ecological units, testing is recommended to assess the significance of such unit. Other approaches to unit identifications should also be explored.

Filed level evaluation

The present field evaluation of performance of the chosen varieties in the identified places is an effort to test the productivity unit map / habitat suitability map generated using Arc view 3.3 GIS software. Field level evaluation for the two varieties namely Seeraga samba (White) and Seeraga samba (Red) was also carried out in one of the places identified by GIS for these varieties by adopting organic farming without addition of artificial fertilizers or any other chemicals.Due to drought, the farmers could not retrieve enough seeds and so they have not been able to spare the required amount of seeds for the field level research purpose in present study for Seeraga samba (Black). They saved the meager amount of seeds left out with them for possible propagation and conservation in their homestead farms itself. Hence, the field level performance could not be evaluated for Seeraga samba (Black).

The following table presents the observations recorded for both of these varieties in the field condition (Table 2 and Table 3). Both varieties have performed well in the newly identified locations with the grain yield of 3.5-4.0 (t/ha) and 3.0-3.5 (t/ha) respectively for Seeraga samba (Red) and Seeraga samba (White) respectively. The farmers have shown interest to cultivate these newly introduced varieties in their fields. It has been noted that Seeraga samba(Red) and Seeraga samba(White) have recorded low 100-grain weight, since the size of the seeds were comparatively smaller than other paddy varieties available in Tamil Nadu. According to rice productivity analysis of Tamil Nadu [10],the rice productivity has been classified into five categories namely:

High productivity(>2.5t/ha),Medium productivity(2.0-2.5t/ha) ,Medium- low productivity(1.5-2.0t/ha),Low productivity(1.0-1.5t/ha) ,Very low productivity(<1.0 t/ha).

Based on the above classification, it has been observed that the yield of these two landraces in the newly identified location appreciably falls in the "high productivity class'- this is black and white evidence for successful establishment and propagation of the landraces of Seeraga samba in the habitats/places located and mapped by habitat suitability analysis done using GIS. Moreover, Seeraga samba (White) is of consumers' top preference for consumption, due to the slender and small shape of grains together with scent. It has been of consumers' top preference for consumption of Seeraga samba (White).Whereas, the Seeraga samba (Red) landrace is blessed with drought tolerance which makes it a hardy crop suitable for dry tracts. Seeraga samba (Black) is used as a base in medications. Whereas Seeraga samba (White) is a favourite variety for special rice dishes owing to its grain qualities.

CONCLUSION

The application of GIS and image mosaicing techniques has emerged with clear maps and distinct identification of possible locations for conservation and propagation of traditional varieties of Seeraga samba in Tamil Nadu. This attempt could serve as a model in implementing conservational programmes for other crops too.

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RESEARCH ARTICLE

Breeding Biology of the Kerala Laughingthrush (*Garrulax fairbanki*) in the Upper Palni Hills, South India.

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ABSTRACT

Kerala Laughingthrush is a globally near threatened species and one of the 16 endemic birds of the Western Ghats. Breeding biology was studied in a 10 ha montane wet temperate forest (shola) plot at Kukkal in upper Palni Hills, southern Western Ghats. Intensive search method was adopted to find the nests in the study plot between April 2002 and June 2005. A total of 76 nests were observed and the nesting period was from February to August. The nesting activities were shared by both the sexes. Mean clutch size was two; incubation period 15 days and nesting period 16 days and nesting success were 51.31%. 95 % nests were placed in the shola forest plants and the remaining are in exotic plants and dominant species was *Psychotria nilgiriensis* var. *astephana* (19) and *Maesa indica* (15). Nest concealment was medium in 42.10 % followed by high in 30.26 % of nests. The ground cover, shade over the nest and nest plant height differed significantly between the unsuccessful and successful nests. The factors like shrub height, shade over the nest and ground cover were determining the nesting success of this species.

Key words: Kerala Laughingthrush, *Trochalopteron fairbanki*, breeding biology, nest-site selection, upper Palni hills.

