ANVESAK
ISSN : 0378 – 4568 UGC Care Group 1 Journal

MEDICAL IMAGE SEGMENTATION USING DEEP LEARNING WITH FASTER RCNN AND U-NET ARCHITECTURES

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Abstract

Image segmentation leads to more granular information about the form of a picture and thus an extension of the concept of Object Detection. We segment i.e. divide the pictures into regions of various colors which helps in distinguishing an object from the opposite at a finer level. It was applied in medical imaging like locating tumors in the brain, measuring tissue structures, studying anatomy, surgery, etc., There are semantic segmentation and instance segmentation. Here we are using the U-Net architecture for semantic segmentation, and overview the Mask R-CNN architecture as an example instance segmentation. Image segmentation has different techniques, like region-based segmentation, edge detection segmentation, and segmentation-supported clustering. Image segmentation creates a pixel-wise mask for every object within the image. This system gives us a much more granular understanding of the object(s) within the image. In this paper, we explained Faster R-CNN and U-Net-based segmentationarchitectures applied for the medical images.

Keywords: Medical Image Segmentation , U-NET, Faster RCNN, Deep Learning

I.INTRODUCTION

In recent years, hardware design and software development have greatly promoted the event of medical imaging [1]. Image segmentation involves a good range of fields, like medical images [3, 4], transportation [5], housing design [6], and so on.

Basically, segmentation may be a process that partitions a picture into regions [2]. It's a picture processing approach that permits us to separate objects and textures in images.

Segmentation is particularly preferred in applications like remote sensing or tumor detection in biomedicine. There are many traditional ways of doing this. Various methods are point, line, and edge detection methods, thresholding, region-based, pixel-based clustering, morphological approaches used for segmentations. New methods are developed for segmentation with convolution neural networks may be a common deep learning architecture, which became

indispensable in tackling more advanced challenges with image segmentation.

The two sorts of image segmentation: Semantic Segmentation and Instance Segmentation. Semantic segmentation will classify all the people as one instance. Now, the image on the proper also has 5 objects (all of them are people). But here, different objects of an equivalent class are assigned as different instances. this is often an example of instance segmentation. we'll be implementing a state-of-the-art image segmentation technique called Mask R-CNN to unravel an instance segmentation problem.

Mask R-CNN is essentially an extension of Faster R-CNN. Faster R-CNN is especially applied for object detection tasks. For a given image, it returns the category label and bounding box coordinates for every object within the image.

In semantic segmentation, each pixel is assigned to an object category; In instance segmentation, each pixel is assigned to a private object; The U-Net architecture are often used for semantic segmentation.

The Mask R-CNN architecture are often used as an example segmentation Medical image analysis is employed by analyzing images obtained by medical imaging systems to unravel clinical problems. the aim is to extract effective information and improve the extent of clinical diagnosis. In recent years, automatic segmentation supported deep learning (DL) methods has been widely used, where a neural network can automatically learn image features, which is in sharp contrast with the normal manual learning method. U-net is one among the foremost important semantic segmentation

frameworks for a convolution neural network (CNN). This is often basically utilized in the medical image analysis domain for lesion segmentation, anatomical segmentation, and classification. The advantage of this network framework is that it can't only accurately segment the specified feature target and effectively process and objectively evaluate medical images but also help to enhance accuracy within the diagnosis by medical images. Therefore, this text presents a literature review of medical image segmentation supported U-net, that specialize in the successful segmentation experience of U-net for various lesion regions in six medical imaging systems. alongside the newest advances in DL, this text introduces the tactic of mixing the first U-net architecture with deep learning and a way for improving the U-net network.

U-Net may be a convolution neural specification that expanded with few changes within the CNN architecture. It had been invented to affect biomedical images where the target isn't only to classify whether there's an infection or not but also to spot the world of infection.

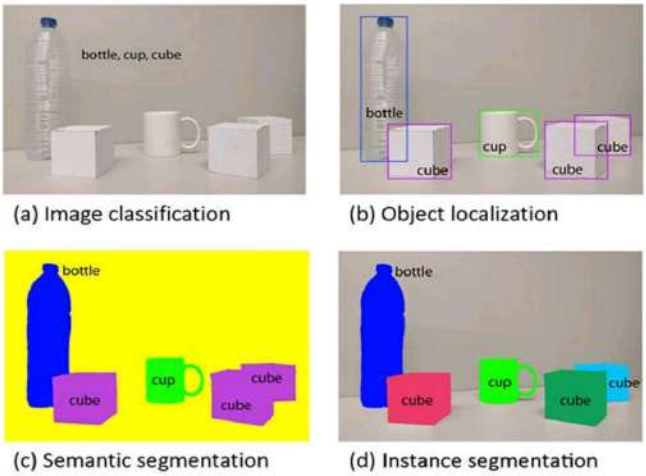

Figure 1: Image classification (*top-left*), object detection (*top-right*), semantic segmentation (*bottom-left*), and instance segmentation (*bottom-right*).

We'll be performing instance segmentation with Mask R-CNN When performing traditional image classification our goal is to predict a group of labels to characterize the contents of an input image (top-left).Object detection builds on image classification, but this point allows us to localize each object in a picture . The image is now characterized by:

1. Bounding box (x, y)-coordinates for every object
2. An associated class label for every bounding box

An example of semantic segmentation are often seen in bottom-left. Semantic segmentation algorithms require us to associate every pixel in an input image with a category label (including a category label for the background).Pay close attention to our semantic segmentation visualization — notice how each object is indeed segmented but each "cube" object has an equivalent color. While semantic segmentation algorithms are capable of labeling every object in a picture they can't differentiate between two objects of an equivalent class.

This behavior is particularly problematic if two objects of an equivalent class are partially occluding one another — we've no idea where the boundaries of 1 object ends and therefore the next one begins, as demonstrated by the 2 purple cubes, we cannot tell where one cube starts and therefore the other ends. Instance segmentation algorithms, on the opposite hand, compute a pixel-wise mask for each object within the image, albeit the objects are of an equivalent class label (bottom-right). Here you'll see that every of the cubes has their own unique color, implying that our instance segmentation algorithm not only localized each individual cube but predicted their boundaries also.

II. REVIEW OF LITRETURE

Qianjin Li et al. used Fully Convolutional Network (FCN) model and max-pooling based U-net model, for the Diagnosis Method for Diabetic Retinopathy supported Improved U-NET Network. The experimental results show that the proposed model are able to do good segmentation and diagnosis results [7].

Zhongming Luo et al. proposed Considering the low contrast between retinal vessels and therefore the background image, complex structural information also as blurred boundaries between tissue and blood vessels, the retinal vessel image segmentation algorithm supported the improved U-Net network [8].

Xiaocong Chen et al. proposed a specific deep learning algorithm for automated segmentation of COVID-19 infection regions. Results demonstrate the better performance in the algorithm for automated segmentation of COVID-19 Chest CT images [9].

Hao Dong et al. proposed brain tumor segmentation, which is developed using U-Net based deep convolutional networks. That method was evaluated on Multimodal brain tumour Image Segmentation (BRATS 2015) datasets, which contain 220 high-grade brain tumour and 54 low-grade tumor cases. Cross-validation has shown that the method can get the segmentation efficiently [10].

Xiaomeng Li et al. proposed UNet (H-DenseUNet), which consists of a 2-D DenseUNet for efficiently extracting intra-slice features and a 3-D counterpart for hierarchically aggregating volumetric the auto-context algorithm for liver and tumor segmentation. The training process of the H-DenseUNet in an end-to-end manner, where the intra-slice representations and inter-slice features are often jointly optimized through a hybrid feature fusion layer[11].

Jun Zhang et al. used the U-Net for the segmentation of in cardiac ventricular an improved U-net named LU-Net by the subsequent three methods. First, so as to enhance the efficiency and effectiveness of extracting the features of the first image, we combine U-net with the SE-Net model. This model reweights the channels of the feature map, which may give higher weight to the useful information and lower weight to the invalid information[12].

Roshan Reddy et al . used Fully convolutional neural network architectures have proved to be very efficient for medical image segmentation, with U-Net inspired architecture because of the current state-of-the-art. Generative adversarial networks (GAN) inspired architectures have recently gained popularity in medical image segmentation with one among them being SegAN, a completely unique end-to-end adversarial neural specification. They investigate SegAN with three different types of U-Net-inspired architectures for ventricle segmentation from cardiac MRI data[13].

Shuyi Li et al. combines densely connected U-Net attentively gates (AGs). It contains an encoder and a decoder. The encoder may be a densely connected convolutional network and therefore the decoder is that the decoder of U-Net integrated with AGs. The proposed method is tested on the general public and authoritative database-Digital Database for Screening Mammography (DDSM) database [14].

Su Yang et al. investigated applying the Faster R-CNN, which has recently shown incredible performance on many public datasets, to cell detection. The Faster R-CNN contains both segmentation and classification. Faster R-CNN model, a series of experiments are performed. Experimental results show that the Faster R-CNN can detect most cells during a microscopic image[15].

Defeng Zhang et al. said First, the Faster R-CNN+ZF model is employed to locate the blind spot via a bounding box (B-box). Second, the most blood vessels within the B-box are removed by Hessian matrix if necessary. Finally, a shape constrained level set algorithm is employed to segment the boundary of the OD[16].

Wei Tang et al. proposed liver detection and segmentation model using DSL. the primary stage uses improved Faster Regions with CNN features (Faster R-CNN) to detect approximate position of liver. The obtained images are processed and input into DeepLab to get the contourof liver[17].

Jeremiah W. Johnson proposed Mask-RCNN are often wont to perform highly effective and efficient automatic segmentations of a good range of microscopy images of cell nuclei, for a spread of cells acquired under a spread of conditions[18].

III. U-NET ARCHITECTURE

The U-Net architecture was invented by OlagRonneberger et al . for Bio-Medical Image segmentation. This architecture consists of two main parts that were encoder and decoder. The encoder is used for all the covenant layers followed by pooling operation. it's wont to extract the factors within the image. The next part decoder uses transposed convolution to allow localization. it's again an F.C connected layers network in the architecture[19].

U-Net is more successful than conventional models, in terms of architecture and in terms pixel-based image segmentation formed from convolutional neural network layers. It's even effective with limited dataset images. The presentation of this architecture was first realized through the analysis of biomedical images.As it's commonly known, the dimension reduction process within the height and width that we apply throughout the convolutional neural network—that is, the pooling layer — is applied within the sort of a dimension increase within the last half of the model in the architecture.

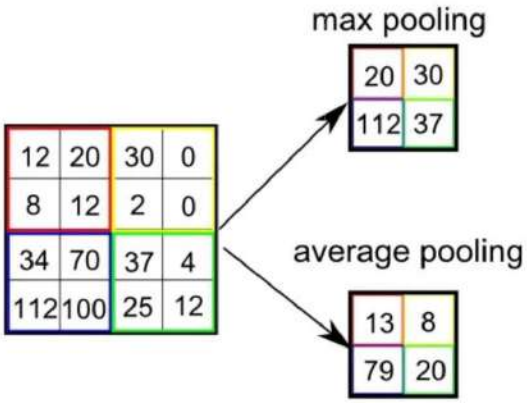

Figure 2: Max Pooling amdAvg Pooling

The pooling layer decreases height and width of data in the image for the number of channels of the input matrix . The calculation may be a step wont to reduce complexity (Each element of the image matrix is named a pixel). The pooling layer refers to a pixel and groups of pixels. Pooling layers can work with many approaches, including maximum, average, or median layers. These layers are intended to extend the resolution of the output. The output is combined with high-resolution features for the model. A sequential convolution layer then aims to supply a more precise output supported this information. Network—that is, the pooling layer — is applied within the sort of a dimension increase within the last half of the model.

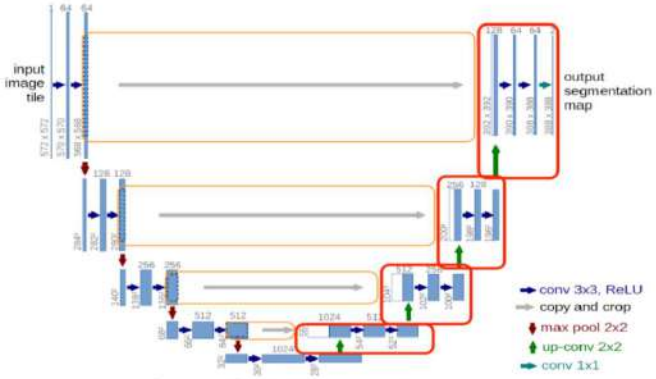

Figure 3 : U-Net Architecture

From this figure, the name "U-Net" is clear, because the architecture diagram shows a U shape. the essential idea of the U-Net is to first obtain a lower-dimensional representation of the image through a standard convolution neural network, then up sample that low-dimensional representation to supply the ultimate output

U-Net takes its name from the architecture, which when visualized, appears almost like the letter U, as shown within the figure above. Input images are processed and obtain a segmented output map. the foremost special aspect of the architecture within the last half. The network doesn't have a fully-connected layer. Only the convolution layers are used. Every standard convolution process is activated by a ReLU is theactivation function.

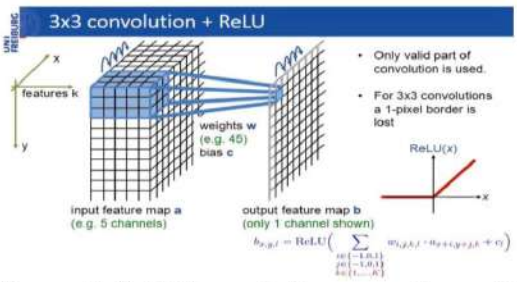

Figure 4: 3x3 Convolution operation with Relu

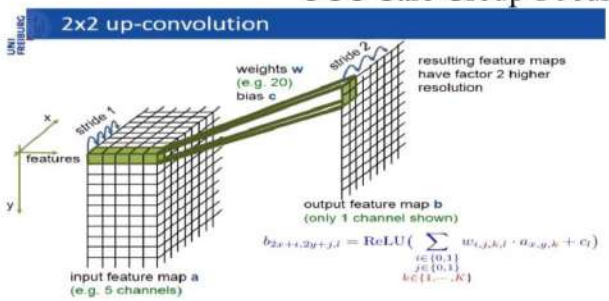

Figure 5: 2x2 Up- Convolution operation

The pixels within the border region are symmetrically added round the image in order that images are often segmented continuously[19]. With this strategy, the image is segmented completely. The padding (pixel adding) method is vital for applying the U-Net model to large images; otherwise, the resolution are going to be limited by the capacity of the GPU memory. The results of padding and segmenting with the mirroring.

IV. FASTER R-CNN ARCHITECTURE

Once you understand how Faster R-CNN works, understanding Mask R-CNN are going to be very easy. So, let's know it step-by-step ranging from the input to predicting the category label, bounding box, and object mask.

- Faster R-CNN first uses a ConvNet to extract feature maps from the pictures.
- These feature maps are then skilled a neighborhood Proposal Network (RPN) which returns the candidate bounding boxes.
- We then apply an RoI pooling layer on these candidate bounding boxes to bring all the candidates to an equivalent size.
- And finally, the proposals are passed to a totally connected layer to classify and output the bounding boxes for objects.

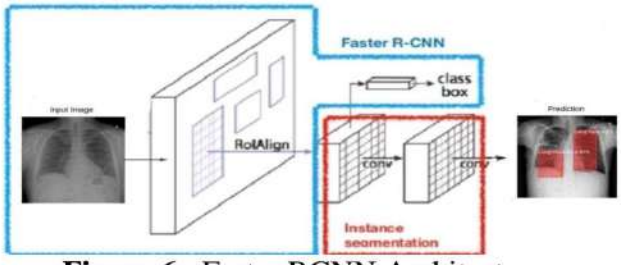

Figure 6: Faster RCNN Architecture

A. Backbone Model

Similar to the ConvNet that we use in Faster R-CNN to extract feature maps from the image, we use the ResNet 101 architecture to extract features from the images in Mask R-CNN. So, the first step is to take an image and extract features using the ResNet 101 architecture. These features act as an input for the next layer.

B. Region Proposal Network (RPN)

Now, we take the feature maps obtained in the previous step and apply a region proposal network (RPM). This basically predicts if an object is present in that region (or not). In this step, we get those regions or feature maps which the model predicts contain some object.

C.Region of Interest (RoI)

The regions obtained from the RPN might be of different shapes, Hence, we apply a pooling layer and convert all the regions to the same shape. Next, these regions are passed through a fully connected network so that the class label and bounding boxes are predicted.

Till this point, the steps are almost similar to how Faster R-CNN works. Now comes the difference between the two frameworks. In addition to this, Mask R-CNN also generates the segmentation mask.

For that, we first compute the region of interest so that the computation time can be reduced. For all the predicted regions, we compute the Intersection over Union (IoU) with the ground truth boxes. We can computer IoU like this:

IoU = Area of the intersection / Area of the union

Now, only if the IoU is greater than or equal to 0.5, we consider that as a region of interest. Otherwise, we neglect that particular region. We do this for all the regions and then select only a set of regions for which the IoU is greater than 0.5.

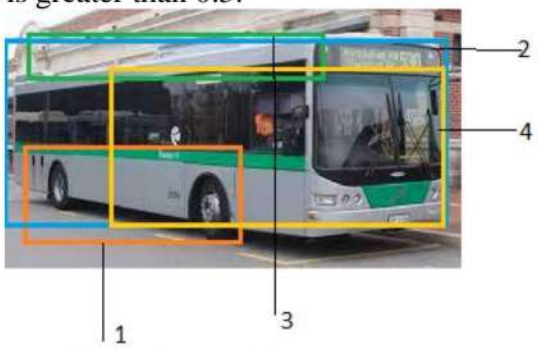

Figure 7: the IoU of Box 1 and Box 2 is possibly less than 0.5, whereas the IoU of Box 3 and Box 4 is approximately greater than 0.5. Hence. we can say that Box 3 and Box 4 are the region of interest for this particular image whereas Box 1 and Box 2 will be neglected.

D. Segmentation Mask

Once we have the RoIs based on the IoU values, we can add a mask branch to the existing architecture. This returns the segmentation mask for each region that contains an object. It returns a mask of size 28 X 28 for each region which is then scaled up for inference.

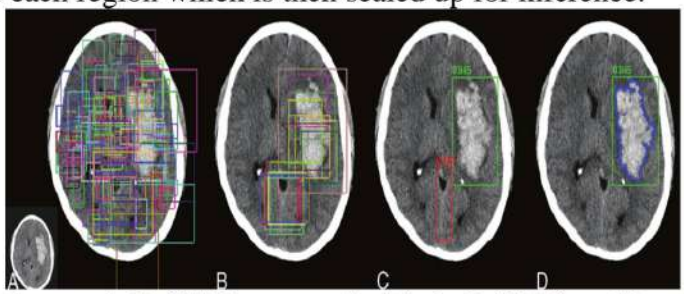

Figure 8: Overview of the mask R-CNN approach. Mask R-CNN architectures provide a flexible and efficient framework for parallel evaluation of region proposal (attention), object detection (classification), and instance segmentation. A, Preconfigured bounding boxes at various shapes and resolutions are tested for the presence of a potential abnormality. B, The highest ranking bounding boxes are identified and used to generate region proposals that focus algorithm attention. C, Composite region proposals are pruned using no maximum suppression and are used as input into a classifier to determine the presence or absence of hemorrhage. D, Segmentation masks are generated for cases positive for hemorrhage.

	R-CNN	Fast R-CNN	Faster R-CNN
Test time per image (with proposals)	50 seconds	2 seconds	0.2 seconds
(Speedup)	1x	25x	250x
mAP (VOC 2007)	66.0	66.9	66.9

Figure 9: Training time and testing time of R –CNN

V.CONCLUSION

The analysis and processing of medical images have great influence on clinical application and research project. the utilization of deep learning can provide new ideas for medical image analysis, allowing detection of image morphology or texture patterns only from data. Since DL has made great progress in several medical imaging systems, it's become the most aim of medical image analysis to further improve its methods. this text reviews the appliance of U-net and R-CNN in medical image processing. The results show that the DL method supported the CNN has been widely known in medical image classification, segmentation, and other fields. U-net is one among the foremost important semantic segmentation frameworks for the CNN. In semantic segmentation, each pixel is assigned to an object category; In instance segmentation.

The U-Net architecture are often used for semantic segmentation; The Mask R-CNN architecture are often used as an example segmentation. However, this method isn't perfect in some imaging systems. Therefore, it must be further improved in future studies for application in various imaging systems.

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PREDICTION OF HEART DISEASES USING NAIVE BAYES ALGORITHM

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Abstract

The medical system is an inevitable task to be done in human life. The business of medical care has become a significant field in a wide area of medical science. The healthcare industry has a large amount of data and hidden information. Effective conclusions are drawn with this hidden information through the use of datamine techniques. Several tests are performed to diagnose heart disease in the patient; However these tests can be reduced by datamine. But there is a lack of analysis of the tool to provide effective test results with hidden information, so a system has been developed using data mining algorithms to classify data and diagnose heart disease.Datamining acts as a solution to many health problems. The NaiveBayes Algorithm is one such database technique that helps diagnose a patient with heart disease. This paper analyzes some parameters and predicts heart disease, and recommends a Cardiovascular Disease Prognosis System (CDPS) based on datamining approaches.

Keywords: healthcare system, datamining, heart diseases, naïve bayes algorithm, Cardiovascular Disease Prognosis System.

1. Introduction

The health system is provided by preventing or treating disease by maintaining mental and physical health. Heart disease is the leading cause of death in the world today. The World Health Organization (WHO) estimates that 12 million deaths worldwide are caused by heart disease each year. More than 80% of deaths in the world are caused by heart disease. Who estimates that in the future, nearly 23.6 million people will die of heart disease. Euro Heart Survey on Heart Disease conducted in 25 countries, including adults with moderate to severe congenital heart disease, Infectious endocardial anterior valve intervention. Heart disease was congenital in 71.9% of patients, with 28.1% having previous intervention. The average age ranged from 64 to 14 years. Degenerative etiologies, where it often occurs in aortic heart disease and mitral regurgitation, while most of the causes of mitral stenosis appear here. Data processing plays an important role in intelligent medical health systems. The processing of medical data is considered an important and complex task in health care, which must be carried out accurately and efficiently. Health data mining seeks to address real-world health issues in diagnosis and treatment. The relationship between disorders and the true cause of disorders and the effects of symptoms that appear spontaneously in patients can be assessed using the cardiovascular prognosis method, which is a computerized method for diagnosing heart disease based on previous data and information.

Heart disease is a term that assigns to a large number of medical conditions related to heart. These medical conditions describe the abnormal health conditions that directly influence the heart and all its parts. Heart disease is a major health problem in today's time. This paper aims at analyzing the various data mining techniques introduced in recent years for heart disease prediction.

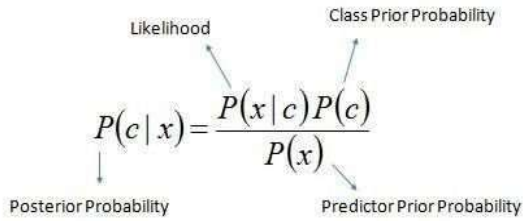
2.METHODOLOGY

Naive Bayes:

Naive Bayes classifiers is a0Tprobabilistic classifiersbased on applyingBayes'theoremTU withstrong(naïvindependence assumptions between the features.A Naive Bayesian model is easy to build, with no complicated iterative parameter estimation which makes it particularly useful in the field of medical science for diagnosing heart patients. Despite its simplicity, the Naive Bayesian classifier often does surprisingly well and is widely used because it often outperforms more sophisticated classification methods.

Bayes theorem provides a way of calculating the posterior probability, P(c|x), from P(c), P(x), and P(x|c). Naive Bayes classifier assumes that the effect of the value of a predictor (x) on a given class (c) is independent of the values of other predictors. This assumption is called class conditional independence

Equations:



$$P(c \mid \mathrm{X}) = P(x_1 \mid c) \times P(x_2 \mid c) \times \cdots \times P(x_n \mid c) \times P(c)$$

Fig

- P(c|x) is the posterior probability of class (target) given predictor (attribute).
- P(c) is the prior probability of class.
- P(x|c) is the likelihood which is the probability of predictor given class. • P(x) is the prior probability of predictor

Where C and X are two events (e.g. the probability that the train will arrive on time given that the weather is rainy). Such Naïve Bayes classifiers use the probability theory to find the most likely classification of an unseen (unclassified) instance . The algorithm performs positively with categorical data but poorly if we have numerical data in the training set.

3.Dataset

The data set used in this work are clinical data set collected from one of the leading diabetic research institute in Chennai and contain records of about 500 patients. The clinical data set specification provides concise, unambiguous definition for items related to diabetes. The diabetes data set is developed to ensure people with diabetes have up to date records of their risk factors, current management, treatment target achievements and arrangements and outcomes of regular surveillance for complications, to help them monitor their care and make informed choices about their management. It will also ensure that when people with diabetes meet health care professionals the consultation is fully informed by comprehensive, up to date and accurate information. The diabetes attributes used in our proposed system and their descriptions are shown in Table 1.

Name	Type	Description
Age	Continuous	Age in years
Sex	Discrete	1 = male 0 = female
Cp	Discrete	Chest pain type: 1 = typical angina 2 = atypical angina 3 = non-angina pa 4 =asymptomatic
Trestbps	Continuous	ng blood pressure (in mm Hg)
Chol	Discrete	Serum cholesterol in mg/dl
Fbs	Discrete	ood sugar > 120 mg/dl: 1 = true
		0 = false
Restecg	Continuous	lectrocardiographic results: 0 = normal = having ST-T wave abnormality =showing probable or define left ventricular hypertrophy by Estes 'criteria
Thalach	Discrete	Maximum heart rate achieved
Exang	Discrete	Exercise induced angina: 1 = yes 0 = no
Slope	Discrete	The slope of the peak exercise segment : 1 = up sloping 2 = flat 3= down sloping
Diagnosis	Discrete	Diagnosis classes: 0 = healthy 1= possible heart disease

Table 2: Parameters of Heart Diseases Prediction System

4.Data mining Tool

Weka is a collection of machine learning algorithms for data mining tasks. The algorithms can either be applied directly to a dataset or called from your own Java code. Weka contains tools for data pre-processing, classification, regression, clustering, association rules, and visualization. It is also well-suited for developing new machine learning schemes. The experiments are conducted using the weka

tool and the results are obtained. We have used the navie bayes method to perform classification by using 70% of percentage split.

5.Data Analysis:

In this system the medical data set is classified based on the classes present/absent.The propo ed naïve bayes model was able to classify 86.4198% of the input instances correctly and the incorrect instances was 13.5802% for 70% of percentage split. With the total of 81 instances 70% was classified as correct and 11% instances was incorrect.The results clearly states that naive bayes provides better results regarding the people affected by heart diseases.

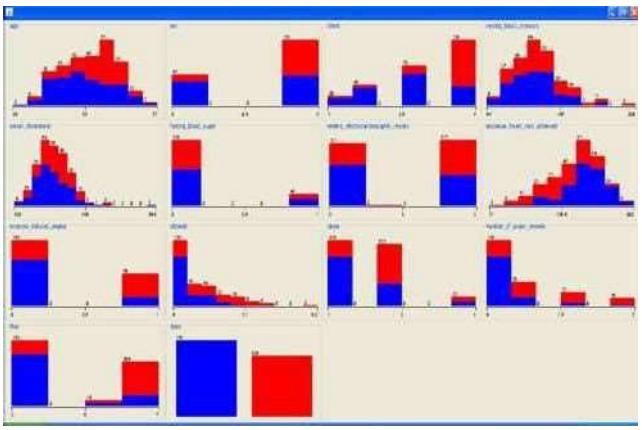


Fig.1. Attribute value distribution The blue colored regions in the graphs in Figure 1 denote high cholesterol values. From the graphs we can see that, most of the diabetic patients with high cholesterol values are in the age group of 45 – 55, have a body weight in the range of 60 – 71, have BP value of 148 or 230, have a Fasting value in the range of 102 – 135, have a PP value in the range of 88 – 107, and have a A1C value in the range of 7.7 – 9.6**.**

7 .Results and Discussions:

The results of our experimentation are shown in Fig2.

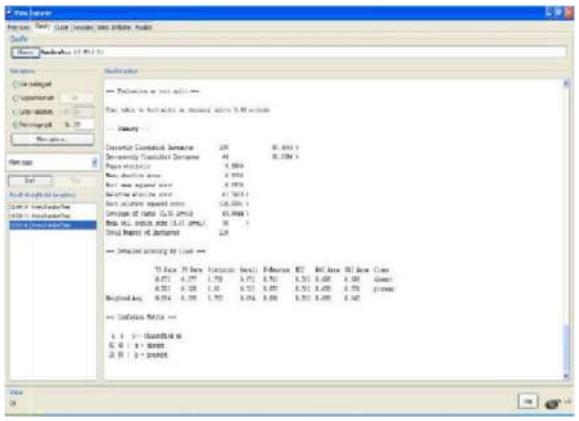


Fig. 3 Result window of the data mining process

The proposed naïve bayes model was able to classify 74% of the input instances correctly. It exhibited a precision of 71% in average, recall of 74% in average, and F-measure of 71.2% in average. The results show clearly that the proposed method performs well compared to other similar methods in the literature, taking into the fact that the attributes taken for analysis are not direct indicators of heart disease.

8 .Conclusion:

Data mining applications are used vastly in the medical field to detect diseases and diagnosis the heart patient based on the data set and the attributes provided. Researchers have been investigating applying different data mining techniques to help health care professionals in the diagnosis of heart disease. In the proposed work navie bayes algorithm is used to classify the data set because navie bayes provides accurate results, with these results heart diseases among people is predicted. Thus heart diseases prediction system successfully diagnose the medical data and predicts the heart diseases.The results thus obtained shows that navie bayes algorithm provides 86.4198% of accuracy with minimum time.