INTRODUCTION

Laughing thrushes, of the genus *Garrulax* lesson, are belonging to family Timaliidae comprising of 46 species [1], of which 26 occur in the Indian subcontinent. Kerala Laughingthrush *Trochalopteron fairbanki is* one of the 16 endemics of the Western Ghats [2-3] and it is listed as a Near Threatened by IUCN [4]. It is found in the hills of Kerala and Western Tamil Nadu from 1200 m to the summit. Apart from description of the species by Ali and Ripley [3] some ecological aspects and geographical variation between other genus *cachinnans* was studied by Islam [5]. Conservation of species depends on knowing their breeding biology and identifies and conserves the habitat features that affect breeding productivity and survival [6]. Breeding success of birds is often influenced by habitat features surrounding nests [7-10]. Nest-site selection typically implies that some nesting site preferred while others are avoided. The probability of successful breeding also varies among nest sites. Thus for the nest site selection to be adaptive, nest sites associated with higher breeding success should be preferred ones [11]. Hence, this study was carried to find out breeding biology and nest site selection of Kerala Laughingthrush in the montane wet temperate forests (shola forests) from April 2002 to June 2005.

Study Area

The study was conducted at Kukkal shola of Palni Hills (10°15' N and 10°18' N and 77°20' E and 77°25' E), a hill range of the Western Ghats, Tamil Nadu, India (Fig 1). The Palni Hills consist of two well-marked topographic divisions, namely Upper Palnis and Lower Palnis. The intensive study was conducted in a 10 ha area in the Kukkal shola in the Upper Palni hills. The Upper Palnis with the elevation ranging between 1500 - 2450 m has a moderate climate with mean temperatures ranging between 12 ^{0}C and 23 °C in summer. In winter, the temperature ranges between 8.3 ^{0}C and 17.3 °C. The average annual rainfall was 165 cm. The vegetation is predominantly of the montane wet temperate forest commonly called as "Shola forest". Common plants in the area include species of *Syzygium*, *Ternstroemia, Sideroxylon, Meliosma, Elaeocarpus, Symplocos, Eurya, Litsea* and *Rhododendron*. The shola forests have adjacent exotic plantations of Wattle, Eucalyptus and Pine [12-14].

METHODS

Intensive search for nests was made on foot in the entire sample area (10 ha) by examining substrates suitable for nesting (shrubs and bushes). A nest was corroborated if adults were observed performing breeding activities such as nest building, incubation, feeding the young at or adjacent to the nest. Regular observations were made on the nest to record various aspects of the nesting cycle. Nest-site selection was studied with the following established methods [15-17].Variables were set at three levels, namely nest, nest - substrate and nest - patch with the following details: Nest- height (m), width (cm), depth (cm), position in the plant (low, middle, top), concealment, shade over the nest.Nest plant – species and its height Nest patch –canopy cover (%), ground cover (%), distance to trek path or road (m), distance to water (m), number of trees and number of shrubs. Nest concealment was estimated by viewing the nest at a distance of 2 m, 5 m, 7 m and 10 m in each of the four-cardinal directions [7]. Based on the number of points where the nest was not seen, the concealment was evaluated as low (0-4 points), medium (5-8 points), high (9-12 points) and very high (13-16 points).

A 15 m radius (0.07 ha) circular plot centered at nest-substrate was laid for every nest to study nest-site selection as suggested by Titus & Mosher [15]. Nest-patch variables were evaluated to identify the microhabitat required for nesting. Distance to road or trek path was used to identify whether the site selection was affected by human activity. Ground and canopy cover was visually quantified in percent.To test for nest site selection, except nest measurements, all other parameters were compared with similar measurements, recorded at randomly selected sites to identify the factors responsible for selecting a nest-site. Random sites were selected based on the place having potential nest-sites and also close enough to the earlier used sites. The 10 ha plot established for nest searching was divided into 100-

grids (20 × 50 m). Grids were plotted on an enlarged topographic map of the study area and numbered. Twenty grids were selected randomly by using lot system and were identified in the study site. Once the approximate grid or site was located, the nearer plant or shrub was made as center of the random plot.

Statistical Analysis

ANOVA, Mann-Whitney U, Wilcoxon sing rank test and other descriptive statistics (Mean and SD), wherever appropriate, were done [18]. Results are reported as significant if they are associated with a value of $P < 0.05$. Principal component analysis was performed on the nest-site characters to determine the most important factors delimiting the habitat niche (nest site selection) of the species. Discriminate function analysis was performed to identify the factors involved in separating the nest sites from the random sites. The SPSS software Version 10.05 [19] was used for data analysis. The Wilcoxon signed rank test was used to compare the successful and unsuccessful nests and to identify the most significant factors that influencing the success of nest.

RESULTS

Kerala Laughingthrush breeding season was observed between February and August. A total of 76 nests were observed, of which 23, 19, 20, 14 nests were located in 2002, 2003, 2004 and 2005 respectively. The cup shaped nest was placed in middle of shrubs / recruits and the nesting materials mainly used were rootlets in the outer line and in the inner feathers, moss, and soft materials. Out of the 76 nests, 84 % (64) nests were observed between first week of April and the third week of May. Only one nest observed with single egg and rests all clutch size were two.The mean incubation and nestling periods were 15.35 $\pm$ 0.69 and 16.29 $\pm$ 0.84 days, respectively. Totally 151 eggs were laid in 76 nests from which 96 chicks fledged out. The hatching success was 61.84 %, fledging success 48.68 % and nesting success 51.31 % during this study.

Nesting Plants

Our study revealed that 94.73 % nests were placed in the shola forest plants and the remaining are in exotic plants; Acacia (3) and Eucalyptus sp (1). Nesting plants of Kerala Laughingthrush identified were *Psychotria nilgiriensis* var. *astephana* (19), *Maesa indica* (15), *Symplocos foliosa* (12), *Lasianthus acuminatus* (7), *Beilschmiedia wightii* (6), *Symplocos cochinchinensis* (6), *Viburnum cylindricum* (4), *Sarcococca saligna* (1), *Xenacanthus pulneynensis* (1) and an unidentified species.

Nest and Nest site selection

Nest-site variables were collected for 76 nests in four successive breeding seasons between April 2002 and August 2005. Nests were mostly placed on the shrubs at the height between 0.5 to 5 m (Fig 2) and position in the plant was middle middle (33.3%) followed by middle edge (20.3 %) and mean nest cup depth and width was 5.01 $\pm$ 1.13 cm and 8.33 $\pm$ 0.96 cm. Nest concealment was medium in 42.10 % followed by high in 30.26 %. Nests were observed dense shrub (18.35 + 8.52), tree (13.01$\pm$3.61) and thick ground cover (60 %) and they are statistically significant also (Table 1.).

The first three principal components were extracted and it showed 54.9% of the total variance and they first component was closely associated with nest plant height, nest height and ground cover. The second component was associated with nest concealment and canopy cover. The third component was closely associated with shade over the nest and nest cup width (Table 2). The factors like nest plant height, nest height, ground cover were directly related to nest plant. The Discriminate Function Analysis (stepwise) resulted two variables canopy cover (0.46) and number of shrubs (0.46) are the crucial factors determine the nest site selection of Kerala Laughingthrush.The successful and

unsuccessful nests nest site characters were analysed to find out the factors crucial for nesting success. The ground cover (Z = 2.504; p 0.012), nest plant height (Z = 2.293; p 0.02) and shade over the nest (Z = 2.805; p 0.005) were significantly differed from the unsuccessful nest (Table 3).

DISCUSSION

Nesting ecology of Kerala Laughingthrush was studied in the shola forest of Palni hills and during the study the number of nests was higher in the drier months (April – May) and decreased with rainfall, which again is similar to that in most of the tropical birds[20-22].

Vegetation structure around the nest and nest plant can also influence the breeding success of the birds [7, 23]. In the present study more than 90 % of nests were on shola shrubs with thick foliage. Kerala Laughingthrush nests in wide varieties of shrubs and there was no species specific preference was observed. The use of wide variety of species by these birds may be a strategy against predator search tactics [24,25]. Several studies have reported that increased amount of canopy cover immediately concealing nests was associated with reduced nest predation [7, 8, 26]. In this study also we have observed shade over the nest and moderate nest plant height had more success. High densities of foliage, when used for nesting can decrease predator efficiency by increasing the number of potential nest sites a predator must search [7,27,28]. After the successful release of fledgling, the nest was destroyed by the birds themselves, similar observation recorded by Islam [5] and in Drongos by Vijayan [29]. The number of trees, number of shrubs and canopy cover were the factors related to one another, when more trees and shrubs with closed canopy gave high concealment to the nests. So, nests could escape from predators and avoid the inclement weather. Similar results were reported by Calder [30], Walsberg [31] and Matsuoka *et al*. [28]. As predation is a powerful selective pressure, birds have evolved various antipredator strategies, which may reduce nest losses [32]. The strategies include selecting a thick vegetation cover for high concealment of the nest and nearness to trek path for escape from predator. The total number of shrubs within a nest patch is a good indicator of the vegetation complexity and it had a significant variation between nesting ad random sites but no effect was observed on successful and unsuccessful nest because montane wet temperate forests have more number of shrubs. Ground litter cover was also playing an important role in the nest site selection of Kerala Laughingthrush, generally these birds fed their chicks more on invertebrate diet during the early stages of fledgling hence the good ground cover supports the invertebrate diversity.