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Research Article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Reduction of Heavy Metals in Steel Industrial Effluent by *Bacillus subtilis* Bacteria

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ABSTRACT

Bio-degradation is a new biological method and various low cost bio-adsorbents are used for maximum removal of heavy metals from waste water.Main importance of the present study was to identify the heavy metals in industrial waste water and to screen the heavy metal degraders. Based on analytical analysis with AAS the concentration of the heavy metals in the effluent is Cu-5.8mg/l, Cd-1.09mg/l, Cr-5.97mg/l, Pb-1.9mg/l. The copper concentrations in the effluent were decreased after the inoculations of the isolated bacterial strains were 2.6mg/l, 1.6mg/l, 2.0mg/l, 1.7mg/l. The Cadmium concentration in the effluent were significantly decreased as 0.76mg/l, 0.6mg/l, 0.17mg/l, 1.1mg/l. The Chromium concentrations in after the inoculation of the bacterial strains were 5.2mg/l, 2.9mg/l, 3.7mg/l, 2.5mg/l. The lead concentration in the effluent after inoculation with bio-degraders were 0.38mg/l, 0.6mg/l, 0.41mg/l, 0.8mg/l. The four bacterial isolate were screened from the waste effluent and isolate no.2 shows efficient degradation that were identified by 16SrRNA sequencing and documented as Bacillus subtilis. Conclude that bio based methods are eco-friendly best solutions for removing toxic to nontoxic from waste water rather than physical and chemical methods.

KEYWORDS: Bio-degraders, heavy metals, chromium, copper, effluents

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INTRODUCTION

Heavy metals from industrial waste are of special concern because, they produce water of chronic poisoning in aquatic animals[9].While some heavy metals are purely toxic with no cellular role[24], other metals are essential for life at low concentration but become toxic high concentration of all heavy inhibits activity of sensitive enzymes [13].

Heavy metal can damage the cell membrane, alter enzyme specificity, disrupt cellular function and damage the structure of the DNA[1], The toxic effect of Arsenic, Mercury and Lead were known to be ancient, but methodical studies of the toxicity of some heavy metal appear to date from only 1868 [26] in human, heavy metal poisoning is generally treated by the administration of chelating agents [27,24]. Some elements otherwise regarded as toxic metals are essential in small quantities [8].

The toxic heavy metals are relatively dense metal or metalloid that is noted for its potential toxicity, especially in environmental context[28].The term has particular application to Cadium, Mercury, Lead and Arsenic all of which appearing the world health organization examples include Manganese, Zinc, Selenium, Silver, Antimony and Thallium[8].Uncontrolled discharges of large quantity of heavymetals containing waste create huge economic and health care burden [25,20].

Thetoxic metal pollutant like Lead, Nickel and Cadium enter to the water bodies through industrial waste water [10].Among the heavy metal, Lead is a nonessential heavy metal and general toxicant[17].The toxicity of these heavy metal occur through the displacement of essential metal from their native binding site or through ligand interaction[22,14]. The toxicity can occur as a alteration in the conformational structure of the nucleicacid and protein and interference with oxidative phosphorylation and osmoticbalance[16,11].In the biosorption mechanisms, the complex structure of microorganisms implies that the manyways for the metal to be taken up by the microbial cell[20.]

The bio adsorption mechanisms are various, they may be classified according to the dependence on the cellmetabolismand bio adsorption mechanism can be divided into: Metabolism dependent and non –metabolism dependent. According to the location where the metal removed from the solution is found bio adsorption can be classified as (1)Extra cellular accumulation/precipitation (2) Cell surface sorption /precipitation: and (3) intracellular accumulation. Heavy metals are not biodegradable and tend to be accumulated in organism and because of numerous disease and disorder[24].To survive under metal -stressed condition, bacteria have evolved several types of mechanism to tolerate the uptake of heavy metal ions[18,19]. These metal mechanisms include the effluxofions outside the cell, accumulation and complexation of metal ions

inside the cell [20]. The objective of this work is to isolate the metal resistant bacteria from the industrial effluent by the determination of physio-chemical properties of the effluent and the biochemical characterization of the metal resistantbacteria.

Heavy metal pollution caused by mining, steel production, and electroplating has induced an adverse impact on the environment, which threatens the health of human beings and the stability of ecosystem[1]. Heavy metal ions can be accumulated through food chain, which is believed to be a risk for human beings even at trace level[29]. Additionally, heavy metal ions can affect cellular organelles, components, and induce oxidative stress. For instance, arsenic can induce DNA hypomethylation gene expression[21]; chromium can induce DNA damage and oxidative stress in Sprague–Dawley rats and in human liver carcinoma cells, copper has been reported to take part in the oncogenic BRAF signaling which is related to various cancers..

As a consequence, numerous diseases such as Menkes disease, Alzheimer's disease and cancers can be induced by the excessive intake of heavy metal ions[4]. Therefore, heavy metal pollution has already become one of the most serious environmental problems and the remediationofactiveheavymetalloidsofgreatimportance.Traditional heavy metal remediation methods have many limitations including production of toxic chemical sludge and not eco-friendly. Therefore, simple and low-cost remediation method is urgently needed.

Nowadays, bioremediation technologies based on microorganisms have attracted scientists' great interest because of their outstanding advantages including high efficiency, low-cost and eco-friendly, especially at low metal concentrations.Therefore, we have summarized the heavy metal remediation and their detoxification pathways of microorganisms to uncover the interrelationship, which will contribute to finding more efficient bioremediation strategies and developing the next-generation bioremediation technology.

MATERIALS AND METHODS

Sample Collection

Industrial waste water sample were collected from a Stainless steel industry in and around Coimbatore in sterile glass bottles and were brought to the lab aseptically in the ice cold box. The samples were stored at 4° C for further use. Heavy metals (Chromium, Copper, Lead and Cadmium) in the sediment were determined. 1g of sediment sample with concentrated HNO3 made up to 50 ml volume. The sediment elutriates were prepared by shaking sediment in water at 1:4 ratio for 24h. The supernatant was separated by centrifuging at 6,000 rpm for 60 min at 4 ºC.

Elutriate was stored at 4 ºC until analysis. Elutriate were acidified and directly used for the estimation of trace metals by Differential Pulse Anodic Striping Voltammetry (**DPASV**) method[15].

Physic-chemical Analysis

These collected water samples were subjected to various analytical analysis of Physicochemical parameters such as temperature, pH, turbidity, colour, odour, electrical conductivity, TDS, TSS, BOD, COD and DO of the water sample were determined APHA [2].

Isolation of metal resistant bacteria

In the present study heavy metal resistant bacterial species were isolated from the industrial effluent by serial dilution and pour plating method using Nutrient agar supplemented with different heavy metal salts. Strains were maintained in agar slants containing nutrient agar. They were characterized morphologically and on the basis of biochemical reactions. They were transferred weekly to new medium in order to keep metabolic activity and checked for purity by microscopic examination.

Screening of metal resistant bacteria

Heavy metals tolerant bacteria were isolated on nutrient agar supplemented with 5µg/ml to 100µg/ml of Cr, Cd, Pb and Cu. The nutrient agar was sterilized at 121°C for 15min and allowed to cool 40- 45°C. Then the metals were added to the nutrient agar and transfer into petri plates. The waste water sample was serially diluted in which 9ml of sterile saline water in 6 tubes and 1ml of samples were added to the first tube to have 10^{-1}repeated up to 10^{-6}. The 0.1ml of the dilution were spread on the surface of the agar plates and incubated at 37 ℃ for 2days colonies differing in morphological appearance were selected for further studies and sub-cultured on same media[12].

Heavy metal analysis in industrial effluent

The 2 ml of treated dye effluent was taken in a boiling tube and was digested using 10 ml of triple acid solution (HNO3, H2SO4 and HCIO4 in 9:2:1 proportion respectively) till the effluentbecomes colorless.The digested sample was filtered using whatman number 1filter paper for two times made up to 50 ml and subjected for heavy metal assay using Atomic Absorption Spectroscopy (Mac: SL 176 Double beam Spectrophotometer) as per the standard method recommended by APHA[2]. The three replications were maintained for each treatment. The

percentage of degradation was calculated from the following equation,

% Degradation=initial amount final amount/Initial Amount×100

Statistical Analysis Heavy metal tolerance

Four heavymetals which were at higher concentrations in the collected effluent, they were viz chromium, cadmium, lead and copper whereas zinc and manganese were considerable low in their presence.So, the heavy metal tolerance of the bacterial consortium was studied with these four heavy metals which were used at the range between 5µg/ml to 100µg/ml with an interval of 25mg/l.Using each of four heavy metals, the tolerance was examined. The used heavy metal substitutes were chromium metal powder, nickel sulfate, lead (II) phosphate and copper sulfate. Heavy metal substitutes were prepared at the concentration using the formula(X);

X = Molecular weight of compound/ Molecular weight of heavy metal × amount of sample required µg/ml.

Heavy metal bioremediation using microbial consortium

The heavy metal contaminated industrial effluent was treated with the bacterial consortium developed in this study with the standardized growth conditions except the heavy metal tolerance parameter. The bacterial consortium was identified for its peak time of heavy metal bioremediation with reference to its cell growth. The enhanced bioremediation was monitored using a portion of cultured broth followed by the separation of cell free supernatant and cell pellet using centrifugation at 3000rpm for 15 min.The fermentation process was monitored for 102hrs with an interval of 6hrs starting from the lag phase to decline phase under batch culture conditions.Individually estimation was monitored for the bacterial growth using the dry weight ofcellbiomass(g/L)as described earlier and heavy metals in the cell free supernatant and cell pellet was determined using AAS as described below.

Estimation of heavy metals using AAS in the treated broth

Heavy metals in the cell free supernatant weredeterminedwitha100mladdedwith3mlHNO3 followed by complete dryness on a hot plate whereas the cell pellet was lyophilized or freeze dried. Further, the same applied conditions in the AAS for the estimation of heavy metals in the effluents were applied here for the determination of four heavy metals viz. Chromium (357.9nm), Lead(283.3nm), Cadmium (228.8nm) and Copper (324.7nm).

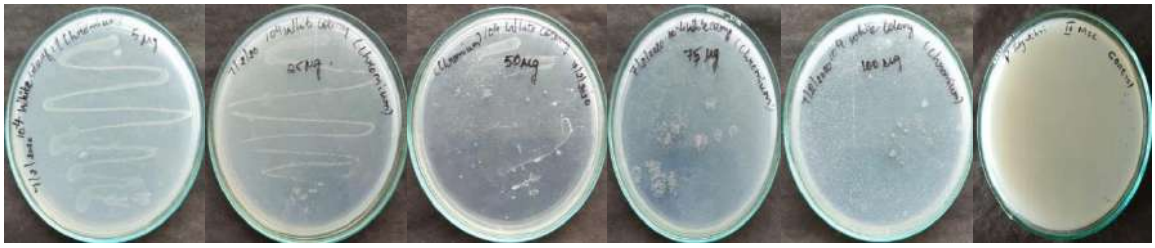
RESULTS AND DISCUSSION

Morphology of the Isolates

The present study deals with isolation, identification and characterization of heavy metal resistant bacteria were isolated from Stainless steel industrial effluent collected in and around Coimbatore. The 24hrs cultures were gram stained and observed under microscope for gram reaction. Violet coloured rod shaped cells were indicated as gram positive rods. Heavy metals are natural constituents of the environment, but indiscriminate use for human purposes has altered their geochemical cycles and biochemical balance. This result in excess release of heavy metals such as cadmium, copper, lead, chromium, zinc etc., into natural resources like the soil and aquatic environments. Prolonged exposure and higher accumulation of such heavy metals can have deleterious health effects on human life and aquatic biota.

Screening and selection of metal tolerant bacteria

Purified isolates were screened for metal tolerance by growing on LB medium amended with varying concentration of different metals (5µg/ml to 100µg/ml in the interval of 25µg/ml) at 28+ 1°C.These plates were incubated for 24hrs. Hence our study is to isolate chromium resistant microbial consortia and to treat the effluent.Growth observed (5µg/ml to 100µg/ml) concentration of heavy metals Cr(III) with different strains:



5µg/ml 25µg/ml 50µg/ml 75µg/ml 100µg/ml Control

Fig No: 1 Screening and selection of Cr (III) tolerant bacteria:

Measurement of physico-chemical parameters

The physico-chemical parameters of the industrial effluent were given in a Table 1. All the samples collected from the area indicated high level of pollution. The physico-chemical of sample collected from industrial effluent show that the milky white in color having pH range in 7.18 may be due to the presence of sodium, potassium and chromium etc. The temperature of the sample was 20-30°C.The sample were found turbid and the turbidity are 86 NTU.

Four heavy metals (Cu, Cd, Cr and Pb) in the industrial effluent were determined using Atomic Adsorption Spectrophotometry (Perkin Elmer) after digestion with microwaves digester following the manual instructions with the addition of nitric acid, hydrofluoric acid and hydrogen peroxide for sample digestion[5,6]. The concentration of the heavy metals in the effluent is Cu-5.8mg/l, Cd-1.09mg/l, Cr-5.97mg/l, Pb-1.9mg/l. The copper concentrations in the effluent were decreased after the inoculations of the isolated bacterial strains are 2.6mg/l, 1.6mg/l, 2.0mg/l, 1.7mg/l. The Cadmium concentration in the effluent there was a significant decrease of cadmium content after the inoculation of bacterial strains are 0.76mg/l, 0.6mg/l, 0.17mg/l, 1.1mg/l. The Chromium concentrations in the effluent were decreased after the inoculation of the bacterial strains from 5.2mg/l, 2.9mg/l, 3.7mg/l, 2.5mg/l. The lead concentration in the effluent after inoculation of heavy metal strains from 0.38mg/l, 0.6mg/l, 0.41mg/l, 0.8mg/l.

Table No:1 Measurement of Physico-chemical parameters

S.NO	PHYSICO-CHEMICAL PARAMETERS	UNIT	RESULTS
1	Colour	Hazen	250
2	Odour	Mg/l	Disagreeable
3	Turbidity	NTU	86
4	pH@25°C	-	7.18
5	Electrical conductivity@25°C	µS/cm	1540
6	Total Dissolved Solids@180°C	Mg/l	1020
7	Biochemical Oxygen Demand(3 Days for 27°C)	Mg/l	3650
8	Chemical Oxygen demand	Mg/l	80,000
9	Total Suspended Solids	Mg/l	596
10	Temperature	°C	30
11	Copper as (Cu)	Mg/l	5.8
12	Cadmium (Cd)	Mg/l	1.09
13	Chromium (Cr)	Mg/l	5.97
14	Lead (Pb)	Mg/l	1.2

Estimation of heavy metals using AAS (atomic absorption spectroscopy) in the treated broth

Atomic Absorption Spectroscopy used for the estimation of the heavy metals in the effluents where inoculated with metal resistant strains and incubated at 37°C for 7 days were applied here for the determination of four heavy metals Cr, Cd, Cu, and Pb.

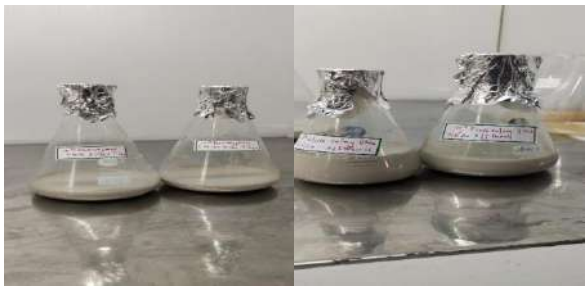


Fig no: 2 Samples for AAS Analysis

Table No: 2Estimation of heavy metals using AAS

S.No	Heavy Metals	Unit	Before treatment	After treatment with isolates			
				Isolate**1**	Isolate**2**	Isolate**3**	Isolate**4**
1	Copper	Mg/l	5.8	2.6	1.6	5.0	1.7
2	Cadmium	Mg/l	1.09	1.1	0.17	0.4	0.76
3	Chromium	Mg/l	5.97	5.2	2.9	3.7	2.5
4	Lead	Mg/l	1.2	0.8	0.41	0.6	0.38

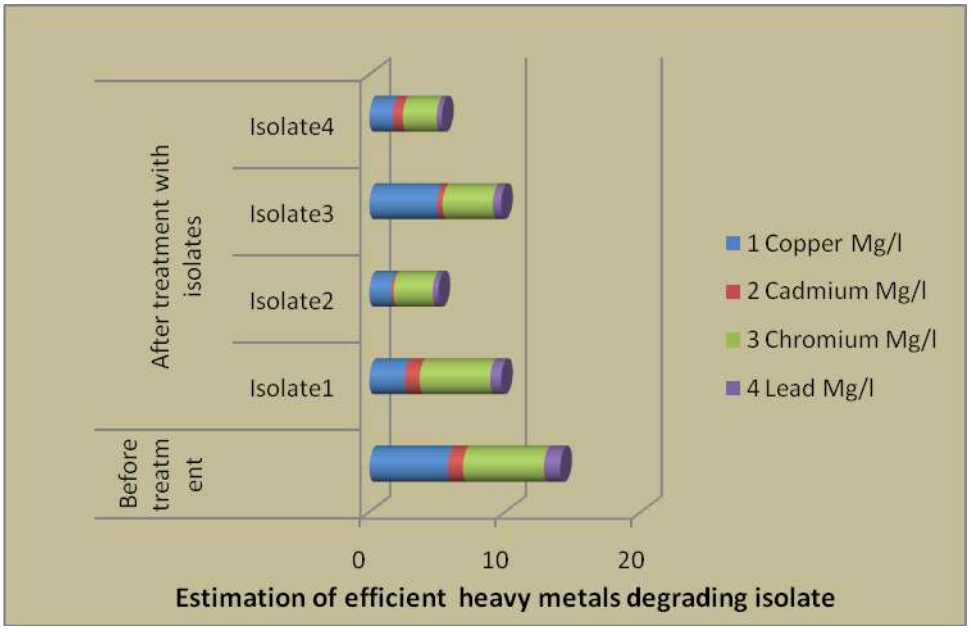


Fig.No:3 Statistical Analysis shows the Efficient Heavy metal degrading Isolate

Based on the statistical analysis of AAS Isolate 2 shows the efficient degradation of heavy metals compare to other 3 isolate. The efficient strains were identified by 16rRNA sequencing.

Identification efficient heavy metal degraders by sequencing

The efficient strain is identified as *Bacillus subtilis* (PGA-10_contig_1) by 16srRNA sequencing at Macrogen. Primer used for PCR 27F 5' AGA GTT TGA TCM TGG CTC AG 3'& 1492R 5' TAC GGY TAC CTT GTT ACG ACT T 3' and for sequencing universal primer 785F5'GGA TTA GAT ACC CTG GTA 3' & 907R 5' CCG TCA ATT CMT TTR AGT TT 3' .The 1344bp were used for sequencing and summation for NCBI. Identification of metal resistant bacteria that are adapted to the new toxic metal environment provides efficient potential candidates for heavy metal bio removal from contaminated media[30,7]. Most efficient heavy metal degraders of Isolate No.2 were selected for 16S rRNA gene sequencing in bacterial identification. Segments from these genes can be amplified by PCR using universal primers and sequenced. Sequence comparison of 16S rRNA has been used as a powerful tool for establishing phylogenetic and evolutionary relationships among organisms. The 16SrRNA gene sequencing and phylogeny analysis revealed that, the isolate were authentically identified as *Bacillus subtilis.*

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
CP020102.1	Bacillus subtilis	4215607	96453	97942	0	2732	0.0	1487/1490	99

Kingdom	Family	Genus	Species
Bacteria	Bacillaceae	Bacillus	Bacillus subtilis

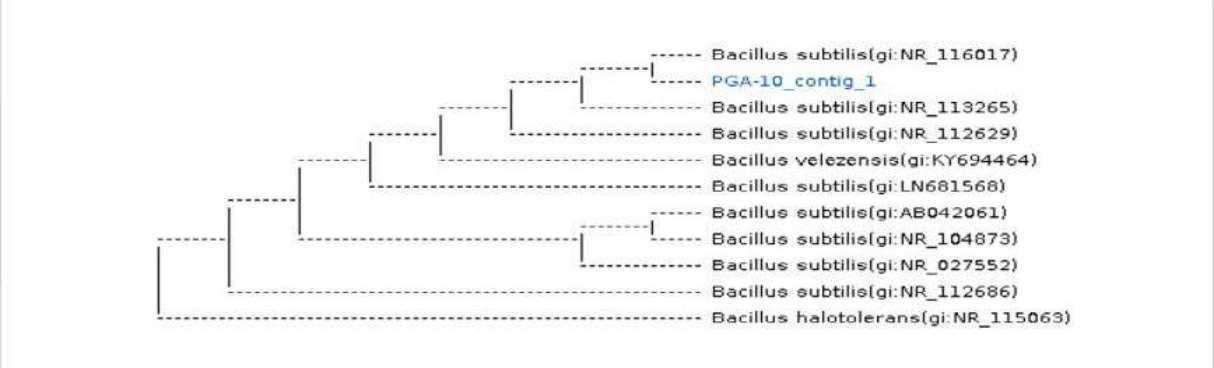


Fig.No:4. Phylogenetic and Evolutionary relationships

>H200317-021_K05_PGA-10_907R.ab1 1344

CACGTCGTCTCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTAAGGG

GCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCA

GGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTT

ACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGC

ATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTC

CCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGA

CTTAAGAAACCGCCTGCGAGCCCTTTACGCCCAATAATTCCGGACAACGC

TTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTT

TCTGGTTAGGTACCGTCAAGGTACCGCCCTATTCGAACGGTACTTGTTCT

TCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGC

GTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCT

CCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTC

TCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTA

GCTAATGCGCCGCGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTA

TGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCC

CGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGT

CCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCAT

GTATTAGGCACGCCGCCAGCGTTCGTCTGACAGAGAGAAAAACCAAAACA

AAAAAAGGAAAAAACTGGGGGGGGGGGGGGGGGGGGGAGGGGGGGGGG

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GGGGGGTGTGGTGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGCGG

GGGGGGGGCGCCGCCCCCCGGGGGCCCGGGGCCCCGCGGGGGGGGGCGCC

GGGGCGGCGGGGGGGGGCCCCCCGGCCGGGGGGGGGGGGGGGGGGCCC

CCGCGCGCCCCCCCCCCCCCCCCGGGGGGGGGCGCGGCCCCCCCGGGGGG

CGGGGGGGGGGGGGGGGGGCGCGGGGGGCCGCGGGGGCGCGGCCGCCCC

CCCCCGCGGGCGGCGGCCGCGCGGCCCCGCCCGGCGGGGGGGGC

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

Author declares no conflicts of interest in this paper.

CONCLUSION

Results of the present study, the capability of bacteria resistance against different heavy metals may offer a beneficial tool for simultaneous monitoring of many contaminants and pollutants in the environment.Bacteria play a very important role in the removal of heavy metals from the industrial effluent. Therefore, the study is very useful to suggest that the possible impact of metal contaminated locations in human life may be greater the direct consequence of the pollution. The characterizations of metal resistant bacteria were done and the four bacterial strains isolated. Based on the result obtained *Bacillus subtilis* were able to tolerate up to 5µg/ml to 100µg/ml of chromium in the interval of 25µg/ml. Growth standardization of consortium in the medium using the highest heavy tolerance were done and reduced after treatment with bacterial consortium it was proved by quantitative analysis of heavy metals cell free supernatant of treated effluent.

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AGENT SATISFACTION IN LIFE INSURANCE

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ABSTRACT

The Insurance broker is a professionally run corporate body, subjected to the stringent regulations and the code of conduct prescribed by the IRDA. Unlike an insurance agent who represents his company, a broker would offer investors a range of products. At an individual level, the broker will play a crucial role of enabling the policyholders to compare rates in basic products. At the corporate level the broker will not only act as an advisor but also help the company in devising a policy to meet his corporate clients requirements.

Independent Financial Advisors (IFAs) are very common in overseas markets, but are new in India. IFAs are qualified professionals who can provide invaluable help to the customers in identifying the product that suits their personal requirements. IFA aid the clients/customers in the selection of insurance products. They charge for their services, instead of contracting for commissions. IFAs are different from their counterpart-brokers, who have interest in maximizing their commission.

INTRODUCTION

The role of agents and intermediaries in insurance marketing whether life or general, is of great significance. An agent is a primary source for procurement of insurance business and as such his role is the corner stone for building a solid edifice of any life insurance organization. Thus, agents play a vital role in insurance marketing. Due to poor insurance awareness on the part of the people, the insurance industry works as a sellers' market instead of being a buyers' market. Free interaction between the buyers and sellers in the insurance industry is not a common occurrence. The development officers who are vital for the internal sale force of the life insurance companies have a heavy schedule in the day to day operations for achieving the target fixed by the company, based on which their increments and perquisites are worked out .

Naturally, they will evince a keen interest in canvassing business of big nature to collect large amounts of premium. They would not care for the potential insurance which covers premiums of small amount. It is not being presumed that they neglect small business, but the constraint of achieving the target marks them in millions of rupees. Another undeniable fact to be noted in this regard is that the development of manpower is not commensurate enough to make the insurance product reach the remotest village of the great Indian subcontinent. Therefore, there is an obvious need for building up a network of external sales force noted as 'agents' to act as intermediaries between the insurance company and the client.

The efficacy of the company in utilizing the agents depends upon the interest shown by it in engaging the agents to procure insurance business of varying nature; and motivation of the development officer who keeps a direct contact with the agents to boost their morale to bring flourishing business.

STATEMENT OF THE PROBLEM

With the entry of private insurance players people have got a host of options to choose from. While the impact of the coming-in of the next generation of life insurance companies on the monolith LIC so far is minimal. The competition has brought in perceptible changes, trends and a slew of new products all favorable to the agents Next, it may face problems in percentage of their payments, allowances and services.On this problem based the study were selected entitled " **Agent Satisfaction in Insurance".**

OBJECTIVES OF THE STUDY

- To analyze the opinion of the agents regarding the training given by LIC.
- To examine the opinion of the agents about the products and services of LIC.
- To measure the job satisfaction of the agents.
- To understand the details of the problems faced by the agents.

RESEARCH METHODOLOGY

Study area: The study area refers to Coimbatore city.

Sources of Data: The study has used only primary data, which was collected using interview schedule. The interview schedule has been framed in such a way that the respondents were able to express their opinions freely and frankly

Tools used for collection of data: An interview schedule was constructed for the purpose of collecting data. A pilot study was conducted on 300 agents, based on that the interview schedule was restructured to meet the accuracy and reliability.

Sources of Data: The study has used only primary data, which was collected using interview schedule.

The following tools are used in the study: Chi-square analysis

LIMITATIONS OF THE STUDY

1. The process of collection of data was a real challenge as it consumed considerable time of the respondents. However adequate care has been exercised to collect the unbiased data.
2. The limitations of tools used in the study are applicable to the analysis also.

ANALYSIS AND INTERPRETATION

Table 1: Association between gender and satisfied behavior of agent

Ho: There is no significant relationship between gender and satisfied the behavior of the LIC agent towards the customers.

Gender	Gender and behavior of the LIC agents			Total
	Very Good	Good	Normal	
Male	108	60	12	180
	60.0%	33.3%	6.7%	100.0%
Female	96	12	12	120
	80.0%	10.0%	10.0%	100.0%
Total	204	72	24	300
	68.0%	24.0%	8.0%	100.0%

H1: There is significant relationship between gender and satisfied the behavior of the LIC agent towards the customers.

Degree of freedom is 2, Chi –square value is 21.569, and Table value is 5.991.

Table 2: Association between age of the respondents and behavior of the LIC agent towards the customers

Ho: There is no significant relationship between age of the respondents and behavior of the LIC agent towards the customers.

Age	**Age and satisfied the behavior of the LIC agent towards the customers**			**Total**
	Very Good	**Good**	**Normal**	
Less than 25 yrs	84	48	0	132
	63.6%	36.4%	.0%	100.0%
25 yrs to 35 yrs	36	24	24	84
	42.9%	28.6%	28.6%	100.0%
35 yrs to 45 yrs	72	0	0	72
	100.0%	.0%	.0%	100.0%
45 yrs above	12	0	0	12
	100.0%	.0%	.0%	100.0%
Total	204	72	24	300
	68.0%	24.0%	8.0%	100.0%

H1: There is significant relationship between Age and satisfied of the respondents and behavior of the LIC agent towards the customers.

Degree of freedom is 6, Chi –square value is 1.118, Table value is 12.592.

Table 3: Association between marital status of the respondents and behavior of the lic agent towards the customers

Ho: There is no significant relationship between Marital Status of the respondents and behavior of the LIC agent towards the customers.

Marital Status	Marital status and behavior of the respondent			Total
	Very Good	Good	Normal	
Married	108	24	24	156
	69.2%	15.4%	15.4%	100.0%
Unmarried	96	48	0	144
	66.7%	33.3%	.0%	100.0%
Total	204	72	24	300
	68.0%	24.0%	8.0%	100.0%

H1: There is significant relationship Marital Status of the respondents and behavior of the LIC agent towards the customers.

Degree of freedom is 2, Chi –square value is 32.278, and Table value is 5.991.

Table 4: Association between educational level of the respondents and behavior of the LIC agent towards the customers

Ho: There is no significant relationship between Educational Level of the respondents and behavior of the LIC agent towards the customers.

Educational Level	Educational level behavior of the LIC agent			Total
	Very Good	Good	Normal	
Informal	12	0	0	12
	100.0%	.0%	.0%	100.0%
School Level	72	12	12	96
	75.0%	12.5%	12.5%	100.0%
College Level	60	60	0	120
	50.0%	50.0%	.0%	100.0%
Others	60	0	12	72

	83.3%	.0%	16.7%	100.0%
Total	204	72	24	300
	68.0%	24.0%	8.0%	100.0%

H1: There is significant relationship Educational Level of the respondents and behavior of the LIC agent towards the customers.

Degree of freedom is 6, Chi –square value is 89.706, Table value is12.592.

Table 5: Association between occupational status of the respondents and behavior of the lic agent towards the customers.

Ho: There is no significant relationship between Occupational Status of the respondents and behavior of the LIC agent towards the customers.

Occupational Status	**Occupational Status of the respondents and behavior of the LIC agent**			**Total**
	Very Good	**Good**	**Normal**	
Agriculture	0	12	0	12
	.0%	100.0%	.0%	100.0%
Business	72	36	24	132
	54.5%	27.3%	18.2%	100.0%
Employed	60	24	0	84
	71.4%	28.6%	.0%	100.0%
Professional	48	0	0	48
	100.0%	.0%	.0%	100.0%
Others	24	0	0	24
	100.0%	.0%	.0%	100.0%
Total	204	72	24	300
	68.0%	24.0%	8.0%	100.0%

H1: There is significant relationship Occupational Status of the respondents and behavior of the LIC agent towards the customers.

Degree of freedom is 8, Chi –square value is1.007, Table value is15.507.

Table 6: Association between income level of the respondents and behavior of the lic agent towards the customers

Ho: There is no significant relationship between Income Level of the respondents and behavior of the LIC agent towards the customers.

Income Level	Income level satisfaction of agent			Total
	Very Good	Good	Normal	
Less than Rs. 5,000	36	12	0	48
	75.0%	25.0%	.0%	100.0%
Rs. 5,000 to 10,000	48	36	12	96
	50.0%	37.5%	12.5%	100.0%
Rs. 10,000 to 15,000	48	12	0	60
	80.0%	20.0%	.0%	100.0%
Rs. 15,000 to 20,000	72	12	12	96
	75.0%	12.5%	12.5%	100.0%
Total	204	72	24	300
	68.0%	24.0%	8.0%	100.0%

H1: There is significant relationship Income Level of the respondents and behavior of the LIC agent towards the customers.

Degree of freedom is 8, Chi –square value is33.382, Table value is15.507.

Table 7: Association between gender and enjoy your competence

H_0: There is no significant relationship between genders and satisfaction level of competence.

Gender	Gender and competence level			Total
	Enjoy more	Normal	Not like	
Male	84	155	12	251
	33.5%	61.8%	4.8%	100.0%
Female	12	37	0	49
	24.5%	75.5%	.0%	100.0%
Total	96	192	12	300
	32.0%	64.0%	4.0%	100.0%

H1: There is significant relationship genders and satisfaction level of competence.

Degree of freedom is 2, Chi –square value is 4.587, and Table value is 5.991.

Table 8: Association between age and enjoy your competence.

H_0: There is no significant relationship between age and competence.

Age	Age and competence			Total
	Enjoy more	Normal	Not like	

Less than 25 years	0	24	0	24
	.0%	100.0%	.0%	100.0%
25 years to 35 years	24	37	0	61
	39.3%	60.7%	.0%	100.0%
35 years to 45 years	24	59	0	83
	28.9%	71.1%	.0%	100.0%
45 years above	48	72	12	132
	36.4%	54.5%	9.1%	100.0%
Total	96	192	12	300
	32.0%	64.0%	4.0%	100.0%

H1: There is significant relationship age and satisfaction level of competence.

Degree of freedom is 6, Chi –square value is 32.474, and Table value is 12.592.

Table 9: Association between marital statuses and enjoy your competence

H_0: There is no significant relationship between Marital Statuses and your competence.

Marital Status	**Marital status and competence**			**Total**
	Enjoy more	**Normal**	**Not like**	
Married	96	168	12	276
	34.8%	60.9%	4.3%	100.0%
Unmarried	0	24	0	24
	.0%	100.0%	.0%	100.0%
Total	96	192	12	300
	32.0%	64.0%	4.0%	100.0%

H1: There is significant relationship Marital Statuses and your competence.

Degree of freedom is 2, Chi –square value is 14.674, Table value is 5.991.

Table 10: Association between educational levels and enjoy your competence.

H_0: There is no association between Educational Levels and your competence.

Educational Level	**How did you enjoy your competence**			**Total**
	Enjoy more	**Normal**	**Not like**	
Informal	12	0	0	12
	100.0%	.0%	.0%	100.0%
School Level	72	144	0	216

	33.3%	66.7%	.0%	100.0%
College Level	0	12	0	12
	.0%	100.0%	.0%	100.0%
Others	12	36	12	60
	20.0%	60.0%	20.0%	100.0%
Total	96	192	12	300
	32.0%	64.0%	4.0%	100.0%

H1: There is significant relationship educational level and your competence.

Degree of freedom is 6, Chi –square value is 82.500, and Table value is 12.592.

FINDINGS

The above table reveals that the calculated chi-square value 21.569 and table value 5.991. Hence the null hypothesis is rejected at 5% level of significance. So there is no relationship between gender and satisfied the behavior of the LIC agent towards the customers.

The above table reveals that the calculated chi-square value 1.118 and table value 12.592. Hence the null hypothesis is accepted at 5% level of significance. So there is no relationship between age of the respondents and behavior of the LIC agent towards the customers.

The above table reveals that the calculated chi-square value 32.278 and table value 5.991.Hence the null hypothesis is rejected at 5% level of significance. So there is no relationship between Marital Status of the respondents and behavior of the LIC agent towards the customers.

The above table reveals that the calculated chi-square value 89.706 and table value 12.592. Hence the null hypothesis is rejected at 5% level of significance. So there is no relationship between Educational Level of the respondents and behavior of the LIC agent Employees towards the customers.

The above table reveals that the calculated chi-square value 1.007 and table value 15.507. Hence the null hypothesis is rejected at 5% level of significance. So there is no relationship between Occupational Status of the respondents and behavior of the LIC agent towards the customers.

The above table reveals that the calculated chi-square value 33.382 and table value 15.507 Hence the null hypothesis is rejected at 5% level of significance. So there is no relationship between Occupational Status of the respondents and behavior of the LIC agent towards the customers.

The above table reveals that the calculated chi-square value 4.587 and table value 5.991 Hence the null hypothesis is accepted at 5% level of significance. So there is no relationship between genders and satisfaction level of competence.

The above table reveals that the calculated chi-square value 32.474 and table value 12.592 Hence the null hypothesis is rejected at 5% level of significance. So there is no relationship between age and satisfaction level of competence.

The above table reveals that the calculated chi-square value 14.674 and table value 5.991 Hence the null hypothesis is rejected at 5% level of significance. So there is no relationship between marital status and satisfaction level of competence.

The above table reveals that the calculated chi-square value 82.500 and table value 12.592 Hence the null hypothesis is rejected at 5% level of significance. So there is no relationship between educational level and satisfaction level of competence.82.500>0.05

CONCLUSION

However, the customer preferences towards life insurance business is found only favor LIC and not in favors of private insurance companies. In view of the advantage that it is a public sector organisation and monolith in life insurance business, LIC can easily win over this problem without losing considerable market provided the very focus of LIC towards service customers remain static.

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IJPSR (2022), Volume 13, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 February 2022; received in revised form, 06 April 2022; accepted, 25 April 2022; published 01 October 2022

IN-VITRO AND *IN-VIVO* NEUTRALIZING POTENTIAL OF *RAUVOLFIA SERPENTINA* ROOT EXTRACT AGAINST *BUNGARUS CAERULEUS* VENOM

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Keywords:

Bungarus caeruleus, *Rauvolfia serpentina*, Direct haemolysis, Proteolytic Acetyl cholinesterase, ATPase, Lethal dose, Effective dose

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ABSTRACT: Snakebite is an international health hazard that needs attention in terms of effective management and better treatment to recover the health of the affected individuals and society. Tribal populations and folk therapists across the world have been using innumerable plants to treat snakebite difficulties. In the present study, the efficacy of aqueous extracts of *Rauvolfia serpentina* root for neutralizing *Bungarus caeruleus* venom by *in-vitro* and *in-vivo* methods was carried out. Various *in-vitro* neutralization tests like Direct and Indirect haemolytic activities, Acetylcholinesterase activity, Proteolytic activity, ATPase activity, and *in-vivo* assessment LD_{50} were carried out. *Rauvolfia serpentina* root extract effectively neutralizes all the venom's toxic effects. The *in-vivo* assessment of venom lethality (LD_{50}) of *Bungarus caeruleus* venom was 0.537 µg/g. *Rauvolfia serpentina* root extract effectively neutralized the venom lethality, and the effective dose (ED_{50}) was found to be10.96 mg/3LD_{50} of *Bungarus caeruleus*. All animals survived and appeared active and healthy throughout the study. The LD_{50} of *Rauvolfia serpentina* root extract was >2000mg/kg. These findings confirmed that *Rauvolfia serpentina* root extract possesses some compounds which inhibit the toxins present in *Bungarus caeruleus* venom.

INTRODUCTION: Snake envenomation is a major health problem that leads to numerous deaths, particularly in India. The majority of the snake bite incidences happened during the monsoons and during the daytime because most rural people heavily depend on cultivation [1, 2].

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DOI: 10.13040/IJPSR.0975-8232.13(10).4054-61

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(10).4054-61

About five million people worldwide are bitten by snakes annually, causing around 1, 25,000 deaths and 4, 00,000 individuals to be permanently disabled or disfigured [3].

The main families of venomous snakes in India are Elapidae which includes Common Cobra (*Najanaja*), King Cobra and Common Krait (*Bungarus caeruleus, Banded krait, Sind krait*); Viperidae includes *Daboia russelli* (Russell's viper), *Echis carinatus* (Saw-Scaled or Carpet viper), Pit viper and Hydrophiidae (Sea snakes). Neuromuscular disorders due to snake envenoming is common, including envenoming by elapid snakes

such as Kraits, Cobras, Coral snakes, Taipans, Tiger snakes, and Death adders. Snake venom-induced paralysis becomes life intimidating with progressive paralysis of the bulbar and breathing muscles which involves rapid airway provision and mechanical ventilation [4].

In tropical regions, snake bites remain an important socio-medical problem. Over one million humans were bitten worldwide every year by various snakes resulting in 70,000 deaths [5]. Elapid snake venoms are mostly neurotoxins or cytotoxins of the three-finger toxin family and phospholipases A_2 (PLA_2) [6].

Viperid venoms, in contrast, are composed mostly of PLA_2S, zinc-dependent metalloproteinases (SVMPs), and serine proteinases (SVSPs) [7]. *Rauvolfia serpentina* is commonly known as Sarpagandha, Chandrabagha, Snakeroot plant, Chotachand, Chandrika, and Harkayaetc [8]. It is used as an antidote against snake bites and bites of other toxic insects [9]. The present investigation explored *Bungarus caeruleus* venom neutralizing activity of *Rauvolfia serpentina* root extracts by *in-vitro* and *in-vivo* methods.

MATERIAL AND METHODS:

Collection and Authentication of Plant Material: *Rauvolfia serpentina* (L.) Benth. ex Kurz. (*Ophioxylon serpentinum* L.) belongs to the family Apocynaceae was collected from Anakkal region, Malampuzha, Palakkad district, Kerala after questionnaire with tribal people and from vaidyas in and around Palakkad district. Dr. Althaf Ahamed Kabeer authenticated it. Scientist 'D'. Botanical Survey of India Southern Regional Centre. Coimbatore (Herbarium voucher specimen number 1160).

Preparation of Extract: 20g of powdered sample of the herb was extracted by soaking in 180ml of distilled water in a beaker, stirred for about 6min, and left-over night. After that, the solution was filtered using filter paper (Whatman No.1), and the extracts were evaporated to dryness under reduced pressure in 40°C. The plant extracts were expressed in terms of dry weight.

Extraction yields (%) = (weight of freeze-dried extract *100 / weight of original sample)

Extraction yields of *Rauvolfia serpentina* is 2.5%

Snake Venom: The freeze-dried snake venom powders of *Bungarus caeruleus* were obtained from Irula's Snake Catchers Industrial Co-operative Society Limited Chennai and were stored at 4° C. Stock solution was prepared by dissolving 1mg of lyophilized venom in 1ml of physiological saline (1mg/ml). (Ethics committee approval number: JSSCP/IAE/PH.D/PH.COLOGY/02/2014-15).

Acute Oral Toxicity: Acute oral toxicity of all the selected plant extracts was performed as per OECD guidelines 423. A limit test of 2000 mg/kg body weight of the extracts was administered. Briefly, two thousand milligrams of the test substance per kilogram of body weight were administered to3 healthy mice by oral gavages. The animals were observed for mortality, signs of gross toxicity, and behavioural changes at least once daily for 14 days. Body weights were recorded before administration and again on Days 7 and 14 (day of termination). Necropsies were performed on all animals at terminal sacrifice.

***In-vitro* Assessment of Venom Toxicity and Neutralization Assays:**

Direct Haemolysis Assay: The hemolytic action of *Bungarus caeruleus* venom and plant extracts was studied *in vitro* by using RBC. Briefly, 5ml of citrated blood was centrifuged for 10minutes at 900rpm. The supernatant was poured off, and the pellet was washed twice with a physiological salt solution. 5ml of physiological saline and 0.5ml of RBC mixture served as a control. 5ml of distilled water with 0.5ml of washed RBC was used for 100% hemolysis. 5ml of venom/extract and 0.5ml of washed RBC served as the experimental sample. The tubes were put in a thermostat for 1hr at 37°C and centrifuged at 2000rpm for 20mts. The supernatant fluid was poured off to separate tubes from measuring the optical density using a spectrophotometer at a wavelength of 540nm against water.

Indirect Haemolysis Assay (PLA_2 activity): Phospholipase A_2 activity was measured using an indirect haemolytic assay on an agarose erythrocyte egg yolk gel plate by the method described by [10]. Increasing concentrations of *Bungarus caeruleus* venom (μg) were added to 3mm wells in agarose gels (0.8% in PBS, pH 8.1) containing 1.2% sheep

erythrocytes, 1.2% egg yolk as a source of lecithin and 10mM $CaCl_2$. Slides were incubated at 37°C overnight, and the diameters of the haemolytic halos were measured. Control wells contained 15µl of saline. The minimum indirect haemolytic dose (MIHD) corresponds to a concentration of venom, which produces a haemolytic halo of 11mm in diameter.

The efficacy of *Rauvolfia serpentina* root extracts in neutralizing the phospholipase activity was estimated by mixing a constant amount of venom (µg) with different amount of plant extracts (µl) and incubating for 30 min at 37°C. Then, aliquots of 10 µl of the mixtures were added to wells in agarose-egg yolk-sheep erythrocyte gels. Control samples contain venom without plant extract. Plates were incubated at 37°C for 20 h. Neutralization is expressed as the ratio mg plant extract/mg venom able to reduce by 50% the diameter of the haemolytic halo compared to the effect induced by venom alone.

Acetyl Cholinesterase Activity: Acetylcholinesterase inhibition assay was carried out according to the modified method of [11] 200 µg of venom (1 mg/ml) was pre-incubated (1 h) with different concentrations of plant extract. The supernatant was added to the assay mixture, which consisted of 100 µl of 75mM acetylcholine iodate in 1 ml of phosphate buffer. The activity was measured by taking the absorbance at 412 nm. Venom without plant extracts was considered as control or 100% activity

Inhibition % = control-test/control ×100

Proteolytic Activity: Proteolytic activity was determined according to method [12]. Using 2%casein as substrate in 0.02 M Tris-HCl buffer (pH 8.5). Venom 200 µg (1 mg/ml) and different dilutions of plant extract were pre-incubated with 1 ml of substrate for 2 h at 37 °C.

The undigested casein was precipitated by adding 1.5 ml of 0.44 M trichloroacetic acid (TCA) and centrifuged. The digested casein in the supernatant was determined using Folinciocalteu's reagent. Venom without plant extracts was considered as a control or 100% activity.

Inhibition % = control-test / control ×100

ATPase Activity: ATPase activity was measured according to the modified method of [13]. *Bungarus caeruleus* venom 200 µg (1 mg/ml) was pre-incubated with different concentrations of plant extract of *Rauvolfia serpentina* root for 30 min. To the reaction, 1 ml of assay mixture (750 µl of 0.1 M Tris pH 7.5,100 µl of 0.1$MgCl_2$, 50 µl of 0.1 M ATP, and 100 µl of BSA) was added with gentle shaking at 37 °C and stopped at a certain time (1 h) by adding 1 ml of SDS solution.

The inorganic phosphate formed was determined by phosphate determination method by taking 400 µl of sample along 600 µl of TCA and incubating separately for 10 min at 37 °C followed by centrifugation at 1500 rpm for 10 min. About 500 µl of supernatant was added together with 500 µl of ferrous sulfate-ammonium molybdate reagent, and the absorbance was measured at 820 nm within 2 h for every 10 minutes of intervals. Reaction mixture without plant sample was referred to as control or 100 % activity. Inhibition reaction was calculated in terms of percentage (100%). Na, K-ATPase was mainly used.

Inhibition % = control-test / control × 100

***In-vivo* Assessment of Venom Toxicity and Anti-venom Effect of Plant Extracts Lethal Toxicity:** The median lethal dose (LD_{50}) of *Bungarus caeruleus* venom was determined according to the method of [14]. Various doses of venom in 0.2 ml of physiological saline were injected into the tail vein of mice, using groups of 3-5 mice for each venom dose.

The LD_{50} was calculated with a confidence limit of 50% probability by analyzing deaths occurring within 24 h of venom injection. The anti-lethal potentials for plant extract were determined against $2LD_{50}$ of *Bungarus caeruleus* venom. Various plant extracts (µl) were mixed with $2LD_{50}$ of venom sample and incubated at 37°C for 30 min, and then injected intravenously into mice. 3–5 mice were used at each antivenom dose. Control mice received same amount of venom without antivenom (plant extracts). The median Effective Dose (ED_{50}) calculated from the number of deaths within 24h of injection of the venom/antivenom mixture. ED_{50} was expressed as µl antivenom/mouse and calculated by probit analysis [15].

RESULTS:

TABLE 1: DIRECT HEMOLYSIS ACTIVITY – VENOM

Sample	OD of hemolysis	OD of Control (RBC + PBS)	OD of D. Water (100% Hemolysis)	% of Hemolysis
Bungarus caeruleus venom	1.06	0.04	1.08	94.44%

TABLE 2: DIRECT HEMOLYSIS ACTIVITY – NEUTRALIZATION ASSAY

Sample	OD of hemolysis	OD of Control (RBC + PBS)	OD of D.Water (100% Hemolysis)	% of Hemolysis
Bungarus caeruleus venom +Rauvolfia serpentina	0. 38	0.04	1.08	32%

Inhibition of Acetyl Cholinesterase Activity: The aqueous extract of the plant was taken in different dilutions starting from 200 µl to 300 µl with triplicate experiments. The maximum of Acetylcholinesterase inhibition (54.41%) occurred at 300 µl concentration of venom and aqueous extract of plant, respectively. The activity was calculated in terms of the percentage of inhibition compared to venom pre-incubated with different amounts of plant extract and venom with the substrate. The enzyme reaction was observed for every 10 min intervals at 412 nm. Acetylcholinesterase activity of the venom was considered as 100%.

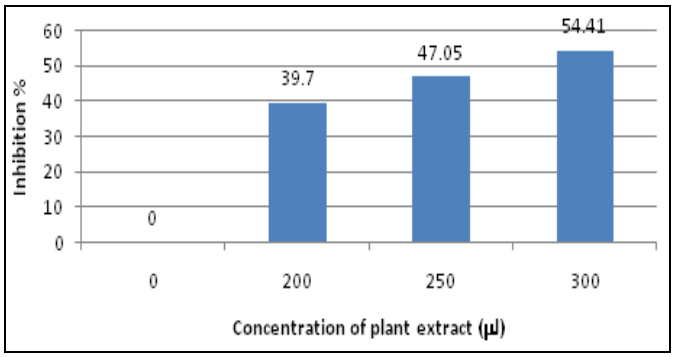

FIG. 1: ACETYLCHOLINESTERASE INHIBITION ASSAY

Inhibition of Protease Activity: To assess the in vitro antagonism of protease, the venom degrade the substrate (casein) into peptide precipitation could be observed at 600 nm.

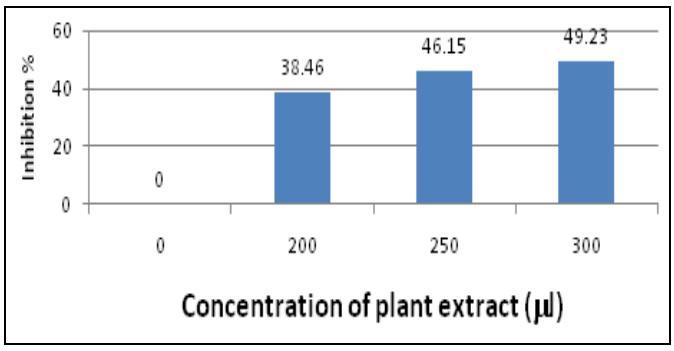

FIG. 2: PROTEASE INHIBITION ASSAY

Maximum of protease inhibition (49.23%) occurred at 300 µl concentration of venom and aqueous extract of plant, respectively. From the results, it was observed that increased plant extract could increase the inhibition of protease of *Bungarus caeruleus* activity.

ATPase Inhibition Activity: ATPase inhibition was calibrated by liberation of inorganic phosphate with of positive control of venom (200 µl) and substrate as ATP (10 µm). Different concentrations of venom and substrate were used for this reaction. The Same concentration of venom 200 µl, with different amounts of active aqueous extract of the plant (200 µl to 300 µl) was pre-incubated for the reaction. Maximum inhibition up to 53.60% has been noted at the highest amount of plant concentration.

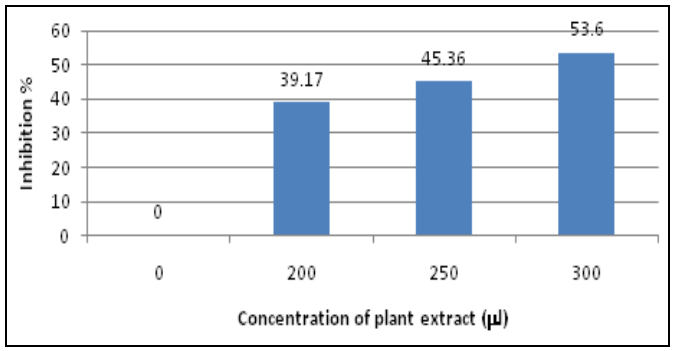

FIG. 3: ATPASE INHIBITION ASSAY

***In-vivo* Methods:** *In-vivo* assessment of venom toxicity (LD_{50}) of *Bungarus caeruleus* venom was assessed by LD_{50} range-finding test and the median lethal dose (LD_{50}) assay using mice (18-20g). LD_{50} of *Bungarus caeruleus* venom was calculated by Miller and Tainter method and was found to be 0.537 µg/g. **Table 4 and Fig. 4**. Venom neutralizing potency test (ED_{50}) using *Rauvolfia serpentina* root extract was carried out by pre incubating constant amount of venom ($3LD_{50}$) with various dilutions of *Rauvolfia serpentina* root extract prior to injection. Calculation of ED_{50} of

Rauvolfia serpentina root against $3LD_{50}$ of venom was done by Miller and Tainter method and was found to be 10.96 mg against *Bungarus caeruleus* venom. **Table 5 and Fig. 5**. All animals survived and appeared active and healthy in acute oral toxicity throughout the study. There were no signs of gross toxicity, adverse pharmacological effects or abnormal behavior. Based on the above findings, the LD_{50} of *Rauvolfia serpentina* root extract was>2000 mg/kg.

TABLE 3: CALCULATION OF LD_{50} OF *BUNGARUS CAERULEUS* VENOM IN MICE RECEIVING VARIOUS DOSES OF *BUNGARUS CAERULEUS* VENOM BY MILLER AND TAINTER METHOD (N=5)

Dose (µg/g)	Adjusted (Dose×100)	Log dose	Death/Total	Dead %	Corrected formula %	Probit values
0.025	*2.5*	0.4	0/5	0	5	3.36
0.05	*5*	0.7	1/5	0	5	4.16
0.1	*10*	1	1/5	20	20	4.16
0.25	25	1.4	2/5	40	40	4.75
0.5	50	1.7	2/5	40	40	4.75
1.0	100	2.0	3/5	60	60	5.25
2.5	250	2.4	3/5	60	60	5.25
5.0	500	2.7	5/5	100	95	6.64

Corrected formula: For the 0% dead: 100(0.25/n) = 100(0.25/5) = 5. For the 100% dead: 100[(n-0.25)/n] = 100[(5-0.25)/5] = 95, n is the number of animals in the group.

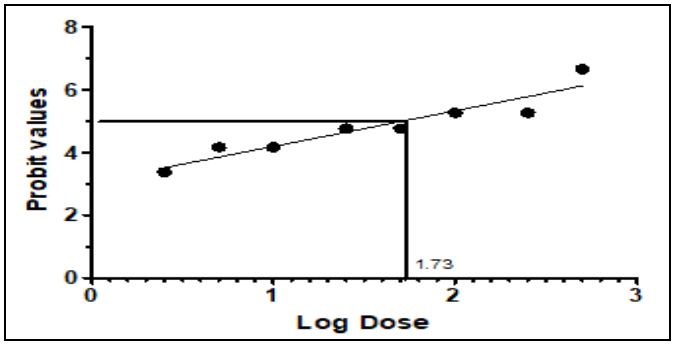

FIG. 4: CALCULATION OF LETHAL DOSE LD_{50} OF *BUNGARUS CAERULEUS* VENOM. LD_{50} of *Bungarus caeruleus* = antilog (log dose)/100 = antilog 1.73/100 = 53.70/100, 0.537 µg/g.

TABLE 4: CALCULATION OF ED_{50} OF *RAUVOLFIA SERPENTINA* AGAINST *BUNGARUS CAERULEUS VENOM* IN MICE BY MILLER AND TAINTER METHOD (N=5)

Dose (µg/g)	Adjusted (Dose×100)	Log dose	Survival/Total	Dead %	Corrected formula %	Probit values
1	*100*	2	0/5	0	5	3.36
2	*200*	2.3	0/5	0	5	3.36
4	400	2.6	1/5	20	20	4.16
8	800	2.9	2/5	40	40	4.75
16	1600	3.2	3/5	60	60	5.25
32	3200	3.5	4/5	80	80	5.84
64	6400	3.8	5/5	100	95	6.64

Corrected formula: For the 0% dead: 100(0.25/n) = 100(0.25/5) = 5. For the 100% dead: 100[(n-0.25)/n] = 100[(5-0.25)/5] = 95, n is the number of animals in the group.

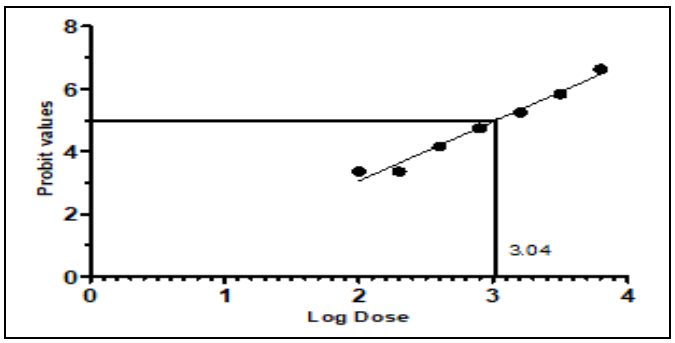

FIG 5: ED_{50} OF *RAUVOLFIA SERPENTINA* AGAINST *BUNGARUS CAERULEUS*. = antilog (log dose)/100 = antilog 3.04/100 = 1096/100= 10.96 mg.

DISCUSSION: Snakebite is constantly considered a chief health threat, leading to a high humanity rate worldwide [16, 17]. Polyvalent antiserum prepared from sheep and horses are effective for systemic envenoming after a bite. They have their own restrictions, such as indulgence and scarcity of accessibility for the rural patients [18]. Restrictions connected to anti-venom serum therapy have made people search for alternative medicines, especially medicinal plants in the past few years. Apart from Indian traditional medicines, Chinese, Greeks, and Egyptians are also identified in the usage of folk and traditional medicinal plants in snake bite treatments [19].

In recent years a number of reports on the use of plants in traditional healing by either tribal people or indigenous communities of India was increasing [20]. However, over the years, the use of antivenom has faced many constraints, notably allergic reactions, high prices, and lack of accessibility, making it challenging for people living in rural areas to access it [21]. Antivenin activities of a few medicinal plants such as *Andrographis paniculata, Andrographis lineata, Andrographis bracteolata, Clerodendrum viscosum,* and *Mimosa pudica* against the crude venom of Indian cobra have been reported using animal models in the previous literature [22, 23, 24, 25].

Elapidaevenoms have higher concentrations of acetylcholinesterase which exert an effect on nervous system [26]. *Thulasi et al* (2017) [27] reported that an aqueous extract of *Cyclea peltata* root is used for treating snakebite. In our present investigation, we checked the antitoxin activity of *Rauvolfia serpentina* root extracts against snake venom *Bungarus caeruleus*. The plant extracts were found to be effective in neutralizing the activity in an effective manner. In one of the reports, 28 *Azimatetra cantha* Lam extract effectively inhibited snake toxic enzymes like phosphomonoesterase, phosphodiesterase, acetyl-cholinesterase, hyaluronidase L-amino oxidase enzymes. In *Rauvolfia serpentina* aqueous rootextract (300µl) against *Bungarus caeruleus* venommaximum, ATPase inhibition was recorded as 53.60% for *Bungarus caeruleus* venom. Shwetha Vasudev [29] reported crude aqueous ethanolic extract cocktail of medicinal plants, *Areca catechu, Azadirachta indica, Butea monosperma, Citrus limon peel,* and *Clerodendrum serratum* for anti-ophidian properties against BIG FOUR venom through *ex-vivo* and *in-vivo* methods. Direct hemolysis using sheep RBC's was studied for *Bungarus caeruleus* venom and we found that snake venom was able to lyse the RBC's. *Bungarus caeruleus* venom showed 94.44% hemolysis. Plant extracts were able to neutralize the venom-induced hemolysis and the hemolysis was reduced below 40%. In phospholipase activity (PLA_2) 15µg of *Bungarus caeruleus* venom were able to produce 11mm diameter hemolytic halo, which is considered to be 1Unit. Plant extracts were capable of inhibiting PLA_2-dependent hemolysis of sheep RBC's induced by snake venoms in a dose-dependent manner.