Declines in shrub birds are associated with decreased availability of suitable breeding habitat was reported worldwide [33-35]. Some shrub birds appear to be ‘‘area-sensitive”, and will not occupy otherwise suitable habitat if the patch does not meet their minimum requirements [36,37]. The Kerala Laughingthrush was observed nesting in the shola shrubs at Kukkal in the Upper Palni hills, shrub density, shade over the nest and nest plant height were determine the nesting success of this bird. The shrub density and ground cover was important in both food availability and production of the nest from the predator. Upper Palni hills is the second most populated area in the Western Ghats [38] and it may be noted that the National Remote Sensing Agency, Hyderabad has reported a loss of 25 % of the forest cover in the Palni Hills between 1972 and1980. The extent of montane wet temperate forests has dwindled in the past century due to mainly conversion of plantations. Current threats are mainly from livestock grazing, fuel-wood harvest and agricultural expansion [39]. During the study period disturbances, namely cutting and clearing of undergrowth for various purposes such as firewood, fencing and poles in agricultural fields were noted and directly affect the habitat of the species and thereby its survival. Human disturbance and birds nesting was coexisting one another [40,41] so such increased pressure may leads to change this near threatened species to threatened species.

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Table 1. Nest-site Characteristics and comparison with random sites of the Kerala Laughingthrush.

Parameter	**Nest site (mean ± SD)**	**Random site (mean ± SD)**	**U**	p
Nest height (m)	2.23 ± 0.81			
Nest width (cm)	8.33 ± 0.96			
Nest depth (cm)	5.01 ± 1.13			
Position in the Plant	Middle middle (33.3%)			
Shade over the nest (%)	84.58 ± 14.79			
Nest concealment	2.08 + 0.80			
Nest plant height (m)	3.18 ± 1.22			
Canopy cover (%)	81.97 ± 9.83	67.44 ± 25.42	85.07	0.001
No. of trees	13.01 ± 3.61	11.71 ± 3.49	83.64	0.020
No. of shrubs	18.35 ± 8.52	12.18 ± 5.53	94.34	0.001
Ground cover (%)	60.76 ± 25.15	48.05 ± 21.90	87.14	0.020
Distant to trek path (m)	11.63 + 33.20	30.98 ± 19.86	45.05	0.001
Distant to water (m)	43.51 ± 35.92	54.42 ± 22.62	67.99	ns

Table.2 Factor loading of various nest site characteristics with the first three principal components for the nest data.

Variables	**PC I**	**PC II**	**PC III**
Nest Height	0.747	-9.43	-0.111
Nest depth	-0.360	0.325	-7.52
Nest plant height	0.887	9.95	-5.48
Canopy cover	0.215	0.743	0.299
No. of trees	-0.136	-0.429	-0.243
Shade over the nest	-0.123	0.260	0.782
Ground cover	0.619	9.34	7.14
Distance to trek path	-4.05	0.238	-0.146
Distance to water	-0.330	0.452	-0.349
Eigen value	2.195	1.878	1.320
% Variance	28.29	15.64	10.99
% Accumulated variance	28.29	43.94	54.93

Table.3. Comparison of successful with unsuccessful nests of the Kerala Laughingthrush in the Upper Palni Hills.