In-vivo venom toxicity (LD_{50}) of *Naja naja* and *Bungarus caeruleus* venoms were assessed by LD_{50} range-finding test and the median lethal dose (LD_{50}) assay using mice (18-20 g). LD_{50} of *Bungarus caeruleus* was found to be 0.537 µg/g. Raphael *et al*., 2014 [30]reported that *Rauvolfia serpentina* root extract was used in neutralizing cobra and krait venom. In the previous report on Rajasree*et al*., 2013 [31] the ethanol extracts of *Rauvolfia serpentina* plant were tested for antivenom activity against *Najanaja* venom.

About 0.14 mg of *Rauvolfia serpentina* plant extract was able to neutralize the lethal activity of 2LD50 of Najanaja venom completely. In the previous work of Thulasi *et al*., 2017 [32] *Terminalia arjuna* bark extract was able to neutralize lethal activity of $2LD_{50}$ of *Naga naja* venom. In another study of *Thushara et al*., 2013 [33] of the *in-vivo* and *in-vitro* neutralizing potential of *Rauvolfia serpentina* plantextract against *Daboia russelli venom, Rauvolfia serpentina* plant extract was *e*ffectively neutralized by the venom lethality, and effective dose (ED) was found to be 10.99 mg/ 3LD of venom. In the previous work of Thulasi*et al*., 2020 [34] about 0.14 mg of *Rauvolfia serpentina* plant extract was able to completely neutralize the lethal activity of 2 LD_{50} of *Najanaja* venom. Timothy *et al.* [35] reported that a literature survey done in multidisciplinary databases revealed that 77 plant species belonging to 65 genera and 42 families are used to treat snakebites in Uganda. The majority of these species belong to the family Fabaceae (31%), Euphorbiaceae (14%), Asteraceae

(12%), Amaryllidaceae (10%), and Solanaceae (10%). These *in-vivo* neutralization assays suggest that plant extracts effectively neutralized the toxins present in both snake venoms. In acute oral toxicity, all animals persisted and seemed energetic and vigorous throughout the study. There were no signs of unfavorable pharmacological effects or unusual activities. Based on the above findings, the LD_{50} of all selected plant extracts was>2000 mg/kg.

CONCLUSION: The result from the *in-vitro* and *in-vivo* analysis indicates that *Rauvolfia serpentina* root extract possesses significant compounds that neutralizes the toxins present in *Bungarus caeruleus* venom. Further investigations are needed to identify and purify the active components involved in the neutralization of the snake venom.

ACKNOWLEDGEMENT: The authors are thankful to Principal Dr. Anirudhan for providing the necessary facilities in the College and sincerely thank Dr. J. Rathinamala, Head, Department of Microbiology, Nehru Arts and Science College, Coimbatore, for her encouragement and support throughout the study.

CONFLICTS OF INTEREST: Nil

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How to cite this article:
Sivaraman T, Sreedevi NS, Meenatchisundaram S and Vadivelan R: *In-vitro* and *in-vivo* neutralizing potential of *Rauvolfia serpentine* root extract against *Bungarus caeruleus* venom. Int J Pharm Sci & Res 2022; 13(10): 4054-61. doi: 10.13040/IJPSR.0975-8232.13(10).4054-61.

European Journal of Biotechnology and Bioscience
www.biosciencejournals.com
ISSN: 2321-9122
Received: 03-06-2022, Accepted: 19-06-2022, Published: 05-07-2022
Volume 10, Issue 2, 2022, Page No. 50-53

A study on herbal face pack antibacterial activity against skin infections and formulation for safety usage

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Abstract
The aim of this work is to plan and assess a natural face pack for gleaming skin by utilizing normal natural ingredients Multani mitti, Gram flour, Sandalwood, Milk powder, Tumeric, Green tea, Orange peel, Avarampoo were obtained from the neighborhood market and were dried, powdered, then these dried powders by utilizing sieve#120 and blended homogeneously in for uniform detailing and assess morphol ogical, physicochemical, phytochemical, irritancy assessment. The dried powder of consolidated structure had tolerable stream property which is reasonable for a face pack. To perform and actually look at antibacterial movement of concentrate of turmeric, sandal wood, orange strip powder against microorganism causing skin infection. The benefit of home grown beauty care products is their non poisonous nature; decrease the hypersensitive responses and dependable handiness of numerous ingredients. Thus in the current work, we found great properties for the face packs and further streamlining studies are expected on this review to find the helpful advantages of face packs on human use as superficial item.

Keywords: multani mitti, physicochemical evalution, phytochemical evalution, irritancy test

Introduction
From the antiquated period individuals are involving spices for cleaning, enhancing and to oversee them from infections. Cosmetics are monetarily accessible items that are utilized to work on the presence of the skin by activity of cleaning, decorating, advancing attractiveness. Herbal face packs supply fundamental supplements to skin. Herbal face pack to decrease acne, pimple, scars, pimple and imprints relying upon its natural ingredients. Face pack is the smooth powder which is utilized for facial application. These arrangements are applied on the face as fluid or glues and permitted to dry and set to frame film giving tightening, strengthening and purging impact to the skin (Rieger MM, 2009). Home grown beauty care products are the item which are utilized to clean and decorate the skin. The extra characteristics of skin can be decreased by joining of fine powders of shoe wood and dried orange peels. Face packs are utilized to build the reasonableness and perfection of the skin. It diminish pimples, kinks, skin break out, and dark circles of the skin (Mithal B M, Saha RN, 2004) [18]. Present examination article manages the definition and assessment of home grown face pack for shining skin at home by utilizing normal materials i.e., Multani mitti, Turmeric, Sandal wood, Lemon strip, Rose petal powder, Majistha, lodhra and Gram flour. The regular face packs are less confounded and easy to use. They help us in taking care of skin and furthermore demonstrate its value by expanding the flow of the blood inside the veins of the face (Saraf S, Saraf SH, 2005).

Materials and Methods
1. Assortment of plant material
- Plant materials was gathered from neighborhoods thrissur, Kerala.
- Plant material was cleaned with refined water and dry at room temperature.
- The dried plant materials were squashed into powder form.
- Bacterial disengages to disengage microorganisms from skin contamination sample. The skin disease microbes detaches of *Staphylococcus* sp, *Pseudomonas* sp, *E Coli, Klebsiella* sp were acquired from the skin disease test of the different emergency clinic in thrissur district. Isolation of pathogenic microorganism

a. Preparation of inoculam
b. Isolation of specific media

2. Morphology and biochemical portrayal of secludes
Extraction of plant materials and Antibacterial helplessness testing
Evaluating for antibacterial action

The ethanol concentrate of turmeric, orange peel, sandal wood and correlation of ethanol concentrate and standard anti-infection plate were utilized for actually looking at the antibacterial action and these were inspected by estimating the measurement of the zone of hindrance by circle dispersion strategy. Arrangement of home grown face pack.

Table 1

S. No	Constituent	Percentage
1	Gram flour(powder)	40(in gram)
2	Multani mitti(powder)	20(in gram)
3	Sandal wood(powder)	10(in gram)
4	Tumeric(powder	8(in gram)
5	Green tea(powder)	4(in gram)
6	Orange peel(powder)	7(in gram)
7	Milk powder	10(in gram)
8	Avarampoo(powder)	1(in gram)

Fig 1

3. Morphological evalution

Natural face pack was assessed for morphological, the shade of plan was light yellow. The smell of arranged plan was wonderful and great adequate which is alluring to corrective formulations.

4. Physicochemical evalution

Home grown face pack was assessed for physicochemical boundaries are pH, ash content. The pH definition was found near neutral.

The debris content and dampness content was inside limit.

5. Phytochemical evalution

Natural face pack was assessed for phytochemical boundaries are viewed as presence of phytoconstituents such a carbohydrates, alkaloids, glycosides, tanins and unstable oil which go about as a decent nourisher for the skin.

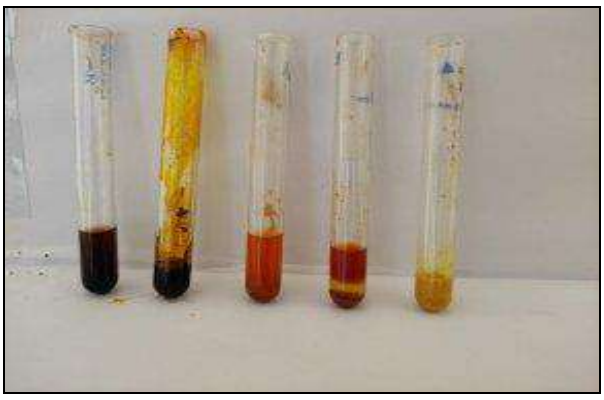

Fig 2

6. Irritancy test

The plan showed nonattendance of irritation, redness and expanding during irritancy studies. This detailing have protected to use on skin.

Table 2

SL No	Parameter	Observation
1	Irritration	No
2	Redness	No
3	Swelling	No

Result and Discussion

Skin possesses both occupant and transient flora. The transient microflora mainly constituents the possibly pathogenic miniature organisms. The significant living beings as pathogens in the skin incorporate *Escherichia coli, Staphylococcus aureus, Pseudomonas* sp, *Klebsiella* sp. In the current review the plant remove curcuma longa, citrus sinesis, santalum collection showed antibacterial movement against clinical organisms *Staphylococcus* sp, *Escherichia coli, Pseudomonas* sp, *Klebsiella* sp exceptionally touchy to natural plant extricates by utilizing ethanol. The zone of restraint has profoundly delicate to microorganisms, similarly the action higher than the standard antibiotics.

Conclusion

Antibacterial action of curcuma longa, citrus sinesis, santalum collection were contemplated and looked at by disc diffusion method against the test organinism, for example, *Staphylococcus* sp, *Escherichia coli*, *Pseudomonas* sp, *Klebsiella* sp. By playing out the strategy it was distinguished that the counter microbial action was more against Curcuma longa, citrus sinesis, santalum album. Ethanol concentrate of plants affects Organism segregated from skin contamination tests.

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International Journal of Computer Science Engineering & Technology
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NATIONAL INSTITUTE OF SCIENCE COMMUNICATION AND INFORMATION RESOURCES (NISCAIR)
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ISSN :2455-9091

A SURVEY ON IMPLEMENTATION SOLUTIONS FOR ATTACK PREVENTION CRYPTOGRAPHY TECHNIQUES IN WSN USING NS2

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ABSTRACT - Because of absence of alters safe equipment and broadcast nature of Wireless Sensor Networks (WSNs), security in sensor networks is one of the significant concerns. In this paper we survey on implementation solutions for attack prevention different cryptography techniques. WSNs comprise of countless sensor nodes and a couple of sink nodes or Storage node is utilized to gather data about the condition of actual world and communicate it to intrigued clients. It utilized in applications, for example, wellbeing checking, natural surroundings observing, military reconnaissance and climate detecting. Sensor nodes have restricted assets in term of handling power, battery force, and information stockpiling. A sensor network that isn't completely believed that is the reason protection is to be safeguarded. A security approaches that utilization secret key cryptography and key administration. To safeguard the trustworthiness, a computerized key is gotten to each node in an organization, every node needs to send their confinement position as scrambled information utilizing advanced marks to the capacity node and it decodes that information and checks the situation by utilizing verification.

Keywords: [Wireless Sensor Networks; Cryptography; Attack Prevention; sensor network.]

1. INTRODUCTION

Wireless sensor networks have arises as cutting edge innovation in data innovation environment and exploration including equipment framework plan, information the executives, security and social factors. Sensor networks allude to a heterogeneous framework joining little sensors and actuators with universally useful processing components. Sensor node is a keen, little, self getting sorted out, low cast, and multi-practical gadget, outfitted with battery, radio correspondence, microcontroller and sensor. It has extremely restricted handling capacity battery force, memory and furthermore a limited field of detecting. The primary reason for WSN is to fill in as an interface to genuine world, giving actual data like temperature, light, radiation and so forth to a PC framework. In this paper to address the basic security issues in WSNs we examine about cryptography. Sensor networks allude to a heterogeneous framework joining small sensors and actuators with universally useful figuring components. These networks will comprise of hundreds or thousands of self-

coordinating, low force, ease wireless nodes conveyed to screen and influence the climate. Sensor networks are regularly portrayed by restricted force supplies, low transfer speed, little memory sizes and restricted energy. This prompts a requesting climate to give security. Classification is concealing the data from unapproved access. In numerous applications, nodes impart exceptionally delicate information. A sensor organization ought not to break sensor perusing to adjoining networks. Simple strategy to maintain delicate information mystery is to scramble the information with a mysterious key that solitary the expected receivers 'possess, henceforth accomplishing confidentiality. As public key cryptography is too costly to ever be American Journal of Engineering utilized in the asset obliged sensor networks, most of the proposed conventions utilize symmetric key encryption methods. For symmetric key methodology the key conveyance component ought to be incredibly powerful. Validation guarantees the unwavering quality of the message by recognizing its origin.

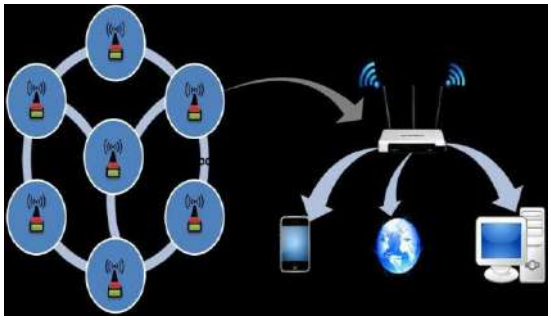

Figure 1.Wireless sensor networks using NS2

In a WSN, the issue of confirmation should address the accompanying prerequisites: imparting node is the one that it professes to be the recipient ought to check that they got parcels have evidently come from the genuine sensor node. For Authentication to be accomplished the two gatherings should share a mysterious key to process Message verification code (MAC) of all conveyed data. The collector will check the validation of the got message by utilizing the MAC key. Uprightness is keeping the data from unapproved modification. Data validation can give information respectability too. Accessibility guarantees that administrations and data can be gotten to at the time they are required. In sensor networks there are numerous dangers that could bring about loss of accessibility, for example, sensor node catching and disavowal of administration assaults. Probably the greatest test with regards to getting MANETs and WSNs is the entirety of the elements that should be represented: unique geographies, asset requirements, no foundation and restricted actual security. As WSNs ordinarily have a greater number of nodes than MANETs, and sensory nodes in WSNs are more assets compelled regarding power, computational capacities, and memory, the security configuration utilized in WSNs must be more explicit for those spaces. Much exploration has been led on directing security, key administration, and trust in MANETs and WSNs; a large portion of it is related with cryptography, validation, approval, encryption, and unscrambling. The definite cycle can be found through different reviews and advancement of cryptography instruments for MANETs/WSNs.

2. LITERATURE SURVEY

1. A. A. Adekunle (2015), et.al proposed A Symmetric Cryptographic Construct for Securing Wireless Sensor Network Communications. AEAD schemes can be delegated possibly one-pass or two pass schemes. In a two-pass plot, the arrangement of secrecy and uprightness security administrations is given in isolated passes. A strategy for building two-pass AEAD schemes is by conventional synthesis; whereby one-pass is a security just symmetric encryption conspire, while the other pass is a message trustworthiness plot. In a one-pass plot, a solitary pass is made through the information, which all the while produces secrecy and trustworthiness security

to the handled information. Typically, one-pass schemes for the most part display higher information handling rate and lower operational expenses.

Merits

Cypher Codex build exhibited favorable execution with respect to a high information handling throughput, low preparing inactivity and a demonstrated low energy use prerequisite across a scope of reasonable WSN conveyed bundle lengths.

Demerits:

Prior to any correspondence, both the sender and the receiver need to concede to a mystery symmetric key. It requires a protected key foundation instrument set up.

2. Constantin Grumazescu, Valentin-Alexandru Vladuta,Andrei Timofte(2018), et.al proposed Hybrid identity based cryptographic scheme optimization using machine learning in WSN to revamp the security foundation to advance force utilization of sensor nodes dependent on qualities gathered by a UAV at every cycle the best up-and-comer calculation we are thinking about here is K-implies. At first considering an irregular or client characterized number of k PKG nodes out of n absolute nodes (with k < n) an orchestrator occurrence can figure the centroids of the security foundation to equitably circulate the force utilization level of sensor nodes. The distance capacity can be viewed as the Euclidean distance between the utilization levels of every sensor node. This information can be accumulated in an old style way by utilizing at least one static sinks or a mobile sink (UAV), totaled, deciphered, prepared and the outcomes directed a similar way.

Merits:

Client prerequisites for both organization life expectancy and security level are accomplished.

Demerits:

This must be finished utilizing AI procedures.

3. Jyothi Metan, K N Narasimha Murthy(2015), et.al proposed Robust and Secure Key Management in WSN using Arbitrary Key-Deployment the space of wireless sensor network is as of now covered with different issues for example energy issues, steering issues, QoS issues, and security issues. Albeit in past there were development of different secure procedures, however dominant part of the method were refined cryptographic executions where reality intricacies were less accentuated. This paper presents a basic and a novel procedure called as MLKS for example Staggered Key Security that applies straightforward set hypothesis and key dissemination tool for getting the data in wireless sensor network. The strategy is likewise material on various security principles supporting 128, 216, 160, or 512 pieces of key size. The result of the proposed framework is contrasted with the main work with discover the proposed framework outflanks the current framework as for computational time and capacity cost.

Merits

This procedure that tends to various degrees of safety tasks for together tending to security in bunch correspondence just as information conglomeration in wireless sensor network.

Demerits

Nonattendance of novel digital signature plan to make the current gathering correspondence safer.

4. Trong-Minh Hoang,Van-Hau Bui,Nhu-Lan Vu(2020), et.al proposed A Lightweight Mixed Secure Scheme based on the Watermarking Technique for Hierarchy Wireless Sensor Networks. Wireless Sensor Network (WSN) is a significant part of the Internet of Things design. It works in antagonistic climate areas to give a ton of

intriguing applications. Nonetheless, WSN is compelled by restricted assets like force or computational expense, making it helpless against numerous sorts of assaults. Henceforth, tracking down a fitting security answer for ensure node validation and sensor information respectability which are two of the most basic security issues has consistently pulled in scientists. In which, the digital watermarking strategy can be utilized to get sensory information while keeping a sensible calculation cost. In any case, these days, node clone assaults can make a conflict of inside assaults which harms vigorously to the exhibition of sensor networks. Henceforth, the classification and trustworthiness of sensor network elements are raised more provokes identified with intricacy and viable issues. This paper proposes a novel lightweight blended secure plan dependent on watermarking strategies to ensure sensory information and guard against node clone assaults. Mathematical outcomes and security examination will be given in the paper to approve the benefit of the proposed security convention.

Merits

A center convention related to an execution algorithm to shield the sensor network node from node parodying assaults while ensuring the information gathered by the sensor network.

Demerits

WSNs network when identifying an assault with both private and worldwide algorithms isn't reacting.

5. Tarek Farah, Safya Belghith (2017), et.al proposed A new chaotic encryption algorithm for WSN and implementation with sensors AS-XM1000. The degree of safety changes starting with one area then onto the next and starting with one application then onto the next relying upon the kind and significance of the information traded in the remote sensors. In this paper we propose to imagine a chaotic encryption algorithm for WSN dependent on S-box and chaotic stages. Correlations other proposed algorithms are introduced in this paper. The aftereffects of the assessments and estimations of the proposed algorithm tests are finished by utilizing the TinyOS working framework, which makes it conceivable to deal with the assets of the WSN. We will likewise introduce an execution of the proposed algorithm on AS-XM1000 sensors. The proposed technique is adaptable; surely the proposed encryption algorithm can be applied to messages of little size or enormous size as on account of pictures

Merits

Encryption technique can monitor the information and correspondence from unapproved disclosure and access of information.

Demerits

The organization or the PC framework can be assaulted and delivered non-useful by a gatecrasher.

6. Pritam Banerjee, Tanusree Chatterjee, Sipra DasBit (2015), et.al proposed Low-overhead Encryption based Node Authentication in WSN. Execution of the plan is basically broke down by utilizing two appropriately picked boundaries like breaking likelihood and breaking time. This assessment guides us in fixing the size of the exceptional id of a node so the plan brings about low overhead just as accomplishes adequate power. The presentation is additionally contrasted a few late works as far as calculation and correspondence overheads and that affirms our plan's matchless quality over contending plans as far as both the measurements.

Merits:

This is light weighted by utilizing computationally light activities like Ex-OR, extraction, bitwise mix.

Demerits:

There is no coordinating message validation with the node verification to make a more complete security answer for WSNs.

7. Chungen Xu, Yanhong Ge(2009), et.al proposed The Public Key Encryption to Improve the Security on Wireless Sensor Networks. Wireless sensor networks (WSN) develops and gains new in our lives. Anyway security in WSN was not painstakingly completed, since just some symmetric encryption based conventions are proposed in writing, under the supposition that the idea of sensor nodes doesn't uphold public key encryption because of the constraint in battery and CPU power. This paper presents a plan of Public Key Infrastructure (PKI) for wireless sensor networks. The plan attempts to take care of the issue of safety in WSN by the utilization of public key cryptography as an instrument for guaranteeing the legitimacy of the base station, and propose a RSA's execution of Public Key Encryption to improve the security on wireless sensor networks.

Merits

The plan attempts to tackle the issue of safety in WSN by the utilization of public key cryptography as an apparatus for guaranteeing the validness of the base station.

Demerits

One hindrance of public-key encryption is that is slower than different techniques, like mystery key encryption. In secret-key encryption, a solitary key gives that best way to encode and decode, improving and accelerating the interaction.

8. Zhang Jing, Ma Chen, Fan Hongbo (2017), et.al proposed WSN Key Management Scheme Based on Fully Bomomorphic Encryption. Improving the safe WSN's continuous, energy utilization, and against spill is a significant exploration issue in view of explicit activities as expansion, duplication, etc. Completely homomorphic encryption can control the cipher text straightforwardly to get the right outcomes without unscrambling. Since completely homomorphic encryption's interaction of the unscrambling can be precluded, the safe WSN's qualities, for example, continuous, etc can be improved adequately. Key administration is a significant issue of completely homomorphic encryption, in which the capacity to hostile to catch in WSN starts things out. In this paper, in light of the estimation calculation of two component even polynomials proposed by Blundo, we propose a pair wise key foundation plot by presenting completely homomorphic encryption, which doesn't just forestall adversary catching data about polynomial connection, yet in addition oppose the huge scope node catch assault effectively. Examination and tests show that this plan can absolutely meet the prerequisites of asset, energy utilization, etc of WSN, and improve the security of WSN at the same time.

Merits

The energy utilization of the nodes has an excellent improvement.

Demerits

The homomorphic encryption requires either application changes or devoted and concentrated customer worker applications to make it work practically.

9. Yan Liu, Xiumei Wu, Xuemin Chen (2015), et.al proposed A Scheme for Key Distribution in Wireless Sensor Network Based on Hierarchical Identity-Based Encryption. Customary technique for key appropriation doesn't fit the need to run in wireless sensor organization (WSN) because of limit on energy, calculation and memory limit of WSN. In this paper, we propose a plan based on Hierarchical Identity Based Encryption (HIBE) to convey encode key. Contrasting and key appropriation based on

Identity-Based Encryption (IBE), the proposed conspire lessens the calculation time and saves memory space of wireless sensor node.

Merits

Wireless sensor networks by embracing the HIBE to save the extra room and to decrease the calculation needed at every node.

Demerits

This requires a more significant level of affirmation and accessibility from PKG side. This is disadvantage of IBE System.

10. Shao-I Chu, Yu-Jung Huang, Wei-Cheng Lin (2015), et.al proposed Authentication Protocol Design and Low-Cost Key Encryption Function Implementation for Wireless Sensor Networks. Wireless sensor networks (WSNs) have been broadly utilized, most outstandingly continuously traffic checking and military detecting and following. Be that as it may, WSN applications could experience the ill effects of dangers and imperil the applications if the reasonable security issues are not mulled over. Subsequently, client confirmation is a significant worry to shield information access from unapproved clients. Rather than generally utilizing a hash work for information security, one of the fascinating parts of this convention is that, with the end goal of information assurance however with a low computational expense, the proposed key encryption work just requires straightforward selective OR (XOR) number-crunching tasks. In addition, the relating equipment design was carried out by utilizing an Altera DE2 board, including an Altera Cyclone II field-programmable gate array (FPGA). At last, the yield waveforms from the FPGA were shown on the 16702A rationale investigation framework for constant check.

Merits

A lightweight common confirmation convention over WSNs is produced for secure validation.

Demerits:

This isn't reasonable for encryption of huge messages as the encryption/decoding throughput is contrarily identified with the key length.

11. A. Karthikeyan, V. Srividhya, Pranjay Gupta, Naveen Rai,(2015), et.al proposed a Discrete cosine and Discrete Wavelet transform based algorithm for simultaneous image compression and encryption is proposed, which is useful for reliable data transmission. The Secure Force encryption algorithm utilized is appropriate to wireless sensor networks (WSN). The adequacy of our proposed algorithm is assessed based on the PSNR (Peak Signal to Noise Ratio), MSE (Mean Square Error), registering time and rate pressure. The proposed algorithm helps in diminishing information repetition altogether which makes it energy proficient while thinking of it as' usage for WSN.

Merits:

An energy effective concurrent pressure and encryption algorithm explicitly for wireless sensor networks to be utilized for military or energy driven applications.
This will reduce the encrypting complexity.

Demerits:

This will decrease the scrambling intricacy.

12. Soufiene Ben Othman, Abdelbasset Trad, Habib Youssef (2012), et.al proposed Performance Evaluation of Encryption Algorithm for Wireless Sensor Networks. With the far reaching development in applications for asset restricted Wireless Sensor Networks (WSN), the requirement for dependable and effective security systems for them has expanded complex yet its execution is a non-minor assignment. Impediments in

preparing speed, battery force, transmission capacity and memory oblige the materialness of existing cryptography Algorithms for WSNs. A few security systems, like TinySec, have been acquainted with address the requirement for security in WSNs. The expense of safety, be that as it may, in any case generally stays an obscure variable. To give a superior comprehension of this expense we have considered three encryption algorithms, AES, RC5 and RC6. We have estimated and looked at their memory and energy utilization on Mica2 sensor bits.

Merits

Cryptographic algorithms are crucial for the protected and proficient improvement of cryptosystem in gadgets with low computational force.

Demerits

Versatile cryptographic components to streamline energy utilization by shifting code boundaries with convenient procurement of asset setting in WSN climate can't be investigated.

13. Jia Chenjun, Liao Yongjian, Chen Kangshen(2008), et.al proposed Secure Encryption in Wireless Sensor Network. Wireless sensor networks (WSN) have gotten a great deal of consideration as of late because of their wide scope of uses in military just as regular citizen activities. It is a moving work to plan reasonable cryptography for wireless sensor networks since the impediments of force, calculation ability, and capacity assets. Numerous plans dependent on symmetric or public key cryptography have been examined. As of late, a reasonable personality based encryption method is proposed.

Merits

Effective personality based encryption plot which has been demonstrated secure in the wireless sensor network climate while decreases the necessity of asset.

Demerits

In the event that you use encryption to secure your data on your PC at work or at home, it could raise doubts.

14. Xueying Zhang, Howard M. Heys, and Cheng Li (2010), et.al proposed An Analysis of Link Layer Encryption Schemes in Wireless Sensor Networks. In particular, we research various variables which influence the energy cost of link layer cryptographic security schemes, for example, the payload size, the wellspring of the introduction vector, and the channel quality. We propose a way to deal with assess the exhibition of cryptographic correspondence schemes by building up an examination model thinking about these components. The propriety of this model is upheld by reenactment results.

Merits:

The cipher criticism conspire accomplishes better execution for a scope of channel characteristics and for little payload sizes gives a huge relative improvement in the quantity of the pieces that can be effectively moved for a given energy.

Demerits

Key circulation and the board are more mind boggling in light of the fact that each bounce gadget should get a key, and when the keys change, each should be refreshed.

15. Mustafa A. Al Sibahee, Songfeng LU (2017), et.al proposed The Best Performance Evaluation of Encryption Algorithms to Reduce Power Consumption in WSN. Wireless Sensor Networks (WSNs), applications are developing quickly, so the necessities to ensure such applications are expanded. Cryptography assumes a principle part in data framework security where encryption calculation is the fundamental segment of the security. On the opposite side, those calculations burn-through a lot of figuring assets, for example, CPU time, memory, and battery power.

Merits

Encryption is utilized to secure delicate information, including individual data for people.

Demerits:

Utilizing encrypted documents that are intended to be opened and shared by at least two individuals can be disadvantageous when at least one member thinks that it's a weight to utilize encryption.

CONCLUSION

Wireless multimedia sensor networks will assume a significant part in the Internet of Things world, since current observing applications will depend on multimedia information for more hearty choices. In this specific circumstance, cryptography will be in WMSN plan. In this paper we surveyed existing cryptography techniques for attack prevention. Late works have proposed many promising answers for give various degrees of safety in those organization, which will impact the manner in which security will be given in current WMSNs. The issue of adding nodes to a current organization is troublesome, even with public key cryptography, as each node in this disseminated network must be educated about recently added and acknowledged public keys. Repudiation is considerably harder, in light of a few entanglements.

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IJRSET AUGUST 2021 Volume 8, Issue 8 Pages: 1-12

ISSN 2394-739X

International Journal for Research in Science Engineering and Technology

REVIEW ON CRYPTOGRAPHY TECHNIQUES IN WSN FOR ATTACK PREVENTION

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ABSTRACT - Scalability of routing protocols used in wireless sensor networks (WSNs) is a critical issue due to the extremely high node numbers and relatively high node density. The analysis of scalability of wireless sensor networks is a challenging performance issue. Complexity is caused by several issues. First, the large number of nodes heavily impacts simulation performance and scalability. Second, credible results demand an accurate characterization of the sensor radio channel. New aspects, inherent in WSN, must be included in simulators, e.g. a physical environment and an energy model, leading to different degrees of accuracy versus performance. A good routing protocol has to be scalable and adaptive to the changes in the network topology. Thus protocols must perform well as the network grows larger or as the workload increases. In this paper, we compare the algorithms in WSN to improve the scalability of the network. This computation showed the limitation and capability of the WSN scalability and specifically for Flooding. In this work, Supervised learning and Unsupervised learning algorithms like k-nn, Fuzzy Logic based has been compared successfully to analyze the efficiency of scalability in WSN.

Keyword: [Wireless Sensor Network, NS2, Fuzzy logic, Cluster Head, Routing, Machine Learning, Neuroscience, LEACH protocol.]

1. INTRODUCTION

Uses of remote sensor organizations (WSNs) have been developing at a quick rate over the recent many years. Since the subject has gotten consideration, the going with innovation additionally quickly upgraded, prompting the plan of little sensors that are equipped for detecting, preparing and imparting information. Sensor networks are normally conveyed at distant destinations. They are accused of the duty of detecting and moving information to the base station for handling and capacity. These tasks require continuous force source, which establishes a test given the areas of the sensors. One approach to take out this issue is to guarantee proficient force utilization. Notwithstanding the force utilization, there are a few different issues that require thought when the constraints of sensors are concerned. Given that the remote sensors are little gadgets with restricted abilities, they are restricted in preparing, force, memory, and correspondence. The sensors are normally

positioned on far off locales notwithstanding their force limits. Thusly, the WSN business centers around expanding the detecting capacity with the base force utilization. Extraordinary conventions are needed to make these sensors work with ideal force utilization. Furthermore, applying standard organization security strategies to WSNs isn't viewed as a feasible arrangement because of their restricted capacities. Besides, use of ordinary organization security methodology would likewise prompt expanded force utilization requiring extra preparing assets. A remote sensor network is a gathering of particular transducers with an interchanges framework for checking and recording conditions at assorted areas. Normally checked boundaries are temperature, moistness, pressure, wind course and speed, brightening force, vibration force, sound force, power-line voltage, substance fixations, toxin levels and crucial body capacities. Remote sensor organizations (WSNs) offer extraordinary guarantee for data catch and handling in both business and military applications. Effective framework plan and arrangement incorporates understanding RF channel attributes, and the decision of adjustment conspires on power utilization. Such factors eventually decide the accessible reach and information pace of a WSN, just as cost and battery lifetime. Remote sensor organizations (WSNs) have acquired overall consideration as of late, especially with the multiplication in Micro-Electro-Mechanical Systems (MEMS) innovation which has worked with the improvement of shrewd sensors. These sensors are little, with restricted preparing and processing assets, and they are economical contrasted with customary sensors. These sensor hubs can detect, measure, and accumulate data from the climate and, in light of some nearby choice cycle, they can send the detected information to the client.

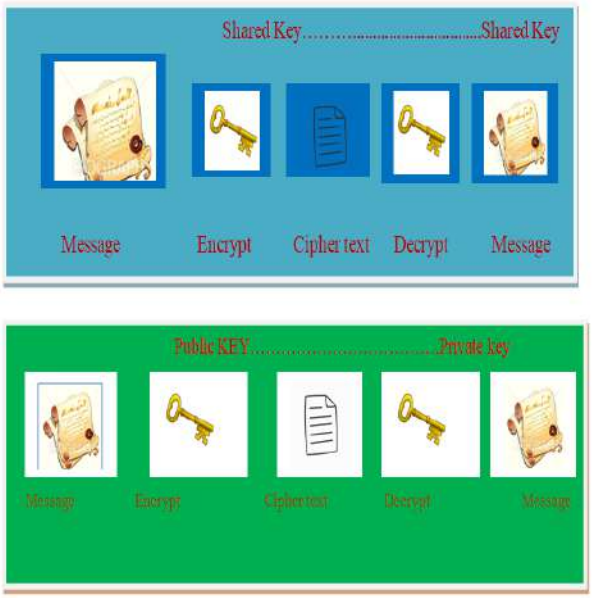

Figure1. Encryption and Decryption process

2. LITERATURE SURVEY

1. Cong Gao, Zhongmin Wang and Yanping Chen (2019), et.al proposed On the connectivity of highly dynamic wireless sensor networks in smart factory. With the advancement of brilliant assembling in Industry 4.0, huge measure of heterogeneous information is created from numerous sources. Different information handling procedures can be applied to these information to separate expected data about the situation with the assembling organization. In this manner, information is significant to the investigation and change of the assembling measures. Because of the variety of items, mechanical production systems and assembling measures are exceptionally powerful. Considering the adaptable sending of remote sensor organizations, they have been generally conveyed to lead information assortment. Notwithstanding, because of the restricted radio scope of a remote sensor hub, the availability of an exceptionally powerful remote sensor network is a central question. Broke down the availability in a remote sensor organization and agreed three sorts of network. At that point, we created two organization availability models from the

viewpoint of one measurement and two measurements. The levels of availability for both two hubs and the organization are expounded. At long last, the connection between the quantity of sink hubs and the normal level of availability of the organization is dissected dependent on exploratory outcomes.

2. Waltenegus Dargie (2020), et.al proposed A Clustering Strategy for Wireless Sensor Networks. The bat algorithm (BA) is a novel metaheuristic search algorithm which simulates the behavior of the bat species for searching prey. Preliminary studies show that it is very promising and could outperform existing algorithms. The BA utilizes a population of bats to represent candidate solutions in a search space and optimizes the problem by iteration to move these agents to the best solutions about a given measure of quality. Cluster Head Selection algorithm is used to put together hubs into productive groups in remote sensor networks works with information accumulation and order dispersal. In any case, grouping is a perplexing and expensive interaction, since it must be done in a dispersed and occasional way. In this paper we propose a basic grouping system utilizing a nearness lattice which encodes hubs' area and availability in an organization. Our methodology empowers the task of bunch sets out toward numerous rounds in a solitary advance consequently restricting the expense of group head political decision and kid hub affiliation.

3. Ahmed Nader al-Dulaimy, and Hannes Frey (2019), et.al proposed Subnet Addressing in Software Defined Wireless Sensor Networks. Software defined networking provides manageability and scalability to networks by decoupling network control and forwarding functions into separate layers. Though the concept is typically a subject for wired networks, recent research activities have also considered software defined networking in the context of wireless networking and especially in wireless sensor networking. Software defined networking in that context is, however, a very challenging task due to limited resources of the sensors. In particular, the number of entries in the flow table and the control message overhead is a limiting factor. This paper introduces a new approach to efficiently manage a software defined wireless sensor networks by means of a hierarchical addressing scheme. It is based on a tree of address masks which are used to split the network into subnets. Each subnet has a range of host addresses and a subnet head which acts as a gateway to its children. With that hierarchical structure, nodes need only to recognize the subnet where a packet is destined to and forward that packet to the subnet head. We evaluated the proposed approach using the Cooja simulation environment of Contiki. Compared to the state of the art our scheme reduces the amount of control messages, the size of path packets, the number of rules needed to be stored in the flow table and hence the processing time for these rules.

4. Babatunde S. Awoyemi, S. Alfa, and Bodhaswar T. Maharaj (2019), et.al proposed Network Restoration in Wireless Sensor Networks for Next-Generation Applications. Profoundly proficient organization reclamation models for remote sensor organizations (WSNs) to be conveyed for future (xG) applications. The created network rebuilding models are planned in view of two principle objectives. The principal objective is to enhance network asset use, and the second is to ensure the organization against disappointments. In understanding the objective of advancing asset utilization, a particular component of WSNs is misused, specifically, their capacity to stay in dynamic assistance in any event, when at least one of their dynamic components (sensor hubs and additionally associating joins) come up short. To accomplish the second objective of organization insurance, we influence the benefit of p-cycle-based rebuilding arrangements - the way that they can furnish ring-like recuperation speeds with network like limit efficiencies - in creating ideal p-

cycle reclamation models that give adequate security to the organization against both connection and hub disappointments. In the reclamation models created, we utilize a choice interaction that together thinks about the briefest lengths, best geographies, and limit necessities of the accessible p-cycles in accomplishing new limit ideal p-cycle-based rebuilding answers for the organization. Near outcomes acquired show that our created choice based limit effective p-cycle rebuilding answers for WSNs beat other comparable methodologies for both organization acknowledgment and assurance, making them especially ideal for xG applications.

5. Nazli Siasi, Adel Aldalbahi, Mohammed A. Jasim (2019), et.al proposed Reliable Transmission Scheme against Security Attacks in Wireless Sensor Networks. Routing protocols in wireless sensor network are vulnerable to various malicious security attacks that can degrade network performance and lifetime. This becomes more important in cluster routing protocols that is composed of multiple node and cluster head, such as low energy adaptive clustering hierarchy (LEACH) protocol. Namely, if an attack succeeds in failing the cluster head, then the entire set of nodes fail. Therefore, it is necessary to develop robust recovery schemes to overcome security attacks and recover packets at short times. Hence this paper proposes a detection and recovery scheme for selective forwarding attacks in wireless sensor networks using LEACH protocol. In general, wireless sensor nodes transmit and receive packets to a centralized base station via a wireless channel. The nodes here often operate with multiple limitations and constraints, in particular limited battery lifetime and hence requirement for low power consumption levels, i.e., resource-constraint nodes. In addition, the wireless channel is also vulnerable to various malicious attacks in the environment, such as selective forwarding and Sinkhole attacks. Hence sensor nodes can suffer from packet loss and also yield network failure when such attacks are present, i.e., yields nodes to selectively drop packets, which also reduces transmission reliability and security. Also, the multicast transmission nature in wireless sensor networks (WSN) further increases susceptibility to security attacks.

6. Runfa Zhou and Roger S. Cheng (2019) proposed Optimal charge planning for energy-compelled wireless-powered network. As an applicant power supply answer for the Internet of Things, radio frequency (RF) energy gathering has pulled in extraordinary consideration as of late. In this paper, they consider an RF wireless-powered network, which comprises of a committed power beacon (PB) and numerous client hubs. The PB is accepted to have constrained energy and moves its power to client hubs wirelessly. The client hubs work just dependent on energy reaped from the PB, and are thought to be in either energy collecting mode, energy utilization mode, or inactive mode. To amplify all out collected energy, they organize the practices of the PB and client hub. They consider a static wireless-powered network whichcomprises of a devoted power beacon (PB) and different wireless powered client hubs. For this energy-compelled wireless powered network, they proposed an ideal charge planning to expand the all-out gathered energy of all client hubs.

7. Craig B. Schindler, Daniel S. Drew, Brian G. Kilberg, Felipe M. R. Campos, Soichiro Yanase, Kristofer S. J. Pister (2019) proposed a bit equipped for 9-hub inertial measurement and low power wireless work networking with the littlest structure factor conceivable; the Micro Inertial Measurement System (MIMSY). Our exhibited stage is planned as a broadly useful wireless sensor bit with a structure factor and value point that makes it manageable to huge scale arrangements over an assortment of segments. The system is completely perfect with the Open WSN wireless sensor networking stack, which empowers the direct usage of principles consistent with 6TiSCH work networks utilizing MIMSY bits. While

the application space of MIMSY is very huge, they present three example usage displaying the open doors managed by a little and moderately ease bit with work networking and inertial measurement abilities. Bits consequently joined the wireless work network once turned on, wiping out the requirement for manual blending and availability support.

8. Basma Mostafa (2019) proposed Binary Integer Programming problem definition. One of the fundamental difficulties for network checking is figuring out where to install (place) the observing hubs. These components ought to have the likelihood for effectively/inactively running observing tests or potentially breaking down the checking results. The tests' arrangement must be upgraded so as to limit the energy cost and observing burden. Additionally, the observing computational cost, battery and memory necessities ought to be insignificant so as to fulfill the ease and energy requirements of IoT devices. Network checking models show that the related advancement is often NP-hard. The authors start by building up a model that focuses on the ideal position of screens while guaranteeing network coverage and computational tractability. The proposed models should work couple with RPL, the graph they use is the Destination Oriented Directed Acyclic Graph (DODAG) built by RPL. The ideal screen position is discovering the base number of observing hubs set on the graph to monitor every one of the connections in the network. The problem can be demonstrated as the great Vertex Cover Problem (VCP). VCP is NP-hard for general graphs. Then again, it is polynomial when comprehended on trees and Fixed-Parameter Tractable (FPT) when illuminated on "tree-like" graphs, likewise called pleasant tree disintegrations, and the jumping parameter here is the treewidth. Considering this information, they proposed calculations, that convert the DODAG speaking to the network topology into decent tree disintegration with solidarity treewidth.

9. Lina Xu and Nuno Pombo (2019) proposed a way to deal with use the sensor organization design and the high privacy saw detecting data to anticipate human conduct. As the supporting advancements for Ambient Assistant Living (AAL) in the Internet of Things (IoT) space have become all the more powerful and increasingly appealing, the related systems will be broadly conveyed and placed without hesitation. With all related implanted IoT detecting devices, how to keep up clients' privacy and data security is an exceptionally concerned issue. There are commonly two ways to deal with secure privacy. One is to actualize complex security protocols to ensure the safety of detecting, storage and data transmission. Another is to forestall the privacy issues and worries from the source. This proposed research will give an idea of a structure that can bolster conduct checking through noninvasive and privacy-protecting detecting. The data gathered, transmitted and utilized for breaking down in this system is detecting information with low extravagance. This structure expects to expand the clients'

10. Paul R. Berger, Miao Li, Ryan M. Mattei, Maimouna A. Niang, Noah Talisa, Michael Tripepi, Brandon Harris, Sagar R. Bhalerao, Enam A. Chowdhury, Charles H. Winter and Donald Lupo (2019) proposed progressions in arrangement process able devices utilizing metal oxides for printed internet-of-things objects. Putting a room light or an indoor regulator on the internet for remote control is viewed as dynamic. In any case, whenever printed gadgets can accomplish execution expands, at that point, IoT articles could be affixed to nearly anything, for example, coffee half and half containers, grain boxes, or that missing sock. Every one of these IoT articles could be driving a sensor, maybe position, temperature or weight, basically a huge number of applications. All together for IoT items to imitate a straightforward postage stamp, with self-powering from energy rummaging and

neighborhood energy storage, all housed in a non-harmful adaptable structure factor.

11. Roshmi Sarmah, Manasjyoti Bhuyan and Monowar H. Bhuyan (2019) proposed SURE-H: A Secure IoT Enabled Smart Home System. The proposed system that empowers to shield homes from robbery or bizarre exercises and parallelly spares power. Our system is created by abusing the highlights of IoT that encourages us to screen an IoT empowered home from anyplace anytime over the Internet when data are put away in the cloud. This system utilizes a movement locator to distinguish a moving article from the environment where the system is sent. The proposed system is assessed utilizing continuous arrangement at KU grounds thinking about 30 spaces for 60 days. It utilizes an Android application that gives exchanging functionalities, where the electrical or electronic devices are observed and controlled remotely. This system includes advantage by taking out the utilization of conventional personal computers (PC) and it is fringe devices during execution. The SURE-H system works dependent on the put-away cloud server data. They store the subtleties of home apparatuses for each room into the server. At first, it sends a solicitation to the server and hangs tight for the endorsement. When it watches any moving object it sends an alert with a detail report against the occurrence. This alert will trigger just when the new article watches.

12. Jernej Hribar, and Luiz DaSilva (2019) proposed a refreshing system fit for gaining from the substance of information gathered to diminish the frequency with which devices transmit their updates, hence improving their energy efficiency. Billions of low-power devices gathering information will be sent in the Internet of Things (IoT) networks. By exploiting the connection displayed in information gathered, it is conceivable to improve sensors' energy efficiency. The proposed instrument gains from the substance of information gathered to improve the energy efficiency of low-power devices, subsequently making IoT organizations increasingly feasible, both financially and environmentally. In our work, the timeliness of information, i.e., the time slipped by since the last transmitted update, has a critical job in the basic leadership process. Evaluate the timeliness of information utilizing the idea of the Age of Information (AoI). Also, they show that depending on information from one connected device can expand the exactness of gathered information on another device.

13. John Fox, Dr. Andrew Donnellan and Liam Doumen (2019), et.al proposed the planned engineering and strategy of a completely working LoRaWan based IoT system. Such a system can be given as a support of a given nearby area, by using an End Device related to a LoRa handset, a LoRaWan Gateway, and a characterized cloud stage. From a system point of view, a total IoT arrangement can be isolated into three classifications, the data gatherer, the communication strategy and the cloud stage administration. The data authority identifies with the inserted system device (or the 'things' component) at the wellspring of the application, the communication strategy identifies with the network protocol used to send or get the data and the cloud stage administration identifies with the office used to store and process the data gathered. LoRaWan and LoRa are 'Long Range' advances, which characterize the communication strategy for such IoT applied systems. LoRa characterizes the tweak technique, that takes into consideration long-range communication, while LoRaWan characterizes the communication and system engineering. The displayed IoT system currently serves the locale of Tallaght (Dublin, Ireland) and its more extensive region.

14. Mustafa A. Al Sibahee, Songfeng LU (2017), et.al proposed The Best Performance Evaluation of Encryption Algorithms to Reduce Power Consumption in WSN. Wireless Sensor Networks (WSNs), applications are growing rapidly, so the needs to protect such applications are increased.

Cryptography plays a main role in information system security where encryption algorithm is the essential component of the security. On the other side, those algorithms consume a significant amount of computing resources such as CPU time, memory, and battery power. This phase provides evaluation of four of the most common encryption algorithms namely: RC4, DES, and AES as a symmetric cipher and RSA for asymmetric cipher. A comparison has been conducted for those encryption algorithms at different settings such as different sizes of data blocks, different key size and finally encryption/decryption speed. Simulation results are given to demonstrate the effectiveness of each algorithm on power consuming. Wireless sensor networks (WSN) are used to monitor environmental and physical changes by means of sensor nodes. Which is becoming a popular ubiquitous computing. They are used in different applications such as health care monitoring, environmental/earth sensing, air pollution monitoring, forest fire detection, industrial monitoring and many more. Since there are only limited resources, WSNs are exposed to many vulnerable attacks such as false message injection, eavesdropping etc., hence more security measures are needed. In recent times many techniques such as random key pre-distribution and random pairwise key distribution has been used. The security in WSN has been enhanced by using a symmetric key encryption technique. The pros and cons of the issues related to WSN have been put forth discussed, compared and evaluated in this research. Cryptographic is a set of algorithms operate in a way to encrypt and decrypt data. Encryption is transform plaintext to cipher text to serve security purposes.

15. Yildirim, G., & Tatar, Y. (2018), et.al proposed Simplified Agent-Based Resource Sharing Approach for WSN-WSN Interaction in IoT/CPS Projects. This phase focuses on the problem of interoperability and resource sharing in wireless sensor networks (WSNs) running under the Internet of Things (IoT) and cyber-physical systems (CPSs). Considering the scale of IoT/CPS projects, conventional WSN virtualization techniques remain incapable because of the hardware/software constraints and heterogeneity. To this end, in this paper, an agent-based server system approach, which improves the resource sharing between heterogeneous WSNs in IoT/ CPS providers, is proposed. In line with this approach, a software agent framework is introduced. With the help of the framework, called Firat Virtual WSN framework (FVWSN), the clients can move the commands/ queries and data fusion/ aggregation algorithms, which use on their local networks, to the provider side and run them remotely or automatically. This process is carried out by logical agent entities, called virtual nodes, which are created with the help of FVWSN. In this way, since the client evaluation mechanism is performed at a closer point to the shared resources, a shorter response time can be achieved in time-critical applications. The most important features that differentiate the developed agent framework from other agent-based technologies are that it is semi-autonomous and uses a specific resource selection/allocation algorithm. With the improvement that FVWSN provides for IoT/CPS WSN providers, it is possible to achieve a shorter response time and allow more client applications to share the same limited WSN resources. In this paper, first, the analysis and the necessity of the proposed system are discussed. Then, the system is simulated in the OPNET Modeler platform to make comparisons with well-known conventional WSN resource sharing mechanisms. Finally, the physical comparison tests of the system are carried out on an OpenStack-based cloud system, and the success of the system is shown.

3. PROPOSED METHODS, MERITS AND DEMERITS

Author Name& year	Proposed Method	Merits	Demerits
Cong Gao, Zhongmin Wang and Yanping Chen (2019),	On the connectivity of highly dynamic wireless sensor networks in smart factory	1. Straightforward and reduced network model.	1. Connectivity scheme for routing cannot be done.
Waltenegus Dargie (2020)	A Clustering Strategy for Wireless Sensor Networks	1. Simple clustering Algorithm has been used.	1.Low scalability
Ahmed Nader al-Dulaimy, and Hannes Frey (2019)	Subnet Addressing in Software Defined Wireless Sensor Networks	1. Compact ability of the system	1. Scalability is less.
Babatunde S. Awoyemi, S. Alfa, and Bodhaswar T. Maharaj(2019)	Network Restoration in Wireless Sensor Networks for Next-Generation Applications	1.The exceptional component of WSNs, which is its capacity to stay in dynamic assistance regardless of whether at least one of its components have fizzled.	1. Span a wide territory, implying that there are numerous sensor hubs that could fill in as a passage to the organization for a vindictive aggressor.
Nazli Siasi, Adel Aldalbahi, Mohammed A. Jasim(2019)	Reliable Transmission Scheme against Security Attacks in Wireless Sensor Networks	1. Vulnerability to attacks is less	1. Cost for the set up is high.
6.Runfa Zhou and Roger S. Cheng (2019)	Optimal charge planning for energy-compelled wireless-powered network	1. Optimal charge planning that can accomplish the system greatest energy gathering efficiency. 2. The complete collected energy is high.	1. It has a bigger bit of inertial measurement and low power wireless work networking.
Craig B. Schindler, Daniel S. Drew, Brian G. Kilberg, Felipe M. R. Campos, Soichiro Yanase, Kristofer S. J. Pister (2019)	A bit equipped for 9-hub inertial measurement and low power wireless work networking with the littlest structure factor conceivable; the Micro Inertial Measurement	High unwavering quality, low inertness communication for modern procedure computerization and control; Long lifetime physical occasion location and movement observing with negligible	1. It has a bigger bit of inertial measurement and low power wireless work networking.

	System (MIMSY)	arrangement time.	
Basma Mostafa (2019)	Binary Integer Programming problem definition	The BIP detailing was compelling in limiting energy utilization. The streamlining guaranteed full network coverage and negligible energy utilization. 3.The lingering battery never fell beneath 74% in all occasions	It very well may be time expending for huge measured or thick networks.
Jernej Hribar, and Luiz DaSilva (2019)	A refreshing system fit for gaining from the substance of information gathered to diminish the frequency	The proposed strategy improving energy efficiency by decreasing the frequency with which the detecting devices transmit their updates. The proposed component exploits connection, removed from information gathered, to improve the energy efficiency without bringing down the exactness of accessible information.	1. The refreshing component should adjust to consistently changing energy levels on devices.
Lina Xu and Nuno Pombo (2019)	A way to deal with use the sensor organization design and the high privacy saw detecting data to anticipate human conduct	It will stay away from data over-assortment and over introduction issues. Sensing data with uninformed wealth to ensure high apparent privacy.	1. Constrained information can be unique from the data.
Paul R. Berger, Miao Li, Ryan M. Mattei, Maimouna A. Niang, Noah Talisa, Michael Tripepi, Brandon Harris, Sagar R. Bhalerao, Enam A. Chowdhury, Charles H. Winter and	Progressions in arrangement process able devices utilizing metal oxides for printed internet-of-things objects	It watches varieties in the optical dielectric capacities and basic properties of the lighted oxide tests. Metal-oxide-based dynamic devices that are incorporated by arrangement handling and low-temperature	1.All housed in a non-harmful adaptable structure factor progresses in arrangement process able devices need to happen.

Donald Lupo (2019)		preparing to understand the up-and-comer discrete devices that will go into a total printed IoT system.	
Roshmi Sarmah, Manasjyoti Bhuyan and Monowar H. Bhuyan (2019)	SURE-H: A Secure IoT Enabled Smart Home System.	IoT-empowered smart home system that expands safeness from robbery and parallelly spares gigantic power cost. Automated switches for every home machine It can be proficient to recognize moving object and produce a secret phrase by joining a client secret phrases and fingerprints. It has ease, least time, exceptionally adaptable, oppose against man-in-the-center and online word reference assaults, and needs least infrastructures.	1.SURE-H can't support the huge scale environment, for example, offices and organizations.
John Fox, Dr. Andrew Donnellan and Liam Doumen (2019),	The planned engineering and strategy of a completely working LoRaWan based IoT system	As assistance, the system has been demonstrated to be fit for supporting a wide scope of IoT based applications. The same execution can be applied to different application necessities of the district.	1. Constraints of the End Device, for example, battery life span.
Mustafa A. Al Sibahee, Songfeng LU(2017)	The Best Performance Evaluation of Encryption Algorithms to Reduce Power Consumption in	1. It is scalable and hence can accommodate any new nodes or devices at any time.	1. It is expensive to build such network and hence can not be affordable by all.

	WSN		
Yildirim, G., & Tatar, Y. (2018)	Simplified Agent-Based Resource Sharing Approach for WSN-WSN Interaction in IoT/CPS Projects	1. Protection against data from theft and Protects the computer from being hacked.	1. The way it distracts can deviate our thoughts and activities towards unproductive activities.

CONCLUSION

In this work, various machine learning techniques which detect outliers WSN have been described. In WSN frameworks, sensors form the crux of generating raw data and are also responsible for detecting environmental changes. Thus, detecting outliers is much needed to analyze error free data generated from sensors. Some works have also been tabulated which are useful in detecting various kinds of outliers in sensor data. It can be easily concluded from the discussion that classification methods are the most extensively used learning methods for detecting outliers in WSN. The existing shortcomings in both WSN require developing more suitable outlier detection techniques for both univariate and multivariate data. Also, while developing new machine learning methods mobility of node and network topology change should also be seriously considered. To make asmall scale usage the examination has been actualized in NS2 using the for the most part accessible conventions.

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How to Cite:
Vijayalakshmi, P. S., & Kumar, M. J. (2022). An improved grey wolf optimization algorithm (iGWO) for the detection of diabetic retinopathy using convnets and region based segmentation techniques. *International Journal of Health Sciences*, *6*(S1), 13100–13118.
https://doi.org/10.53730/ijhs.v6nS1.8330

An improved grey wolf optimization algorithm (iGWO) for the detection of diabetic retinopathy using convnets and region based segmentation techniques

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***Abstract*---**Diabetic Retinopathy is a retinal eye disease that affects people with Diabetes Mellitus. DM is metabolic disorder and it is caused by the high glucose level in blood. This leads to eye deficiency called Diabetic Retinopathy (DR). In this paper, we proposed iGWO algorithm for the detection and diagnosis of DR stages and Region growing technique is used for segmentation of the images. Various preprocessing techniques are followed for the better enhancement of the images. An improved Grey Wolf Optimization (iGWO) algorithm are proposed to obtain the global optimum. Convnets are utilized for DR categorization. The iGWO-FFOCNN model is compared with existing technologies like SVM-GSO (Support Vector Machines – Glowworm Swam Optimization), PSO-CNN (Particle Swarm Optimization-Convolutional Neural Networks), CNN (Convolutional Neural Networks), DCNN-EMFO (Deep Convolutional Neural Networks-Enhanced Moth Flame Optimization) and MACO-CNN (Modified Ant Colony Optimization–CNN). The APTOS DR Dataset utilized for the proposed metodology. The effort is made for identifying the DR stages by using iGWO. Finally, the results confirm that iGWO-FFOCNN technique yields good performance than compared to the existing techniques in relation to F-measure, precision, recall and also in DSC (Dice Similarity Coefficient), JSC (Jaccard Similarity Coefficient) and time period.

***Keywords*---**Gray wolf optimization, improved gray wolf optimization algorithm, Convnets, Region growing, segmentation, enhanced crow search algorithm.

Introduction

Diabetic retinopathy (DR) is among the most common reasons for blindness in people of age of employment. It is one of the most recognized dreaded diabetic challenges. The primary difficulty with DR is that it is incurable once it has progressed to an advanced degree, thus early detection is critical. Many variables contribute to DR, including oxidative damage, enzymatic activation, cell osmoticlysis, rise in toxins, stimulation of Hormones, various growth hormones, and carbonic metabolites. Pods, optic neuritis, flashes and abrupt vision impairment are all frequent symptoms of DR [1]. Nonvascular pathologies, such as hypertension, diabetes, and cardiovascular disorders, are also affected [2]. All of these methods injure retinal cells, causing micro aneurysms, cotton wool patches, hemorrhages, and a variety of other abnormalities. This results in morphological alterations in the vascular system, just like changes in dimension, height, and highly branched ratios. As a result, veins of blood supply essential points that may be utilized to diagnose and evaluate circulatory and ophthalmic illness. In most other occurrences, newly formed blood vessels begin to form as in macula, resulting in depreciation of eyesight [3].