Parameter	**Successful nest (m ± SD)**	**Unsuccessful nest (m ± SD)**	**Z**	**p**
Nest height (m)	2.41 ± 0.83	2.49 ± 0.70	-0.70	0.48
Nest width (cm)	8.31 ± 0.79	8.58 ± 0.94	-1.54	0.12
Nest depth (cm)	5.06 ± 1.2	5.09 ± 1.05	-0.67	0.50
Shade over the nest (%)	86.80 ± 11.11	76.48 ± 15.01	2.80	0.05*
Nest concealment	2.08 + 0.80	1.96 + 0.92	0.69	0.12
Nest plant height (m)	3.38 ± 0.88	3.77 ± 1.48	2.29	0.02*
Canopy cover (%)	87±6.75	86.34 ± 7.99	-.378	0.70
No. of trees	11.60 ± 3.47	12.72 ± 3.40	-0.14	0.88
No. of shrubs	14.70 ± 2.71	18.34 ± 10.44	-0.10	0.91
Ground cover (%)	64.40 ± 23.62	75.00 ± 24.71	-2.50	0.01*
Distant to trek path (m)	7.05 ± 7.21	14.56 ± 36.86	-0.35	0.72
Distant to water (m)	51.10 ± 44.96	51.70 ± 44.05	-0.66	0.50

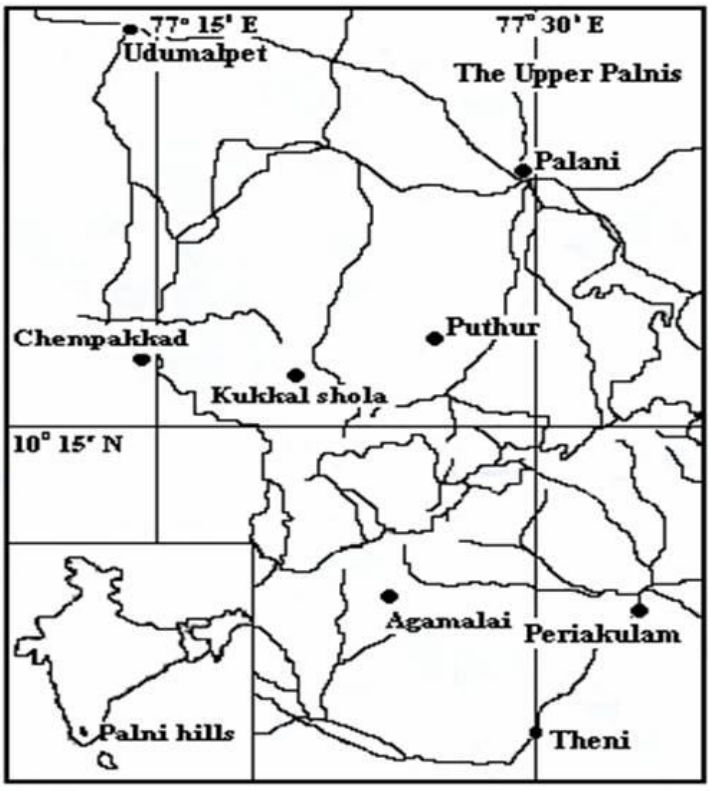

Figure 1. Map of the Palni Hills with the study location of Kukkal shola (scale 1:250,000).

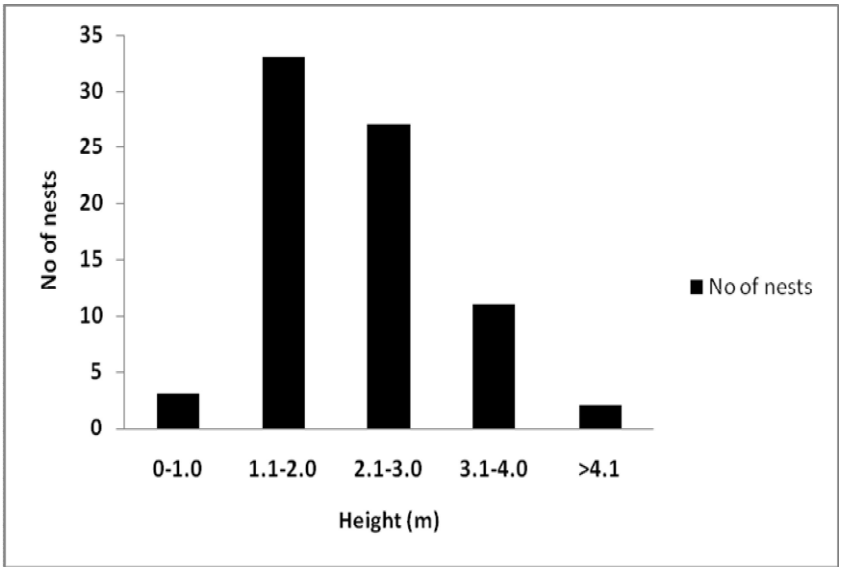

Figure 2. Nest height of Kerala Laughingthrush in the upper Palni Hills.