Macular Edema, Hard Exudates, Micro aneurysms, Hemorrhages, Cotton Wool Spots and Neovascularization are all symptoms of DR. DR is divided into five categories based on these five clinical characteristics. According to Wilkinson et al. [4], there are five grades of DR: Level 0 is healthy with no indications of DR, level 1 is light DR, level 2 is medium, level 3 is acute, and level 4 is described by new artery growth and vision loss concerns such as bleeding into the ocular vascular separation. Micro aneurysms arise as a result of revascularization or aberrant permeability of blood vessels in the retina are the early stages of retinal injury. Micro aneurysms are little blood vessels that are smaller than 125 microns in diameter and appear in the fundus imaging as a crimson patch with sharp borders. The leaking of lipids and other enzymes via dysfunctional arteries of blood causes hard exudates to accumulate within the retina's outer surface. White or pale yellow little patches sharpness borders appear [5]. Cotton Wool Spots [6] are an Ocular Nerve Fiber Layer has a foamy white lesion caused by debris deposition and are caused by an arteriole occlusion. Hemorrhages are red patches with an uneven edge caused by weak capillaries leaking blood, and they are larger than 125 microns. Neovascularization is the unusual development capillaries of blood in the retina's innermost layer that leak into the hollow translucent, producing impaired sight [7].

Deep Learning (DL) solutions for effective DR detection and categorization have become possible because to recent breakthroughs in Artificial Intelligence (AI) and increased computer resources and capabilities. GPUs have sparked interest in strategies for deep learning, that have demonstrated remarkable achievement in a variety of visual technology applications and even have decisively defeated classical methods based on manual. Numerous deep-learning (DL) algorithms

have been developed for diverse tasks have furthermore been created to assess retinal fundus pictures in order to build automatic computer-aided diagnosis systems for DR.

The paper is ordered as the following sections: literature review in the Section 2, proposed methodology shows in the section 3 and it describes the preprocessing methods, segmentation of lesions, classification and feature extraction. The experimental outcomes are discussed in section four. Lastly, in section 5, conclusions are provided.

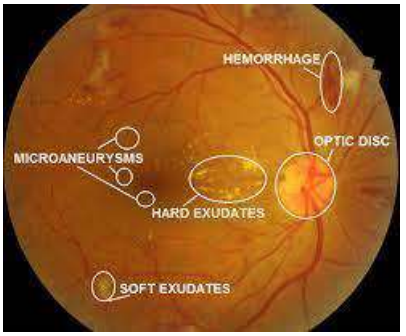

Figure 1: Retinal fundus image

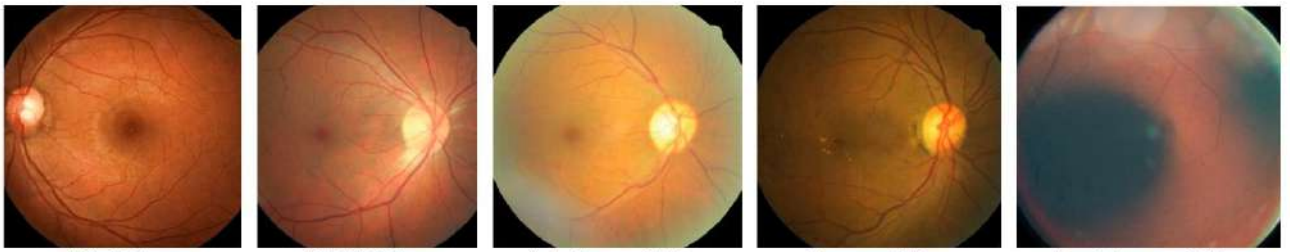

Figure 2: Stages of DR

Review of Literature

Various studies have been conducted on identifying and categorizing diabetic retinopathy, however, there are always improvements to be made in terms of accuracy and application to other types of datasets. Antal and Hajdu [8] developed a DR-levels and diagnosing abnormalities soit allows for numerous feature extraction and pre-processing approaches. Highlighted, intensity improvement, localized distribution, vessel elimination is some of the pre-processing techniques. Diameter closure techniques, tophat transformation algorithm, Inter reflect, circle Hough transformation technique, and multiple Gaussian filters fitting are among the feature extraction methods. Finally, three classifiers are tested for lesion detection: Random forests, k-nearest neighbor and SVM.

A TensorFlow framework was used to construct a smartphone application for real-time diabetic retinopathy detection. The CNN model was Mobile Nets, which include 28 convolutional layers and are tailored for mobile devices. The result is a

label, such as diabetic retinopathy or no diabetic retinopathy. This concept was created with mobile devices in mind [9]. Vimala et al. presented a technique for noticing maculopathy disease in retinal pictures using morphological procedures. Contrast Limited Adaptive Histogram Equalization improves the quality of retinal pictures (CLAHE). Morphological processes are used on a regular basis to shape structures. To aid in the discovery of the illness, a Support Vector Machine (SVM) is used in the categorization process [10].

Karthikeyan et al. suggested a novel algorithm-based technique that combined association rule data mining with an upgraded FPG growth algorithm that is comparable with ACO. CACO, or Continuous Ant Colony Optimization for exudate segmentation, is the algorithm employed in this approach [11]. Naluguru et al. [12] offer EC approaches for segmentation. In automated DR, it is a form of feature extraction approach that includes blood vessel segmentation. They are extracting the blood vessels, texture, optic disc, and entropies from the retina using GA with SVM and Bacterial Foraging Algorithm (BFA). It starts with segmentation and then extracts features from pictures using bifurcation points, texture, and entropy before moving on to statistical feature extraction. After extracting statistical features, the authors employ GA and BFO with a neural network to categorize the pictures into three categories: normal, NPDR, and PDR. They then determine the best classifier for retinal lesions and grade them as mild, moderate, or severe.

Bajeta et al. [13] built a model for blood vessel segmentation in which they used ACO in fundus pictures and had ACO extract features. To extract characteristics from retinal pictures. Mateen et al. showed asymmetrically optimized result employing a Gaussian mixture model (GMM), visual geometry group network (VGGNet), singular value decomposition (SVD), principal component analysis (PCA), and softmax for region segmentation, high dimensional feature extraction, feature selection, and fundus image classification. According to the authors, the VGG-19 model outperformed AlexNet and the spatial invariant feature transform in terms of classification accuracy and processing time (SIFT) [14].

Gu et al. proposed a retinal image segmentation method. For the segmentation of medical pictures, deep learning has been used. For retinal pictures, a context encoder network performs this segmentation approach. The basic components of the context encoder network are a feature encoder, a context extractor, and a feature decoder module are all included. As a fixed feature extractor, a pre-trained ResNet is used [15].

Deep learning with transfer learning models for medical diagnosis of DR were researched by Khalifa et al.[16] The numerical experiments were carried out using the 2019 dataset from the Asia Pacific Tele-Ophthalmology Society (APTOS). GoogleNet ,Res-Net18, SqueezeNet, VGG16, AlexNet and VGG19 were the models used in this study. These models were chosen because they have a smaller number of layers than bigger models like DenseNet and InceptionResNet. A method called computer-assisted retinal vascular segmentation is used to diagnose eye disorders. They told using an establishment inter artificial bee colony algorithm, an unsupervised retinal vessel identification approach was developed. (EMOABC) [17]. The curve is utilized in this energy to discover a set of

characteristics worth considering choose thresholds this will divide the vessels. while also reducing noise. The EMOABC method is basic and straightforward compared to various approaches.

According to Shankar et al. [18], using the feature extraction with Inception-ResNet v2 model and the deep learning neural network with moth search optimization (DNN-MSO) method, a successful DR classification approach has been provided. The Messidor dataset was used to validate the provided model, and the findings showed that it produced better outcomes. For diabetic retinopathy classification, Glowworm Swarm Optimization and utilized with Support Vector Machine with [19] was employed in conjunction with a Genetic Algorithm. The classifier performance of the Support Vector Machine is controlled by the parameters C and gamma. The Support Vector Machine, in combination with the GSO-GA with chromosomes, will also steer the GA's search in orbit. This GSO has no memories, and glow worms do not have any information stored in their memories. This strategy has been shown to enhance detection accuracy. SVM combined with hybrid GSO is employed to classify DR in this case. To update the location depending on the population, a hybrid GSO is employed, and that will give a superior result. Heterogeneous GSO employs a local GA search technique to find optimal values.

Proposed Methodology

The following phases make up the newly introduced work. Preprocessing comes initially, followed by localization, segmentation, classification, and prediction. Global minimum is obtained by using iGWO. CNN's Softmax Classifier performs the classification. The outcomes of these work approaches are the most effective procedure.

Selecting Dataset

The model will be trained by using the APTOS (Asia Pacific Tele-Ophthalmology Society) 2021 Blindness Detection (APTOS2019 BD) dataset [20]. The dataset includes 3662 Images of the fundus taken from a range of patients in rural India. Images are available in a range of sizes and from one of five categories: Absent DR (level 0), Slight (level 1), Medium (level 2), Extreme (level 3), and Cell proliferation DR (level 4).

Preprocessing techniques

Images of the retinal fundus in various sizes and aspect ratios are included in the dataset. Before training with Deep Convolutional Neural Networks, we performed a number of preprocessing processes on the original images in our dataset. Preprocessing is a technique for removing unwanted noises and improving image quality. There are a few visual options for image processing, as well as a variety of pre-processing approaches.

Rescaling, also known as resampling, is a process for creating a new version of an image with a certain size. Upsampling is the process of increasing the image's dimensions, whereas downsampling is the process of decreasing the image's

dimensions. Every clipped picture was then scaled to a standard input image size of 256x256 pixels.

- **Min-MaxNormalization**

To avoid the convolutional neural network from learning the image's underlying ground noise, each image was modified using Min-Max normalization. Image normalization is a typical image processing technique that changes the intensity range of pixels. Min-Max normalization is used to scale the image values between 0 and 1. A picture's pixel intensities will be scaled within a range 0 and 1:

$X'=x-min(x)/max(x)-min(x)$ _____ (1)

where x is the initial value and x0 the new value standardized.

- **Image Resolution Enhancement**

Imaging filters are used to intensify the retinal characteristics of blood vessels, optic disc, MA, HM, and exudates in the retinal fundus image. In clinical applications, medical images play an essential role. The clarity of visuals for human seeing is improved via image enhancement. Enhancement procedures include removing blur and noise from a picture, as well as increasing contrast and revealing features in the image.

- **Contrast Limited Adaptive Histogram Equalization (CLAHE)**

CLAHE works on tiles, which are tiny areas of a picture rather than the complete image. This approach may be used to boost the image's local contrast. HSV conversion of a BGR (blue, green, and red channel) images (Hue, Saturation, and Value channel). This allows us to solely use the value channel for the CLAHE algorithm. CLAHE is based on the following variables:
ClipLimit is a number in the range [0, 1] that determines the contrast enhancement limit.
NBins is a 256 positive integer value that represents the number of histogram bins required to construct a contrast enhancement transformation.

- **Median filter**

The algorithmic program's workings are as follows: median filtering determines the output value of each pixel, the pixel values are taken inside a range of spatial windows with a minimum size of 3x3, and the current values are sorted in ascending order [21]. Mathematically, things went like this:
$G(x,y) = med \{ fmg (x-m,y-n) , (m,n) € ω \}$_____(2)
where g (x, y) is an image created from the image of fmg (x, y), with w as the window in the image field and (m, n) as the window element.

- **Gaussian Filter**

The final picture is Hue Saturation Density was transformed from RGB color space then histogram equalization filtering by median. Gaussian noise is reduced from retinal pictures simply rearranging the result with distribution equalization with a Gaussian kernel for improved feature extraction. The Gaussian kernel is defined as,

$$G(x,y) = \frac{1}{2\pi\sigma 2} \; e\frac{-a^2+b^2}{a2} \quad \text{(3)}$$

The variation of the picture pixel values σ is shown here. The value of is selected adaptively using the procedure below.

Algorithm 1: An algorithm is proposed for evaluating adaptively based on image intensity.

Input: fundus Image of Retina R-FIMG Result: Standard deviation σ
Step 1) Transformation of RF_IMG into HE image H-IMG
Step 2) H-IMG to HIS and is to convert RGB color space.
Step 3) RGBcolor into HSBcolor space
Step 4) Compute the mean of value of HIS-I
Step 5) Computer σ as σ = $\sqrt{mean\ /HIS-I}$

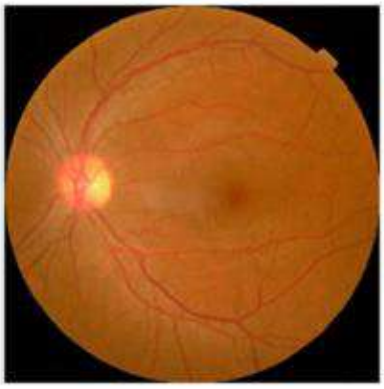

Figure 3: Input image

Figure 4: Grey scale image

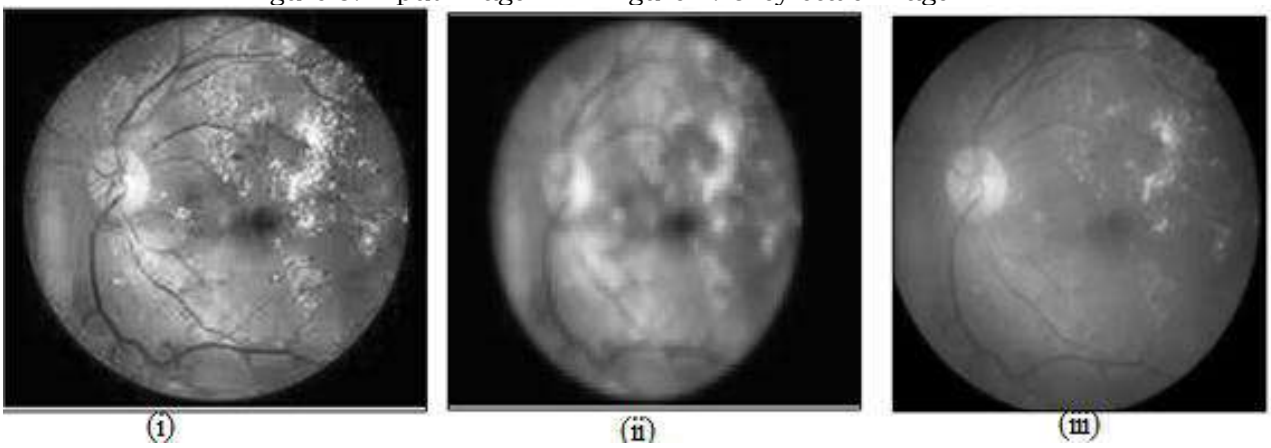

Figure 5: Preprocessed image i) CLAHE image ii) Gaussian filter iii) Median filter

Segmentation of the Image

The technique of segmenting a retinal fundus image into meaningful regions with homogenous attributes is known as image segmentation. Identifying the

lesions with the appearance of Fundus ocular images on the optical disc and blood vessels looks to really be a a difficult issue. As a result, the problem is to locate and remove the and even a circle optics discs to detach the blood capillaries that follow. The following steps are involved in image segmentation for this proposed system. i) Optic disc finding, (ii) Optic disc removal, iii) Blood vessel extraction, (iv) microanurysms, Hemorrhages, and Exudate Segmentation.

Optic disc detection and Elimination

Considering one of the aims of the proposed system is to identify the lesion, it's essential to remove the optic disc before the feature extraction operation can begin. In contrasted to the rest of the fundus picture, the optic disc has consistent intensity levels, uniform contrast, and uniform color. When viewing a retinal image in colorspace, the circle optical disc appears white or pale yellowish in color. The optic disc is distinguished as a large circular area with great contrast. Entropy [22] was determined mathematically for a high intensity fundus picture for oculat disc localization as in the

$$H(M) = -\sum_{h \in IP(M)} \boldsymbol{P_h}.log_2(P_h) \quad (4)$$

Along with all M pixels in a patched frame IP(M), P_h refers to the quantity of units in a distribution I the patchframe and h∈IP(M).
The actions of morpo-dilation and morpo-erosion are essential in morphological image processing methids. Dilation increases the amount of pixels on the boundaries of objects in a picture, whereas erosion reduces the number of pixels on the edges of objects. The image is processed in this study using a mix of dilate-erode procedures.
The dilation of P by Q denoted P ⊕Q is denoted as,

$$P \oplus Q = \{ R \mid (\hat{Q}) R \cap P \neq \phi \} \quad (5)$$

where Q seems to be the structuring element and ϕ represents the empty set. In these other aspects, dilatation P by Q is the set of elements the origin positions of all the structural components.
Erosion, on the other hand, diminishes boundaries and expands the size of holes. If any of the structural component does not entirely ON weighted pixels merge, it will set ON pixel to OFF [22]. P is being eroded by Q denoted P ⊖ Q, is described this way:

$$P \ominus Q = \{ R \mid (\widehat{Q}) R \subseteq P \} \quad (6)$$

Blood vessels extraction

For the extraction of objects of interest, the matching filters (MF) technique convolves the picture with numerous matched filters. Thus, constructing multiple filters to identify vessels with varying orientations and sizes plays a key role in obtaining vessel outlines. The computing burden is affected by the size of the convolution kernel. Following the MF, various image processing processes such as thresholding are frequently performed to get the final vessel outlines. A thinning technique is performed first to find vessel centerlines.

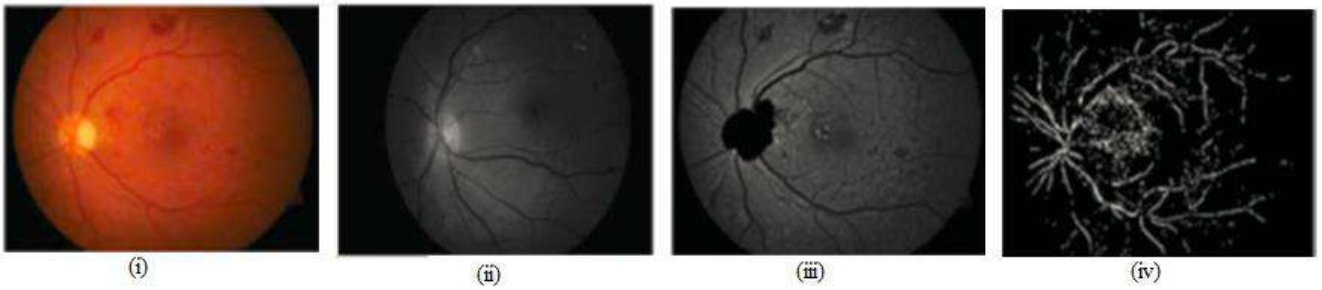

Figure 6:i) Fundus image ii) Green Channeled imaged iii) Optic disc removal iv) Blood vessel extraction

RBT for Segmentation of Lesions in DR

The Lesions of DR are Microanurysms, Hemorrhages, and Exudates are segmented by using Region Based Techniques (RBT) called Region growing. Region growing [23] is a method for extracting a linked region of an image using predetermined criteria. This criterion is based on information about intensity. A method of picture segmentation called region expanding examines nearby pixels and adds them to a section class with no boundaries discovered. This method is repeated for each pixel in the region's boundaries. If nearby areas are discovered, a region-merging algorithm is applied, wherein the weakest edges are dissolved and robust edges are preserved. This studies indicate a new region-growing depending on technique the vector angle color similarity measure. The method for expanding regions is as follows:

Step 1: Pick a few seed pixels from the sample.
Step 2: Create a region from each seed pixel.
Step 3: Change the seed pixel for the area prototype.
Step 4: Determine how similar the regional design and the selected pixel are.
Step 5: Determine the degree of similarity between the applicant and its closest regional neighbor;
Step 6: If both similarity measurements are greater than the experiment's predefined criteria, include the candidate pixel.
Step 7: Calculate the new principal constituent for the region prototype;
Step 8: Move onto another pixel to be evaluated.When there are no more pixels that meet the criteria for inclusion in that region, the region will be stopped expanding.

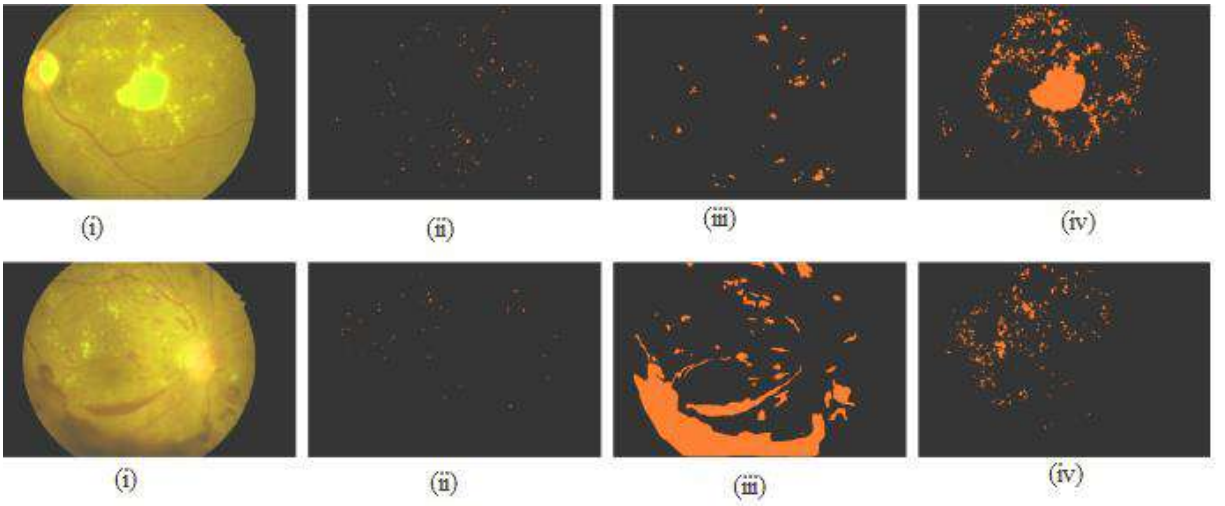

Figure 7: i) Retinal fundus image ii) Microanurysms iii) Hemorrhages iv) Exudates

Convnets for Classification

Convolutional Neural Networks (CNN) or Convnet is a viable method for categorizing retinal fundus images. It is a two-dimensional model that is built and used to recognize image datasets. Its first step is to specify all of the functions that will be used to construct the model. TensorFlow is a lovely computational graph that aids in the creation of these functions and variables by just defining their shape or size rather than putting data in them. Using a 256x256 input image, a filter is being used on all of the of the images, capturing the data. This information is transmitted to the pooling layer, which conducts a mathematical calculation and returns a specified result. The whole model, including the layers required for classification, may be used for training, testing, and classification.

Grey Wolf Optimization Algorithm (GWO)

The GWO algorithm [24] is a novel metaheuristic population-based stochastic method for picking the optimal solution from a set of solutions. The GWO algorithm is centered on how grey wolves behaved, and it replicates the social hunting mechanism in 3 stages: tracking, surrounding, and attacking. Grey wolves are four groups were formed: alpha, beta, delta, and omega. Each group has a rigorous social dominating structure. The α grey wolf is a dominating wolf who makes judgments about sleeping time and hunting.

Other subservient grey wolves have accepted him. The β grey wolf, who aids in decision-making, is regarded to be at the second rung of the hierarchy. It obeys and dominates the instructions of other grey wolves. When gets too old or dies, the following most qualified grey wolf to flourish is β. δ is the next third tier in a grey wolf hierarchical order that either listens to and submits to wolves or dominates ω wolves.If the wolf is α/ β/ δ/ω, ω is the lowest level in the grey wolf hierarchy that submits to other powerful grey wolves [26]. Team hunting is an

intriguing social activity of grey wolves, which includes the social hierarchy of wolves. The main hunting techniques used by grey wolves are listed below.

i. Getting close to the prey
ii. Surrounding and pestering it until it stops moving.
iii. Striking out the prey

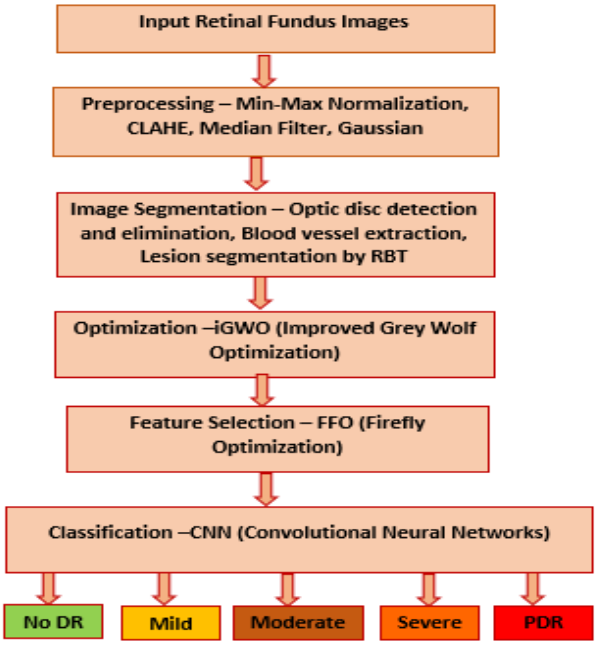

Figure 8: Workflow of proposed method

Improved Grey Wolf Optimization (iGWO)

For the better value of global minimum, the Improved Grey Wolf Optimization Algorithm (iGWO) is chosen. The improved global minimum is found using this population-based optimization strategy. The exploration entails looking into a potential area in order to find a better solution amongst some of the available options, whereas exploitation entails using the solutions found during the exploration phase. Exploration and exploitation stability aids convergence to the global optimum. The trade-off system for exploration and exploitation has to be enhanced. Therefore, in addition to address the shortcomings of the existing GWO method, the improved Grey Wolf Optimization (iGWO) approach is presented.

Different developments are made in the suggested iGWO algorithm in order to come up with solutions to the efficiency and convergence rate difficulties. vectors and they might also be used to change the algorithm's convergence rate. With the goal of simulating grey wolf hunting behavior, it is assumed that the fittest wolf has a better understanding of the probable position of prey, and hence only such fittest solution is archived. The iGWO method is suggested to improve performance in terms of avoiding convergence speed, convergence rate, and accuracy by avoiding premature convergence.

The search agents are activated and dispersed throughout the search space. Local minima are used to classify the whole search space. Finally, the data is used by the people, who identify the global minimum. The convergence speed is determined in this method, so each individual fitness(optimal) is calculated for the local optimum solution. This approach achieves population-based fitness estimations. Eventually, the exploitation goes off without a hitch. Locally, possible solutions are investigated, and metaheuristic optimization is used to obtain exact optimization.

Algorithm 2: iGWO Algorithm

Restore and Initialize the grey wolves(search agents) population X_i *(i=0,1,2,….,n)*
generate randomly position of each search agent
Restore and Initialize a,A,C
Computer the fitness value of each search agent in the pack
Set $X_\alpha, X_\beta, X_\delta$ *according to the fitness*
X_α *= the best search agent*
X_β *= the second best search agent*
X_δ *= the third best search agent*
t=1
while(t< Max_Number_Iterations)
    for each search agent
        update the position of each search agent
    end for
    update a,A,C
    Compute the fitness value of each search agents
    Update $X_\alpha, X_\beta, X_\delta$ *and* X_ω
t=t+1
end while
return X_α

This iGWO yields the 94% accuracy. We think this performs will be better from the existing algorithms.

Feature Extraction Using Firefly Optimization (FFO)

The Firefly algorithm is a swarm-based metaheuristic algorithm that imitate the way fireflies communicate by flashing their lights [25]. The method assumes that almost all fireflies are unisex, meaning that another firefly can be attracted to another firefly; a firefly's appeal is proportionate to its brightness, which is determined by the objective function. A brighter firefly will attract another firefly. In addition, the brightness diminishes with distance according to the inverse square rule. Basis of performance mostly in objective function, a randomly generated viable solution termed firefly will be assigned a light intensity. The brightness of the firefly, which itself is directly proportional to its light intensity, will be calculated using this intensity. For minimization tasks, the maximum light intensity will be awarded to the solution with the least functional value. Each firefly will follow fireflies with higher light intensity that once intensity or brightness of the solution has been assigned. The brightest firefly will conduct a local search by travelling about randomly in its neighborhood. As a result, if

firefly x is brighter than firefly y, firefly x will use the update to migrate towards firefly y.

Results and Discussion

Five distinct phases of DR pictures were taken from the APTOS dataset for this suggested experiment [20]. Diabetic Retinopathy is divided into five categorizations: NPDR normal, NPDR mild, NPDR moderate, NPDR severe, and Proliferative DR. The collection is made up of 3662 photographs taken from patients in rural India. The whole dataset is separated into training and testing sets with a 70:30 ratio and images with No DR 1805, mild 370, moderate 999, severe 193, and PDR 295. This suggested iGWO-based DR detection is implemented in Python using the Google Colob tools Keras and Tensorflow. Various standard metrics like that as f1 measure, recall, accuracy and precision were computed, as well as segmentation measures such as DSC, JSC, and time period.