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RESEARCH ARTICLE

Studies on the Present Status of Singanallur Pond with Special Reference to Physico-Chemical Parameters, Coimbatore, Tamil Nadu, India.

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ABSTRACT

The water sample of the Singanallur pond was analyzed to assess the pollution status of the pond. The physico-chemical parameters were analyzed by the standard methods of APHA, 1995. The results obtained were showed fluctuations in concentration of parameters which gave an idea about the intensity of pollution. The present investigation revealed that discharges of domestic and industrial waste in to the pond cause the deterioration of water quality. Therefore it is concluded that there is an urgent need to assess the water quality and to protect the pond.

Key words: pollution status, physico-chemical parameters, domestic and industrial waste.

INTRODUCTION

Water is essential for the well being of mankind and for sustainable development. In general water commodities are used for domestic and agricultural purposes. In this view an attempt has been made to study pond environment with special reference to physical and chemical characteristics. The number of natural and manmade lakes, ponds and reservoirs having some important services to nearby urban and rural populations as large lakes but unfortunately are more susceptible to pollution.It is suggested that water samples are highly polluted so some effective measures are urgently required for periodic water quality monitoring and maintenance of water source. Human and ecological use of in-stream water requires to be considered for both the quantity and the quality of water [1,2]. Water quality is important to assess the health of a watershed and to make necessary management decisions to control current and future pollution of receiving water bodies [3,4]. The information on water quality and pollution sources is important for implementation of sustainable water –use management strategies [5-10].

The dense growth of *Eichhornia crassipes* along with water bloom formation by *Microcystic aeruginosa* has been observed to reach the limit of nuisance which may further deteriorate the lake ecosystem by onset of anaerobic condition in the pond. The present investigation provides information to highlight the features of Singanallur pond. In India Still now several researchers have done Study on Physico-chemical and Biological characteristic of Standing and Running Water Resources [11-13].

MATERIALS AND METHODS

Study Area

The Singanallur pond is located 0.5 km. south west of Singanallur village in Coimbatore district. It is situated at an altitude of 450m from the Mean Sea Level. The pond receives water from Noyyal river and Singanallur canal during rainy season. The total depth of the pond is 13.95 feet and the area is about 1.143sq.km, the water storage of the pond is about 14.80 million cubic feet.

Sample collection

Water samples of Singanallur pond was collected during early hours for three months from October to December (2011-2012) (Before mixing pollution and after mixing pollution). The samples were uniformly maintained throughout the study period .Then the samples were analyzed for physico- chemical parameters and nutrient status like water temperature, color, suspended solids(SS) dissolved solids(DS), totalsolids(TS), pH ,alkalinity, dissolvedoxgen(DO), dissolved carbondioxide(DCO_2), phosphates, nitrates ,calcium, magnesium, sulphates and chloride using standard methods of American Public Health Association[14].

RESULTS AND DISCUSSION

The present investigation of Singanallur pond shows vulnerable changes in the water quality parameters. The physico- chemical parameters and nutrient status of the pond were analyzed and the results obtained were presented in tables (1-2). The water temperature fluctuated from 25^0C to 30^oC. Thermal conditions of the layers of water bodies are influenced by atmospheric temperature. The color of the water noticed to be green, which indicate the concentration of algae, dissolved and suspended substances in the water. The suspended solids level was maximum during rainy month (November). This may be caused by silt, clay and organic materials suspended or dissolved in it. The pH level was alkaline during October to December. High pH is normally associated with high photosynthetic activity in water; during this maximum consumption of CO_2 by phytoplankton is seen [15].Dissolved carbondioxide values were obtained as minimum in October and as maximum in November were reported that total hardness was high during summer than monsoon and winter.