Table 1: Performance results of various models

S.No	Techniques	Results %							
		FALSE PR	TRUE NR	FALSE NR	PRECISION VALUE	RECALL VALUE	F-measure VALUE	Accuracy VALUE	Error%
1	SVM-GSO	66.66	33.34	22.21	77.77	77.77	77.77	66.66	33.34
2	PSO-CNN	56.13	43.87	16.76	84.56	82.77	83.65	75.00	25
3	CNN	49.20	50.80	15.32	85.21	88.87	87.00	80.00	20
4	DCNN-EMFO	41.35	58.65	14.42	87.11	89.11	88.09	82.00	18
5	MACO-CNN	31.22	69.78	9.83	93.43	92.21	92.81	91.32	8.68
6	**Proposed iGWO-FFO**	**29.12**	**70.88**	**8.45**	**94.11**	**93.02**	**92.05**	**94.11**	**6**

Table 2: Experimental results of DSC, JSC and time period

S.No	Techniques	DSC	JSC	Time period
1	SVM-GSO	0.51	0.56	4.8
2	PSO-CNN	0.56	0.61	4.5
3	CNN	0.64	0.68	3.5
4	DCNN-EMFO	0.68	0.73	2.8
5	MACO-CNN	0.78	0.78	2.4
6	**Proposed IGWO-FFO**	**0.89**	**0.89**	**1.9**

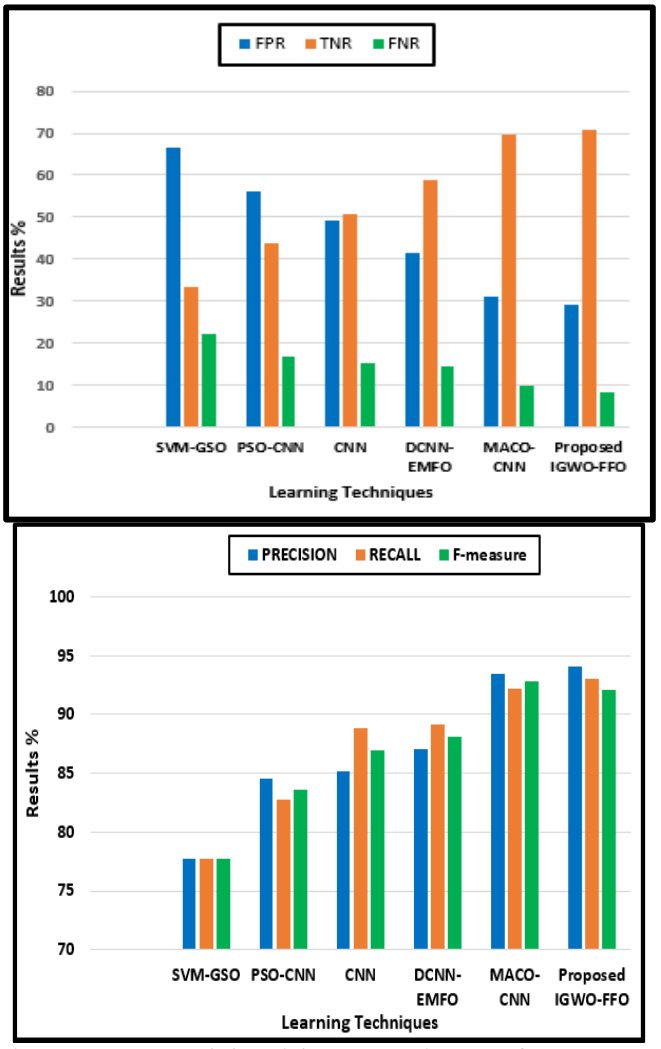

Figure 9: Learning system Models with comparisons of outcomes (False PR,True NR, False NR)

Figure10: Learning system Models with outcomes (precision values, recall and F-measure values)

The performance of six different classifiers, SVM-GSO [26], PSO-CNN [27], CNN [28], DCNN-EMFO [29], MACO-CNN [30], and the suggested IGWO-FFOCNN, is shown in the figure9. It shows that the suggested IGWO-FFOCNN has a higher TNR outcome percentage and a lower FPR, FNR. The SVM-GSO approach yields percentages of 66.66 %, 33.34 %, and 22.21 % for those criteria, respectively. The result percentages for the PSO-CNN classifier are 56.13 %, 43.87 %, and 16.76 %, respectively. According to CNN, the results are 49.20 %, 50.80 %, and 15.32 %. The DCNN-EMFO model yields values of 41.35 %, 58.65 %, and 14.42 %, respectively. The outputs of a deep learning model MACO-CNN are 31.22 %, 69.78 %, and 9.83 %, respectively. The outcomes of the suggested IGWO-FFOCNN model are 29.12 %, 70.88 %, and 8.45 %, respectively. When compared to existing methodologies, it indicates that the proposed IGWO-FFOCNN has a higher TPR.

In addition to accuracy, recall, and F-measure, the figure 10 depicts the additional performance measures of various classifiers' outputs. It is demonstrated that the suggested IGWO-FFOCNN has a higher F-measure, accuracy, and recall. The 77.77 %, 77.77 %, and 77.77 %, respectively, are obtained using the SVM-GSO approach. 84.56 %, 82.77 %, and 83.65 % for the PSO-CNN classifier, respectively. According to the CNN model, 85.21 %, 88.87 %, and 87.00 % are correct. The DCNN-EMFO approach produces results of 87.11 %, 89.11 %, and 88.09 %. Accordingly, the MACO-CNN model receives 93.43 %, 92.21 %, and 92.81 %. The outcomes of the suggested model IGWO-FFOCNN are 94.11 %, 93.02 %, and 92.05 %, respectively.

The additional performance measures Accuracy and error outcomes of those classifiers SVM-GSO, PSO-CNN, CNN, DCNN-EMFO, MACO-CNN, and proposed IGWO-FFOCNN are shown in the figure 11. It reveals that the projected IGWO-FFOCNN has a 94.11 % accuracy rate and a 6% error rate, indicating that it leads to better outcomes.

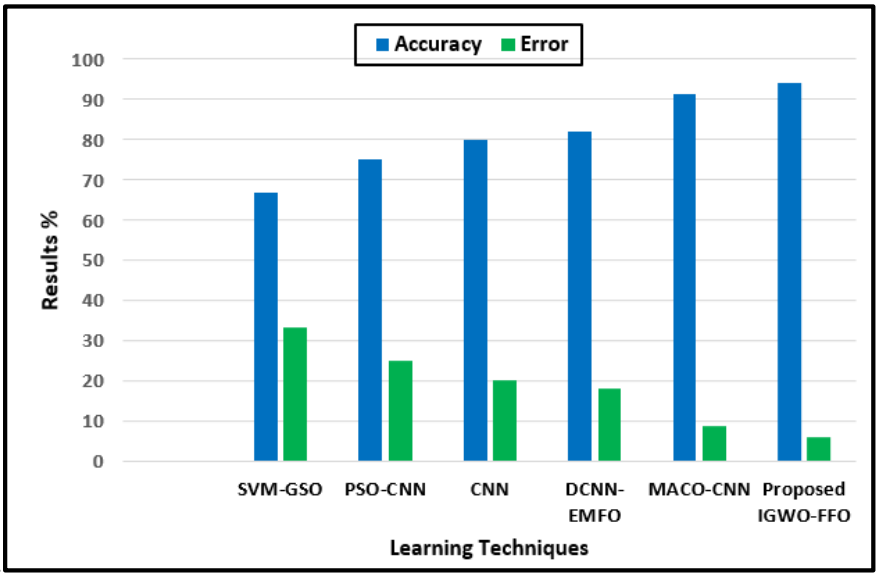

Figure 11: Learning Techniques and outcomes (Accuracy and Error)

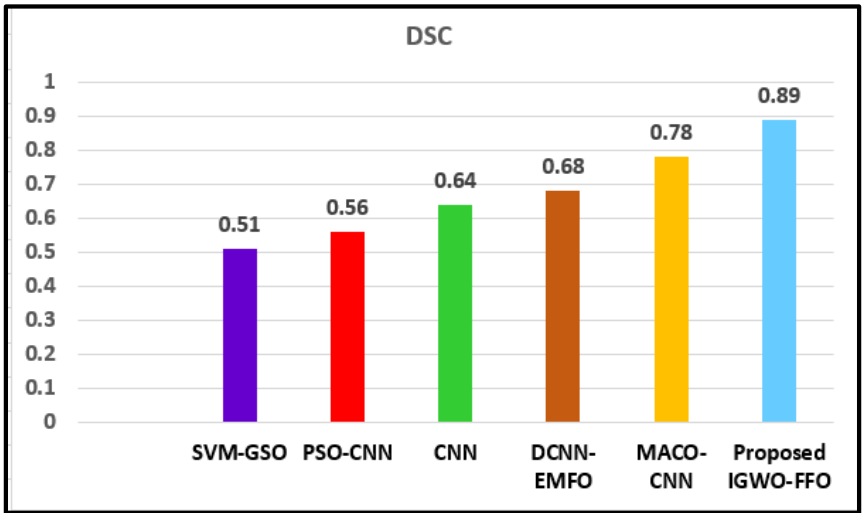

Figure 12: DSC of various learning models

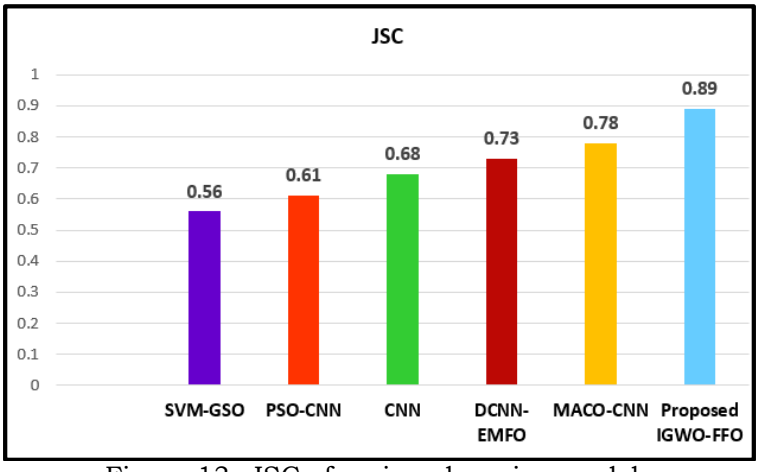

Figure 13: JSC of various learning models

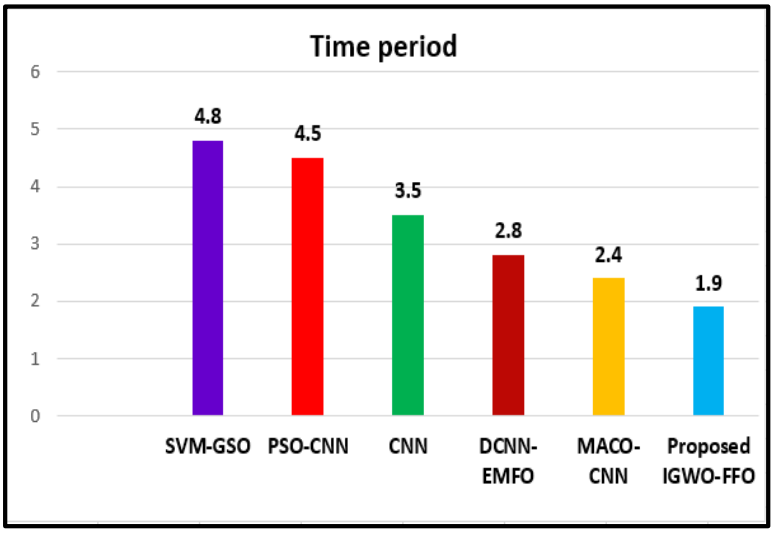

Figure 14: Time period of various learning models

The DSC, JSC, and time period of classifiers SVM-GSO, PSO-CNN, CNN, DCNN-EMFO, MACO-CNN, and proposed IGWO-FFOCNN are shown in the figures 12-14. It demonstrates that the presented IGWO-FFOCNN achieves superior DSC and JSC outcomes of 89 % and 89 percent, respectively, with a time period of just 1.9 percent.

Conclusion

Improved Grey Wolf Optimization with Firefly-based Convolution Neural Networks (iGWO-FFOCNN) is used to classify retinal fundus images in this research. Min-Max normalization, CLAHE, Median, and Gaussian filters are used for preprocessing. All of the samples came from APTOS patients in rural India. Five distinct classifiers are used to analyze performance: SVM-GSO, PSO-CNN, CNN, DCNN-EMFO, MACO-CNN, and iGWO –FFOCNN. In this case, the iGWO-FFOCNN classifier has a higher accuracy and error rate. It reveals that the suggested iGWO –FFOCNN approach achieves a higher accuracy of 94.11 % to existing technologies. The detection performance is increased in the future by utilizing hybrid embedded with cloud-based approaches such as the Hadoop cluster integrated smart algorithm for DR detection.

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Journal
of the
Oriental Institute
M.S. University of Baroda
ISSN: 0030-5324

PERFORMANCE OF NEW GENERATION PRIVATE SECTOR BANKS IN INDIA

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ABSTRACT

Role of private sector banks in delivery of banking and financial sectors to customers and public are very effective and well organized with the help of technology. Modern banking facilities and online banking system were initiated by private banks for the purpose of meeting competition and sustain their customers. Therefore, understanding the performance of private sector banks particularly new generation banks are more important. With this view, this paper made an attempt to discuss performance of new generation private sector banks in India based on secondary data which have been collected from the trends and progress of banking in India published by RBI.

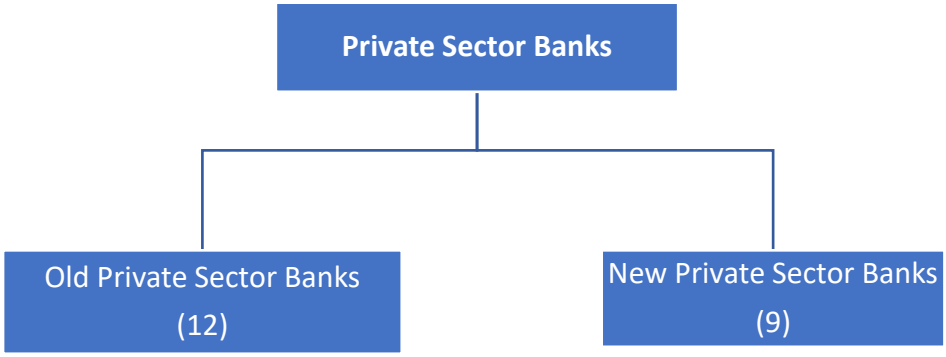
KEY WORDS: Banking sector, public sector banks, private sector banks, new generation banks, foreign banks**,** financial services, information technology

INTRODUCTION

Banking is one of the nerve systems of economic development of the nation which help to smooth flow of money from individual to industrial sectors. Structure and organization of banking sector leads to innovative and speedy delivery of financial services to all. In India, banks are classified into public private and foreign sectors based on shareholding pattern. Private Sector Banks are solely owned by major public shareholders, who tend to take the entire profit that the Private Sector Banks make. These private sector banks are more focused on meeting the needs of their customers than government-owned public banks. Private sector banks also offer additional services to attract customers and persuade them to invest. Role of private sector banks in delivery of banking and financial sectors to customers and public are very effective and well organized with the help of technology. Modern banking facilities and online banking system were initiated by private banks for the purpose of meeting competition and sustain their customers. Therefore, understanding the performance of private sector banks particularly new generation banks are more important. With this view, this paper made an attempt to discuss performance of new generation private sector banks in India based on secondary data which have been collected from the trends and progress of banking in India published by RBI.

PRIVATE SECTOR BANKS

On the basis of ownership pattern of commercial banks, it classified into public sector, private sector and foreign banks. Among these, private sector banks play a key role in economic development of the country. Private Sector Banks refer to those banks where most of the capital is in private hands. In India, there are two types of private sector banks viz. Old Private Sector Banks and New Private Sector Banks. Old private sector banks are those which existed in India at the time of nationalization of major banks but were not nationalized due to their small size or some other reason. After the banking reforms, these banks got license to continue and have existed in India along with new private banks and government banks.



OLD PRIVATE SECTOR BANKS

At present, there are 12 old private sector banks in India as follows: Catholic Syrian Bank, City Union Bank,,Dhanlaxmi Bank, Federal Bank, Jammu and Kashmir Bank, Karnataka Bank, Karur Vysya Bank, Lakshmi Vilas Bank, Nainital Bank, Ratnakar Bank, South Indian Bank and Tamilnad Mercantile Bank

NEW PRIVATE SECTOR BANKS IN INDIA

The new private sector banks were incorporated as per the revised guidelines issued by the RBI regarding the entry of private sector banks in 1993. At present, there are nine new private sector banks as follows: Axis Bank, Development Credit Bank (DCB Bank Ltd), HDFC Bank, ICICI Bank, IndusInd Bank ,Kotak Mahindra Bank, Yes Bank, IDFC and Bandhan Bank of Bandhan Financial Services.

ADVANTAGES OF PRIVATE SECTOR BANKS

- Customers are offered **quick service by** private sector banks.
- These banks also provide tailored services based on the customer's financial requirements.
- Banks in the private sector have a **streamlined management system**.
- Private sector banks can make quick financial decisions.

DISADVANTAGES OF PRIVATE SECTOR BANKS

- Private sector banks charge additional fees for all financial services.
- These banks only operate in cities, making them inaccessible to the rural population.
- Employees in private sector banks have no job security.

LITERATURE REVIEW

Mohi-ud-Din; Nazir, (2010) stated that sound financial health of a bank is the guarantee not only to its depositors but is equally significant for the shareholders, employees and whole economy as well. In this paper, an effort has been made to evaluate the financial performance of the two major banks operating in northern India. This evaluation has been done by using CAMEL Parameters, the latest model of financial analysis.

Anurag & Priyanka (2012) conducted a comparative study of financial performance between SBI and ICICI bank. The purpose of the study is to evaluate the financial performance of SBI (Public sector bank) and ICICI Bank (private sector bank. This study was descriptive and analytical in nature.

Ruchi (2014) has evaluated the performance of public sector banks in India using CAMEL model. Like other sectors, banking sector plays a very important role for development of economy. Banking sector helps in capital formation, innovation and monetization in addition to facilitation of monetary policy. It is essential to evaluate and analyse the performance of banks to ensure a healthy financial system and an efficient economy.

Srivatstava, et al. (2015) conducted a study to Analysis the performance of Indian Banks – A Comparative Study. Authors evaluated the comparative study on some selected banks with reference to some selected parameters. The descriptive and conclusive research designs were used by the researcher during this analyzation of the banks.

Mahesh et al. (2019) conducted a comparative study between private and public banks in India through the camel rating system. Axis Bank and Kotak Mahindra Bank from Private Sector and Bank of Baroda and State Bank of India from the public sector are taken for this study.

Kajal Chaudhary and Monika Sharma,(2011) The last decade has seen many optimistic developments in the Indian banking sector. Public banks must give concentration on their functioning to compete private banks. Banks should be well versed in appropriate collection of borrower/project and in analyzing the financial statement

Padma.D and V. ArulmathiS (2013) BI and ICICI banks are maintaining the required standards and running profitably. This comparative study of SBI and ICICI Bank demonstrates that there are significant differences on the performance of SBI and ICICI Bank in terms of Deposits, Advances, Investments, Net profit and Total assets. Based on the study, it can be said that SBI have an extensive operation than ICICI Bank .

Nishit V.Davda (2014) All new private sector banks shifted their focal point from service to customer. If any bank needs to acquire success in this field they have to know the customer and focus their requirements. And also they have to work based on the current market development .

Jaiswal A and Jain C,(2016) The SBI is performing well and financially sound than ICICI Bank. Also, the market position of SBI is better than ICICI in terms to earning per share, price ratio per share and dividend payout ratio, but on the other hand ICICI bank is performing well in terms of NPA and provision for NPA in comparison of SBI bank.

Annapurna.V and G. Manchala,(2017) HDFC bank performance is good in financial perspective of the BSC. The two banks ICICI and Axis performance is moderate in the variables in customer satisfaction perspective of the BSC. Finally, the performance of ICICI bank is best followed by Axis and HDFC banks during the study period in the BSC framework. It is recommended the banks to improve performance in all perspectives of the BSC as well as to improve overall performance .

Priyanka Jha, "Analyzing (2018) Bank customers have a lot of belief in public sector banks compared to private sector banks. Individuals wellliked PNB bank to necessitate loans and advances as compare to ICICI bank. However, PNB bank has lower operational efficiency comparatively than ICICI bank. In case of dividend payout ratio, debt-equity ratio and Interest expended to interest earned, ICICI bank has performed sounder as compare to PNB bank.

M. Selvakumar, H. Janani, V. Sathyalakshmi and R. (2019), While go through the existing studies relating to performance analysis, particularly in banking sector, most of the studies analyzed either public sector banks or private sector banks or both. A limited study is made in New Generation Private Sector banks; however, such banks contribute more to Indian economy.

Prakash M, Senthil Kumar A , Girish S (2020), Based on the study also suggest that mergers of small sized as well medium sized banks are encourage for the stability in the banking sector. Mergers of small banks having can improve their performance along with strong banks because of financial health and improvised methods. Regulators would need to be cautious in approving mergers of these banks and also there is a need to analyse the impact of economy.

Axis Bank

Axis Bank is one of the first new generation private sector banks to have begun operations in 1994. The Bank was promoted in 1993, jointly by Specified Undertaking of Unit Trust of India (SUUTI) (then known as Unit Trust of India), Life Insurance Corporation of India (LIC), General Insurance Corporation of India (GIC), National Insurance Company Ltd., The New India Assurance Company Ltd., The Oriental Insurance Company Ltd., and United India Insurance Company Ltd. The shareholding of Unit Trust of India was subsequently transferred to SUUTI, an entity established in 2003.

DCB Bank

DCB Bank is a modern emerging new generation private sector bank with 228 branches across 18 states and 2 union territories. It is a scheduled commercial bank regulated by the Reserve Bank of India. It is professionally managed and governed. DCB Bank has contemporary technology and infrastructure including state of the art internet banking for personal as well as business banking customers. DCB Bank's business segments are Retail, micro-SME, SME, mid-Corporate, Agriculture, Commodities, Government, Public Sector, Indian Banks, Co-operative Banks and Non Banking Finance Companies (NBFC). DCB Bank has approximately 450,000 customers.

HDFC Bank

The HDFC Bank was incorporated on August 1994 by the name of 'HDFC Bank Limited', with its registered office in Mumbai, India. The Bank at present has an enviable network of over 1416

branches spread over 550 cities across India. All branches are linked on an online real–time basis. Customers in over 500 locations are also serviced through Telephone Banking. The Bank also has a network of about over 3382 networked ATMs across these cities.

Indusind Bank

Indusind Bank incorporated in April 1994 derives its name from the Indus Valley civilisation. The bank was the vision of Srichand P Hinduja, a Non–Resident Indian businessman and head of the Hinduja Group. A decade after its incorporation, June 2004, the bank was merged with Ashok Leyland Finance. IndusInd Bank, which began in 1994, boats of 573 branches, and 1055 ATMs spread across 392 locations of the country. We also have representative offices in London and Dubai, catering to every need of the customer. It is the first Indian bank to receive ISO 9001:2000 certification for its corporate office and its entire network of branches.

Kotak Mahindra Bank Ltd.

Kotak Mahindra Bank is the flagship company of the Kotak Group. It is one amongst the fastest growing banks and the most admired financial institutions in India. The Bank offers transaction banking operates lending verticals manages IPOs and provides working capital loans. The principal business activities of the Bank are organised into consumer banking commercial banking corporate banking treasury and other financial services. As of 31 December 2020 the Bank had 1603 branches and 2573 ATMs across the country.The Bank along with its subsidiaries offers a comprehensive range of financial products and services to its customers.

Yes Bank Ltd

Yes Bank Ltd was incorporated on November 21 2003. The bank was founded by Rana Kapoor. Yes Bank Ltd is engaged in providing a range of banking and financial services. The Bank operates in four segments: Treasury Corporate/Wholesale Banking Retail Banking and Other Banking Operations. The Treasury segment includes investments all financial markets activities undertaken on behalf of the Bank's customers trading maintenance of reserve requirements and resource mobilization from other Banks and financial institutions. The Corporate/Wholesale Banking segment includes lending deposit taking and other services offered to corporate customers.

IDFC First Bank Ltd

Headquartered in Mumbai IDFC Bank is a universal bank offering financial solutions through its nationwide branches Internet and mobile. As on March 31 2021 the Bank has built a national footprint through the operation of 596 branches592 ATMs and 85 recyclers across the country. IDFC was granted an in-principle approval by Reserve Bank of India (RBI) on 9 April 2014 to set up a new bank in the private sector under Section 22 of the Banking Regulation Act 1949. On 31 March 2016 IDFC Bank announced a partnership with Uphold the world's fastest growing cloud-based financial platform for instant easy and affordable inward remittances to India.

Bandhan Bank Ltd

Bandhan Bank is a commercial bank focused on serving underbanked and underpenetrated markets in India. Bandhan Bank is the first instance in India of a microfinance entity transforming into a universal bank. The bank's total banking outlets as on 31 March 2021 stood at 5310. The network consists of 1147 branches and 4163 banking units and the total number of ATMs stood at 487 across the country. Bandhan Bank has significant presence in under-penetrated East and North East India. The Bank began operations on August 23 2015 when Bandhan Financial Services Limited ('BFSL') its ultimate parent company transferred its entire micro finance business to the bank and the bank simultaneously commenced general banking activities.

Table No – 1 : Branches of New private sector Banks (at end - March 2021)

Sl.No	Name of the Bank	Branches				
		Rural	Semi-Urban	Urban	Metropolitan	Total
1	Axis Bank	756	1409	1098	1446	4709
2	Bandhan Bank	1811	2022	977	500	5310

3	DCB Bank Ltd	67	93	89	103	352
4	HDFC Bank Ltd	1052	1742	1159	1651	5604
5	ICICI Bank	1097	1537	1063	1542	5239
6	IDFC Bank	47	160	218	329	754
7	IndusInd Bank Ltd	290	430	513	628	1861
8	Kotak Mahindra Bank	256	292	341	715	1604
9	Yes Bank Ltd	148	302	235	385	1070
	Total	5524	7987	5693	7299	26,503

Source: Trend and Progress of Banking in India 2020-21

Table no-1 Branches of New private sector Banks, Indicates that branches of New Private Sector Banks as on March 2021, There are 26503 branches of New Private Sector Banks of which Axis Bank Ltd consists of 4709 branches, Bandhan Bank Ltd consists of 5310 branches, DCB Bank Ltd consists of 352 branches, HDFC Bank Ltd consists of 5604 branches, ICICI Bank Ltd consists of 5239 branches, IDFC Bank Ltd consists of 754 branches, IndusInd Bank Ltd consists of 1861 branches, Kotak Mahindra Bank Ltd consists of 1604 branches, Yes Bank Ltd consists of 1070 branches in scheduled commercial banks.

Chart No – 1 :Branches of New private sector Banks (at end - March 2021)

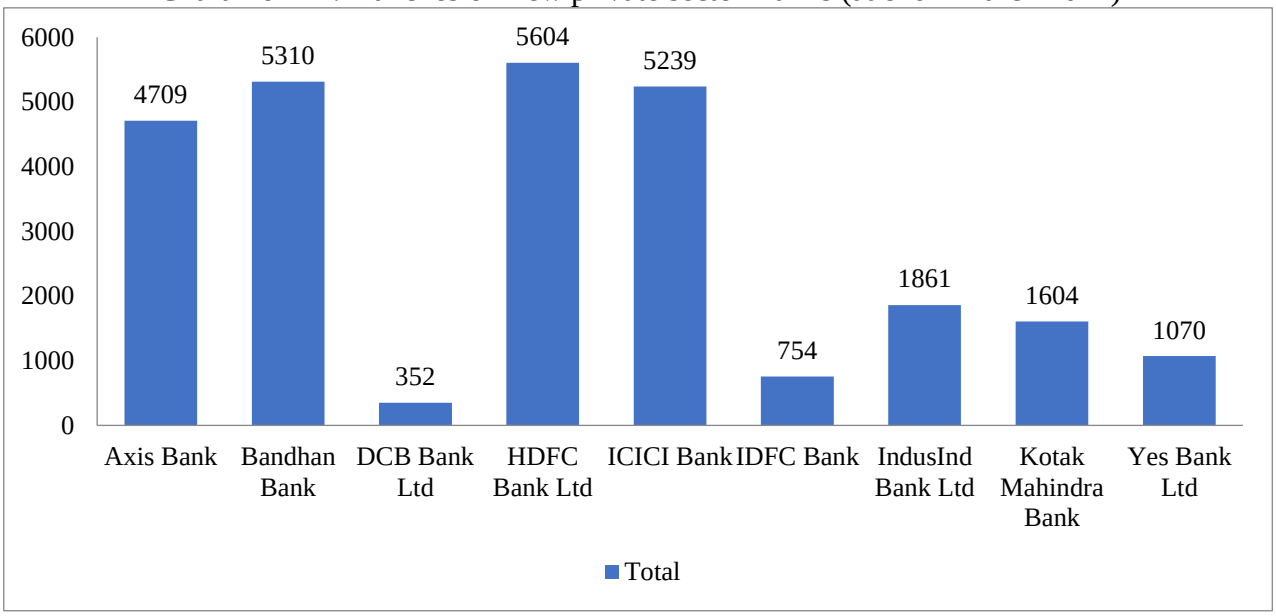


Table No – 2 : ATMs of New private sector Banks (at end - March 2021)

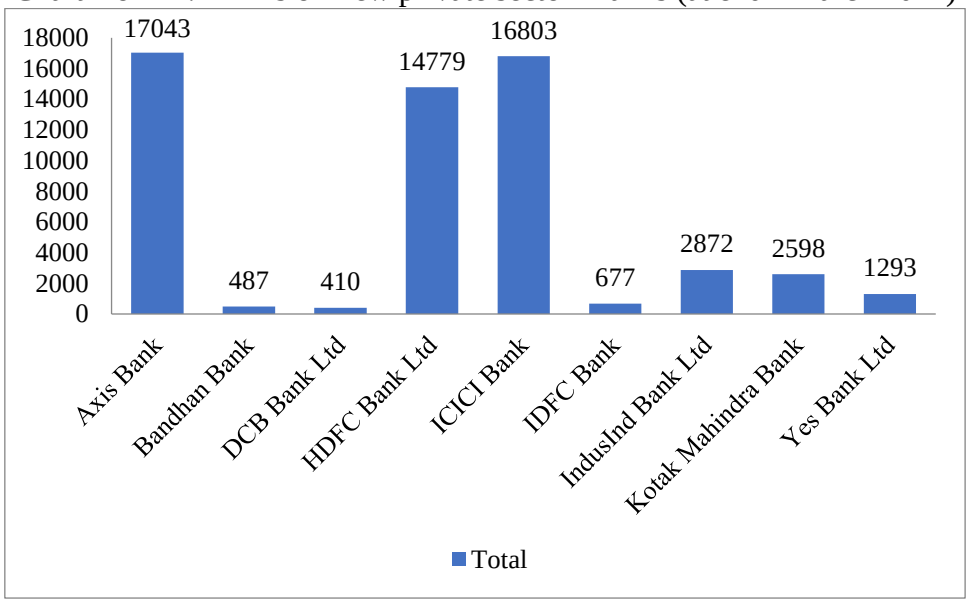
Sl.No	Name of the Bank	ATMs		
		On-Site	Off-Site	Total
1	Axis Bank	5598	11445	17043
2	Bandhan Bank	485	2	487
3	DCB Bank Ltd	308	102	410
4	HDFC Bank Ltd	6552	8227	14779
5	ICICI Bank	8161	8642	16803
6	IDFC Bank	507	170	677
7	IndusInd Bank Ltd	1393	1479	2872
8	Kotak Mahindra Bank	1325	1273	2598
9	Yes Bank Ltd	995	298	1293
	Total	25324	31638	56962

Source: Trend and Progress of Banking in India 2020-21

Table no-2 ATMs of New private sector Banks as on 2021, As regards ATMs of New Private Sector Banks, Axis Bank Ltd have 17043 ATMs, Bandhan Bank Ltd have 487 ATMs, DCB Bank Ltd have 410 ATMs, HDFC Bank Ltd 14779 ATMs, ICICI Bank Ltd 16803 ATMs, IDFC Bank Ltd 677 ATMs, IndusInd Bank Ltd 2872 ATMs, Kotak Mahindra Bank Ltd 2598 ATMs, Yes Bank Ltd 1293

ATMs. On the whole on, there are 56962 ATMs owned by New Private Sector Banks, of which 25324 are on-site ATMs and 31638 are off-site ATMs.