As the number of phytoplankton increases through winter and summer month's free carbondioxide disappears because of greater utilization of free CO_2 for photosynthetic activity [16].During the sampling period the amount of nutrients was higher due to washing, dumping, religious offerings, restaurant activities and other anthropogenic activities. The values obtained for phosphates were maximum during October and minimum during December. Phosphate and nitrates are the basic nutrients which determine the productivity of static water. The values obtained for nitrates were higher during winter and low during summer. The nitrate depletion in summer may be due to the photosynthetic activity of algae and macrophytes. Sulphate values obtained were maximum during November and minimum during December. Sulphate is a naturally occurring ion found almost in all types of water its concentration occurs in wide ranges in nature. Chloride had maximum values during the rainy season and this increase in concentration of chloride is probably due to the heavy rainfall might have supplemented its source from catchment areas. These parameters are reactive components that are partially of anthropogenic origin [17].

CONCLUSION

Based on the above investigation, it was concluded that the responsible factors for eutrophication are mainly the localized human activities in the form of cloth washing, bathing, cattle wading. Sewage and run off. All these factors have brought about several changes in disturbing the balance and the ecosystem is leading towards further deterioration. The unplanned activities resulting in the fact to development of water hyacinth and makes unfit for human consumption. So, a periodical survey of the lake is necessary to maintain the quality of water for future uses.

Table1:Physico-chemical and nutrient status of Singanallur pond (before mixing of pollution)

S.No	Parameters	October	November	December
1	Water Temperature (^{0}C)	25	27	30
2	Colour	Green	Green	Green
3	Suspended solids (mg/l)	431.0±6.44	640.3±4.163	536.2±1.56
4	Dissolved solids (mg/l)	558.3±3.012	360±4.321	324.34±3.231
5	Total solids (mg/l)	532.4±6.260	874.0±4.315	732.2±2.613
6	pH	8.2	8.0	8.1
7	Alkalinity(mg/l)	23.5±1.003	32.23±0.342	36.7±0.940
8	Dissolved oxygen(mg/l)	5.6±0.042	3.95±0.102	7.10±0.076
9	Dissolved CO_2 (mg/l)	3.44±0.54	4.86±0.045	4.412±0.062
10	Phosphate(mg/l)	5.43±0.045	4.45±0.076	4.36±0.321
11	Nitrate(mg/l)	11.02±0.012	14.78±0.023	17.24±0.032
12	Calcium(mg/l)	150.2±0.002	165.02±1.875	143.2±0.654
13	Magnesium(mg/l)	69.20±0.872	39.8±0.982	36.4±0.876
14	Sulphate(mg/l)	35.90±0.765	56.3±2.459	40.60±3.10
15	Chloride(mg/l)	80.0±0.983	98.3±1.002	76.98±1.53

Mean±SD Period :2011-2012

Table2: Physico-chemical and nutrient status of Singanallur pond (after mixing of pollution)

S.No	Parameters	October	November	December
1	Water Temperature (^{0}C)	26	24	25
2	Colour	Green	Green	Green
3	Suspended solids (mg/l)	511.2±5.14^d	614.0±4.062	436.2±1.36
4	Dissolved solids (mg/l)	438.3±3.320	410±4.210	442.14±2.031
5	Total solids (mg/l)	976.4±4.260	894.0±4.525	932.2±3.413
6	pH	8.2	8.5	7.7
7	Alkalinity(mg/l)	31.5±1.001	41.23±0.842	37.7±0.440
8	Dissolved oxygen(mg/l)	5.2±0.082	4.75±0.072	6.30±0.046
9	Dissolved CO_2 (mg/l)	4.54±0.624	4.26±0.055	5.41±0.073
10	Phosphate(mg/l)	5.72±0.035	6.32±0.062	4.42±0.324
11	Nitrate(mg/l)	18.02±0.035	21.78±0.053	25.24±0.18
12	Calcium(mg/l)	142.2±0.671	157.12±1.750	151.2±0.340
13	Magnesium(mg/l)	59.20±0.72	42.8±0.762	46.4±0.576
14	Sulphate(mg/l)	47.70±0.735	53.70±0.845	57.60±3.30
15	Chloride(mg/l)	93.0±0.943	99.3±0.402	87.86±0.54

Mean±SD Period :2011-2012

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Illustrations: Tables - Should be typed on separate sheets of paper and should not preferably contain any molecular structures. Only MS word table format should be used for preparing tables. Tables should show lines separating columns but not those separating rows except for the top row that shows column captions. Tables should be numbered consecutively in Arabic numerals and bear a brief title in capital letters normal face. Units of

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