Chart No – 2 : ATMs of New private sector Banks (at end - March 2021)



CONCLUSION

Private sector banks play a significant role in the banking system of the country which is also challenging to private and foreign banks with respects to infrastructure, delivery mechanism, innovative products and universal information. When new economy policy initiated in 1991, many liberalization process has taken particularly in banking and financial sectors. RBI revised its policy to allow private banks at par with all other commercial banks. There are 21 private sector banks of which nine banks are considered as new generation private sector banks which play a effective role in economic development of the country. Operational and financial efficiency of new generation private sector banks are performing in a consist manner.

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Buying Behaviour of Consumers in Organized Retail Outlets

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Abstract : This study has been undertaken to investigate the buying behaviour of the consumers in organized retail outlets. Consumer buying behavior refers to the actions taken (both on and offline) by consumers before buying a product or service.This process may include consulting search engines, engaging with social media posts, or a variety of other actions. Consumer behavior helps organizations decide what products and services to manufacture or offer. When they know what customers buy and how they go about buying those products, organizations can more easily spot a need that has not yet been satisfied. With centralized buying organizations, growth in market coverage and turnover, retailers have become gateways to the consumer markets. Therefore, knowledge about retailers' and trade buyers' buying behaviour has become important to producers. A retail stores is a place where people can buy all things under one roof. It satisfies the need of the people.

Key Words- buying behavoiour, consumer, organized retail, traders and retailer.

I. INTRODUCTION

Indian Retail Industry is a booming industry whose growth level and Expansion circle is behind ones imagination. The abnormal growth of this sector is in such a way that it affects the Country's GDP and plays a major role in eradicating Country's unemployment sin. The comfort zone and the facilities like one stop purchase, more choices, ambience, hospitality and service level made way for an enormous growth of this sector. The wrapping support from the Indian retail consumers open up the international retailer's sight and focus towards Indian retail market. Due to this a number of various retail formats like Super markets, Department stores, Hyper markets and Malls were originated and made the Indian consumers to experience a leisure and mind blowing shopping time.

The adoption of government principles like Demonetization and GST has also paved way for the expansion and percentage growth rate Progress in Indian retail market. The GST application definitely needs proper and legal billing methods which can't be followed by unorganized retailers. The digital India system also serves as a major boon for the Organized retailers whom encourages various mode of payment associated with latest updations like google pay, phonepay, Tez, Pay tm and so on., The various promotional offers and their awareness make the organized retailers to reach the nook and corner consumers and to succeed more than what they planned. This massive growth and wide market made the international retail giants like Walmart and Amazon to lay foundation in India and tasting the success.

II. STATEMENT OF THE PROBLEM

The favorable outcome of any format of retail industry merely depends on how it performs in the market place at a given point of time. The performance in turn depends on how efficiently the retailer persuade the consumers in to their basket which requires the retail stores to understand the behaviour of the buyer. But, understanding the consumer behaviour is strenuous, as it is related to the psychology of consumers and also depends on various social factors which have a direct impact on consumer behaviour. Cultural, personal and economic factors are also largely depends on the buying behaviour. This in turn, led the retailers to redraft their existing marketing strategies and introduce appropriate changes to establish and sustain themselves in the industry. Thus, the consumers‟ buying behaviour becomes an indispensable part of the marketing strategies of the organized retail stores. In this context, it is vitally important to study the consumers‟ buying behaviour towards organized retail stores and the present research work has enormous relevance to the retailers in formulating their strategies in the market.

III. REVIEW OF LITERATURE

Review of previous study is highly important for every research to carry on the investigation effectively and successfully. Hence, the present research is also based on the following reviews:

ArpithaKhare (2011) in her study titled “Mall shopping behaviour of Indian small town consumer”, she analyzed about the behavior of the small city consumers towards shopping malls whom were not much aware about the malls and organized retail stores. These consumers experiences functional and experiential benefits which have an impact over the buying behavior. The different group of consumers experiences different merits r benefits based on the consumers age and gender.

JamaliahMohd. Yusuf, Rosidah Musa & SofianAbd. Rahman (2012) through their study “The Effects of Green Image of Retailers on Shopping Value and Store Loyalty” analyzed about the influence of green image on shopping value and store loyalty. The study states that the green image and store loyalty won’t have any significant association where the green images have influence over shopping value. The output of the study provides source material for the researchers and practitioners.

VineetaGangaland Kumar (2013) through their study titled “Consumer Behaviour towards organized Retail”, they mentions that the shopping behavior and purchase style of the Indian consumers have experienced a drastic change in the past ten to fifteen years. This study involves in tracing these new Indian consumers, their purchase style, what are the causes that made them to move towards shopping malls and on what basis they count their shopping destination.

RupaGunaseelan& R. Chitra (2014) under their study titled “Customer’s expectation towards shopping behaviour in retail”, they pointed out the problems and difficulties faced by the retailers while tried to develop or maintain a store image in order to improve their market standards.

Nisha Rathore (2015) in her Study on “Consumer behavior towards organized and unorganized retailing” she clearly points out the cause for the retail revolution in India and its effect. It describes how retail revolution affects the buying behavior of the consumers, income growth of middle class, infra structure

development and customer options. The article states the impact of organized retailers over unorganized retailers and how the consumers get benefitted by the organized retails.

K. Ramya (2016) in this study under the topic "A study on customer perception towards organized retailing in Coimbatore city", she tried to find out the cause behind the customer's choice of organized retail outlets and the effective measures taken by the organized retailers to get and retain their consumers. The study concludes with the consumer's perception towards retail stores and their promotional activities.

Ranjana Thakur, Leena Devi (2017) through their study under the topic "Consumer inclined buying behaviour towards organized retailing", they have identified that emerging factors which affects the consumers inclined buying behavior in organized retail store.

Saba Inamdar (2019) the aim of this study titled "An empirical study on customer preference and buying behaviour with reference to retail outlets", is to determine customer preference and buying behaviour in retail business. The study was analyzed by psychological prospective of the customer also. The changing life style pattern of the city is the key factor which determines satisfaction level towards retail stores.

IV. OBJECTIVES OF THE STUDY

The specific objectives of the study are as follows

1. To study the buying behaviour of the consumers in retail outlets
2. To identify the factors which are affect the buying behaviour
3. To discover the impact on organized retail outlets.

V. RESEARCH METHODOLOGY & DATA COLLECTION

Methodology is a action plan for the research and it is provide enough background on the technical aspects of the study. This includes research design, sample size, sampling method, area of sampling source of data, framework and tools used for analysis.

Research Design

Descriptive research is used to identify buying behaviour of the consumers in the organized retail outlets. Descriptive statistics provide simple summaries about the sample and about the observations that have been made.

Sample Size

Sample size is for the consumer's survey is 784.

Sampling Method

Simple random sampling method was adopted for the study with a sample size of 784 respondents from the consumers of organized retail outlets.

Area of Sampling

Coimbatore city is selected to conduct the survey.

Source of Data

The data were collected from primary and secondary sources. Primary data are collected by conducting direct structured questionnaire. Secondary data were collected from various journals, magazine and internet.

Framework and Tools used for Analysis

Statistical tools such as percentage analysis, Chi - Square test, Friedman Rank test and ANOVA are used to find out the results.

VI. Preference of the Consumers

Association between the socio economic factor of the consumer and their level of preference towards retail outlets

Table 1 - Problem Level Of Consumers Towards Socio Economic Factor: Chi-square Analysis

Socio Economic Factor	Degrees of Freedom	Chi-Square Table Value	P Value	Significant/
Area of Residence	4	31.496	.000	Significant
Age	4	9.475	.050	Not Significant
Gender	2	2.957	.228	Not Significant
Occupation	14	27.110	.019	Significant
Earning Members in the Family	4	20.081	.000	Significant
Non - Earning Members in the Family	4	10.912	.028	Significant
Total Family Members	4	14.676	.005	Significant
Type of Family	2	5.949	.051	Not Significant
Status in Family	2	.051	.164	Not Significant
Education	6	7.909	.245	Not Significant
Monthly Family Income	4	50.499	.000	Significant
Monthly Family Expenditure	4	17.061	.002	Significant

Source: Primary Data. ***S-****Significant****; NS-*** *Not Significant****; Level of Significance-****0.05;*

By applying chi-square test, it is found that there is a significant relationship between area of residence, occupation, earning members in the family, non - earning members in the family, total family members in a family, monthly family income, monthly family expenditure and their preference level. By applying chi-square test, it is also found that there is an insignificant relationship between the age, gender, type of family, status in family, education and their preference level.

Table - 2 Garrett's Ranking Technique – Factors which have induced you to visit the store

Reasons	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**	**Total Score**	**Mean Score**	**Rank**
	83	72	66	60	56	52	47	43	39	33	27	16				
Availability of all products	154	97	69	94	71	50	47	49	53	43	21	36	784	45481	58.01	1
	12782	6984	4554	5640	3976	2600	2209	2107	2067	1419	567	576				
Multi Brands	39	118	99	87	72	52	76	53	42	79	30	37	784	41721	53.22	3
	3237	8496	6534	5220	4032	2704	3572	2279	1638	2607	810	592				
Discount offers	85	91	104	64	68	59	69	50	38	40	76	40	784	42074	53.67	2
	7055	6552	6864	3840	3808	3068	3243	2150	1482	1320	2052	640				
Proximity	40	41	38	72	68	90	93	66	64	75	76	61	784	36796	46.93	8
	3320	2952	2508	4320	3808	4680	4371	2838	2496	2475	2052	976				
Easy Shop in	58	75	65	75	136	77	63	68	49	45	39	34	784	41502	52.94	4
	4814	5400	4290	4500	7616	4004	2961	2924	1911	1485	1053	544				
Self-Service	48	51	64	88	89	82	71	53	68	58	60	52	784	39042	49.80	6
	3984	3672	4224	5280	4984	4264	3337	2279	2652	1914	1620	832				
Comfort	72	50	112	79	59	81	81	74	50	49	40	37	784	41452	52.87	5
	5976	3600	7392	4740	3304	4212	3807	3182	1950	1617	1080	592				
Store Reputation	26	83	43	46	50	75	62	73	85	82	85	74	784	35985	45.90	10
	2158	5976	2838	2760	2800	3900	2914	3139	3315	2706	2295	1184				
Store Attraction	81	55	54	54	56	62	64	57	99	86	72	44	784	38653	49.30	7
	6723	3960	3564	3240	3136	3224	3008	2451	3861	2838	1944	704				
Feel at Home	52	37	67	57	45	43	81	96	68	71	90	77	784	36170	46.14	9
	4316	2664	4422	3420	2520	2236	3807	4128	2652	2343	2430	1232				

Neighbor Buys	27	45	35	34	32	51	39	98	83	93	109	138	784	31779	40.53	12
	2241	3240	2310	2040	1792	2652	1833	4214	3237	3069	2943	2208				
Open On Week Ends	99	44	35	38	45	55	40	47	80	67	83	151	784	35244	44.95	11
	8217	3168	2310	2280	2520	2860	1880	2021	3120	2211	2241	2416				

GARRETT'S RANKING TECHNIQUE

Factors which have induced you to visit the store

Garrett's Ranking Technique has been used to ascertain the factors that induce a customer to visit retail outlet. Under the Garrett's ranking technique the percentage position is calculated by using the following formula:

Percentage Position $= 100 (R_{ij}-0.5) / N_j$

Where R_{ij}=Rank given for i^{th} variable by the j^{th} consumers

N_j = Number of variables ranked by the consumers.

The consumers are asked to rank the twelve questions relating to reason for visiting retail outlet.

By referring the Garrett table the per cent position is converted into scores. Then for each factor, the scores of each customer are added and then mean value is calculated. The factors having highest mean value is considered to be the most important. Scale values as per Garrett ranking technique for first to twelve ranks are as: 83, 72, 66, 60, 56, 52, 47, 43, 39, 33, 27 and 16 respectively. The percentage position of each rank is made into score by referring factors is summed up for assigning rank. The following table exhibits the reason for visiting retail outlet by consumers. From the analysis it is inferred that majority of the consumers have visited retail outlet due to availability of all products followed by availability of discount offers, multi brands etc.

Organized retail outlets are continuously impacting the consumers by availing the benefits performed by them, giving cash discounts and offering happy shopping Experience. Hence the outlets are always valuing the importance of customer. Based on the consolidated views and opinion of the respondents, presenting the results in the form of tables with suitable interpretations. Friedman Rank Test was performed.

Table 3

Friedman Rank Test – Consumers Opinion on Benefits

Particulars	**SA**	**A**	**NO**	**DA**	**SDA**	**Total**	**Mean Score**	**Rank**
Cash Discount	295	309	126	37	17	784	6.46	1
	37.60	39.40	16.10	4.70	2.20	(100.0)		
Exchange Offer	275	279	122	74	34	784	6.01	2
	35.10	35.60	15.60	9.40	4.30	(100.0)		
Free Offer/Gifts	241	233	184	88	38	784	5.49	5
	30.70	29.70	23.50	11.20	4.80	(100.0)		
Maximum Guarantee	236	280	164	80	24	784	5.71	4
	30.10	35.70	20.90	10.20	3.10	(100.0)		
Cash back offer	195	237	167	110	75	784	4.87	10
	24.90	30.20	21.30	14.00	9.60	(100.0)		
Seasonal Price Reduction	244	319	139	55	27	784	6.03	2
	31.10	40.70	17.70	7.00	3.40	(100.0)		
Contests/games/ Prize Schemes	204	221	164	125	70	784	4.89	9
	26.00	28.20	20.90	15.90	8.90	(100.0)		
After sales service	209	274	166	94	41	784	5.39	6
	26.70	34.90	21.20	12.00	5.20	(100.0)		
Price-pack/Bonus packs deals	174	277	172	112	49	784	4.98	8
	22.20	35.30	21.90	14.30	6.20	(100.0)		
Sampling	213	256	166	87	62	784	5.17	7
	27.20	32.70	21.20	11.10	7.90	(100.0)		

Cash Discount

Two hundred and ninety five (37.60) consumers strongly agreed that availability of cash discount facility at retail outlets induce them to prefer retail outlets; 309 (39.40) consumers agreed that availability of cash discount facility at retail outlets induce them to prefer retail outlets; 126 (16.10) consumers neither agreed nor disagreed that availability of cash discount facility at retail outlets induce them to prefer retail outlets; 37 (4.70) consumers disagreed that availability of cash discount facility at retail outlets induce them to prefer retail outlets and 17 (2.20) consumers strongly disagreed that availability of cash discount facility at retail outlets induce them to prefer retail outlets. Thus, most of the consumers agreed that availability of cash discount facility at retail outlets induce them to prefer retail outlets.

From the Friedman rank test, it is inferred that majority of the consumers prefer retail outlets due to availability of cash discount facility followed by facility of exchange facility, Seasonal Price Reduction and the like.

Table 4

Friedman Rank Test – Level of importance for your happy shopping

Particulars	SA	A	NO	DA	SDA	Total	Mean Score	Rank
Proper Window displays	337	336	78	26	7	784	8.00	1
	43.00	42.90	9.90	3.30	0.90	(100.0)		
Store Ambience	219	365	135	50	15	784	6.67	9
	27.90	46.60	17.20	6.40	1.90	(100.0)		
Packing-delivery	328	304	108	32	12	784	7.81	2
	41.80	38.80	13.80	4.10	1.50	(100.0)		
Providing adequate security	290	286	133	46	29	784	7.10	4
	37.00	36.50	17.00	5.90	3.70	(100.0)		
Enabling in-store hygiene	278	301	144	45	16	784	7.06	5
	35.50	38.40	18.40	5.70	2.00	(100.0)		
In store Stacking	227	314	173	55	15	784	6.50	12
	29.00	40.10	22.10	7.00	1.90	(100.0)		
Customer care Activities	307	304	97	47	29	784	7.36	3
	39.20	38.80	12.40	6.00	3.70	(100.0)		
Valette parking	270	308	124	55	27	784	6.95	7
	34.40	39.30	15.80	7.00	3.40	(100.0)		
Rest room Cleanliness	297	261	138	62	26	784	6.96	6
	37.90	33.30	17.60	7.90	3.30	(100.0)		
Executional excellence	263	272	165	59	25	784	6.63	10
	33.50	34.70	21.00	7.50	3.20	(100.0)		
The soothing music and good colors of the store.	273	281	134	70	26	784	6.75	8
	34.80	35.80	17.10	8.90	3.30	(100.0)		
The convenient operating hours	243	310	156	59	16	784	6.63	10
	31.00	39.50	19.90	7.50	2.00	(100.0)		
The prices are fair and suit my pocket.	255	282	150	65	32	784	6.57	11
	32.50	36.00	19.10	8.30	4.10	(100.0)		

Proper Window Display

Three Hundred and Thirty Seven (43%) consumers strongly agrees that the proper window display of a store determines the level of importance of their happy shopping experience; Three Hundred and Thirty

Six (42.9%) consumers agrees that their level of happy shopping experience depends on the stores window display; Seventy Eight (9.90%) Consumers neither agree nor disagree the proper window display as a factor for their happy shopping experience; Twenty Six (3.30%) Consumers disagree the proper window display as a cause for their happy shopping; Seven (0.90%) consumers strongly disagree proper window display as a factor for their happy shopping.

Thus most of the consumers strongly agree proper window display as a major cause for their shopping experience as a happy one.

From the Friedman rank test it is clearly proven that more than 80% of the consumers admits that the proper window display of a store is the major cause for their shopping experience as a happy one followed by that store's in-store hygiene, customer care activities, security measures, rest room cleanliness, parking facility etc.,

Table 5

Friedman Rank Test – Factors influencing visit to the Store

Particulars	**SA**	**A**	**NO**	**DA**	**SDA**	**Total**	**Mean Score**	**Rank**
Overall the goods sold in this store are with better Quality	374	344	56	7	3	784	14.14	1
	47.70	43.90	7.10	0.90	0.40	(100.0)		
Stores own brands (private labels) are reasonably priced	229	389	110	49	7	784	11.60	6
	29.20	49.60	14.00	6.20	0.90	(100.0)		
Prices at this store are comparatively cheaper	226	305	186	50	17	784	10.62	20
	28.80	38.90	23.70	6.40	2.20	(100.0)		
The store offers latest fashion trends	274	325	128	45	12	784	11.92	5
	34.90	41.50	16.30	5.70	1.50	(100.0)		
The store provides wide range of products in all sizes and styles	320	285	128	35	16	784	12.40	3
	40.80	36.40	16.30	4.50	2.00	(100.0)		
The store displays trendy products	259	309	164	38	14	784	11.48	9
	33.00	39.40	20.90	4.80	1.80	(100.0)		
The store offers all branded and non-branded Products	282	280	158	49	15	784	11.55	7

Particulars	SA	A	NO	DA	SDA	Total	Mean Score	Rank
	36.00	35.70	20.20	6.20	1.90	(100.0)		
The store pays attention to fashion trends	246	294	166	58	20	784	10.89	18
	31.40	37.50	21.20	7.40	2.60	(100.0)		
The store concentrates on interior and exterior displays	306	335	104	26	13	784	12.64	2
	39.00	42.70	13.30	3.30	1.70	(100.0)		
The advertisement made by the store is extensive and informative	238	342	145	50	9	784	11.30	13
	30.40	43.60	18.50	6.40	1.10	(100.0)		
The store uses celebrities in advertisement	276	248	170	64	26	784	11.00	15
	35.20	31.60	21.70	8.20	3.30	(100.0)		
The store offers variety of advertisement according to seasons	294	287	150	39	14	784	11.93	4
	37.50	36.60	19.10	5.00	1.80	(100.0)		
The store offers well-assured rest area (toilets, seating etc.,)	239	316	143	65	21	784	10.78	19
	30.50	40.30	18.20	8.30	2.70	(100.0)		
The store offers safe and easy way to pay	268	313	143	45	15	784	11.55	7
	34.20	39.90	18.20	5.70	1.90	(100.0)		
The store provides an atmosphere of happiness while shopping	257	320	143	42	22	784	11.32	12
	32.80	40.80	18.20	5.40	2.80	(100.0)		
The store offers luxurious atmosphere while shopping	231	316	145	65	27	784	10.52	21
	29.50	40.30	18.50	8.30	3.40	(100.0)		
The store concentrates more on attractive lighting and catchy interiors	257	277	175	56	19	784	10.97	16
	32.80	35.30	22.30	7.10	2.40	(100.0)		
The pleasing personality of sales personnel	252	294	166	61	11	784	10.93	17
	32.10	37.50	21.20	7.80	1.40	(100.0)		
The adequate knowledge of sales personnel about products	251	337	143	40	13	784	11.52	8
	32.00	43.00	18.20	5.10	1.70	(100.0)		

Particulars	SA	A	NO	DA	SDA	Total	Mean Score	Rank
The personal attention of sales personnel with consumers	260	313	154	40	17	784	11.35	11
	33.20	39.90	19.60	5.10	2.20	(100.0)		
The willingness of sales personnel to help consumers.	262	316	148	42	16	784	11.47	10
	33.40	40.30	18.90	5.40	2.00	(100.0)		
The efficiency of sales personnel to respond to customer's request	257	310	133	57	27	784	11.14	14
	32.80	39.50	17.00	7.30	3.40	(100.0)		

Overall, the goods sold in this store are with better Quality

Nearly fifty percent Three Hundred and Seventy Four (47.7%) consumers strongly agrees that the quality of the product sold in that store influences them to make their purchase in that store; Three Hundred and Forty Four consumers agrees that the best quality of products sold decides their purchase in a store; Fifty Six (7.10%) consumers never agrees nor disagrees the quality of the product as an inducing factor for their retail out purchase; a negligible percentage i.e., less than one percent 0.90% [7 nos] and 0.4% [3 nos] respectively didn't agree and strongly disagrees the quality of the product as an influencing factor for their organized retail store purchase.

Thus the Quality of the Product plays as the major influencing factor for the Consumers Store Choice.

From the Friedman Rank test it is clearly justified that the Quality of the Product serves as a major influencing for the consumers followed by the importance of stores interior and exterior displays, range of products, seasonal advertisements, latest trend updation, reasonably priced own brands, availability of multi brands, sales persons product knowledge, attitude, store atmosphere and so on.

VII. Suggestions

To the Retailers

- The basic facilities like parking, locating in centre of the city, providing locker facility to keep the things safely are quite lacking in the stores which has to be improved to make stick the customers regularly.
- The people are slowly adopting the organized retail store culture, hence it is their duty to educate the customers in every aspect and make them feel at least somewhat satisfied to visit the store again.
- As the society is becoming digital, it is the responsibility of the store to move the customers towards digital transactions and make them to feel secure in spending money.
- The youngsters are visiting the store most often to satisfy their needs from 'Pin to Plane' so there may be update to stores regularly to satisfy such customers.

To the Consumers

- The consumers purchasing option or shopping preference of an organized retail outlet provides them proper bill (which lacks in unorganized retail outlet) which makes the retailers to pay proper tax to the government which supports the governments revenue.
- The Organized retail outlets offers their consumers a number of various payment options like card payment, online payment, RTGS, NEFT etc,. which were safer and secure methods.
- As particularly speaking about the city (Coimbatore), the person are expecting the more organized stores which will be convenient to visit the store often and requires wallet parking in the stores.

VIII. Conclusion

Consumer expectations are very high from the organized retail stores and such expectations have also rubbed off on the conventional retailers. While insisting on value for money and cost effectiveness, today consumers want a better shopping experience, recreation, friendly interactions and a wide choice of products and services. Retail stores have to live up to these expectations in order to flourish, prosper and grow in the Indian market. The retailer in order to satisfy customer needs must have a thorough understanding of how customers make store choice and purchase decisions. Customer's behavior provides some valuable insights into the process and therefore is useful for retail management decision making. It is important to realize that the purchase of product involves motivational, social, psychological and economic factors. There are also important stages involved in the purchase process and the type of purchase and the users of the purchase that will affect the buying behavior.

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Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [5] April 2021 : 215-222

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Photocatalytic Degradation of Methylene Blue Dye using Green Synthesized Iron Oxide Nanoparticles (Fe_3O_4) from *Gracillaria edulis*

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ABSTRACT
The present study was carried out to assess the photocatalytic degradation capacity of Fe_3O_4 nanoparticles using various concentrations of dyes and to assess the degradation processes with best fitted kinetic model.Fe_3O_4 Nanoparticles (NPs) were bio synthesized from Gracilaria edulis seaweed, photocatalytic (presence of UV irradiation) activities of green synthesized Fe_3O_4 nanoparticles were studied for degradation of methylene blue dye at different concentrations of aqueous dye, parallel with Nano particle concentration, time and pH. The dye samples before and after photo catalysis, were subjected to HPLC analysis and FTIR analysis and the changes that has occurred during photo catalysis were observed. In the presence of UV irradiation, the rate of decolourization increased with increase in Fe_3O_4 Nanoparticles and here 9 mg was the optimum concentration. When effect of time was checked with 90 mg/ml aqueous dye solution and 9 mg Fe_3O_4-NPs, it was noticed that after 18th hr, there was no change in rate of degradation, indicating that 18 hrs. was the required optimum time . The optimal pH was found to be 6 for methylene blue. In HPLC analysis the presence and absence of different types of aromatic compounds, hydroxyl and amide groups before and after treatment represents that constituents of the dye had undergone mineralization. FTIR analysis shows the presence and absence of many functional groups before and after treatment. The green synthesized Fe_3O_4-NPs catalyst from G. edulis was found to promote Photocatalytic degradation of methylene blue and fitted with various kinetic models. The Fe_3O_4 Nanoparticles size and its anatase phase property was conducive for effective removal of dye.
***KEYWORDS**: FTIR, Green synthesis, iron oxide nanoparticles, Photocatalytic degradation, textile dye, UV irradiation and Kinetic studies.*

Received 09.12.2020 Revised 04.03.2021 Accepted 24.03.2021

INTRODUCTION

It is known that clean water is becoming scarcer. The main pollutants of surface water were dyes. There are more than 10000 commercially available dyes, they are used in various business most often in the textile industry. Most dyes were not biodegradable, even little concentration as 1 µg can be more harmful. Convectional technologies relating to UV radiation and hydrogen peroxide oxidation are not effective with degradation of dyes as dye pollutants are chemically stable. Nowadays, photocatalytic dye degradation method have got extensive attention due to its effective decolourization of dyes [1].

Photocatalytic degradation is relatively easy; it can be reused and also release less toxic waste than adsorbents [2].Photocatalytic degradation can be carried out effectively with various forms of Nano particles [3]. Due its large surface area [4], Nanoparticles provide cost effective solution to some of the most challenging Environmental cleanup problem. The most commonly used compound were Iron oxide nanoparticles, as it is relatively cheap and safe for use. The nanoparticles shows efficient degradation of methylene blue dye under sunlight irradiation [5]. These Nanoparticles are stable and reusable, so can be ascertained many times for potential photocatalytic activity and remediation of polluted water [6]. Magnetic properties of iron oxide nanoparticles allow active adsorption, and effective removal of dye from aqueous system [7].

Biosynthesized Nanoparticles using Seaweed was found to be impressive in degrading dyes [8]. To improve degradation, recovery and safety Nanoparticles were immobilized with a solid structure [9]. When compared to other irradiation techniques, photocatalytic degradation was found to be faster in decolorizing methylene blue with the presence of Nanoparticles [10]. The self-degradation ability of methylene blue dye under visible light was found to be negligible in the absence of photo catalyst [11].

The aim of this study was to assess the ability of photocatalytic degradation of methylene blue dye by green synthesized Fe_3O_4 nanoparticles under UV irradiation conditions. To assess the adsorption and degradation reaction rate and kinetic models.

MATERIAL AND METHODS

Green synthesized Fe_3O_4 nanoparticles, methylene blue dye were purchased from sigma Aldrich. All glassware's were cleaned with sterile distilled water and rinsed with deionized water.

Preparation of extract:

Gracilaria edulis seaweed were collected, washed with sterile distilled water and dried, then made to powder. 1g of powder was mixed with 100 ml of water and kept on orbital shaker at 120 rpm for 12hrs. Then, the extract were filtered through WhatMann No1 filter paper and stored at 40°C in refrigerator for further use [12].

Synthesisof Iron OxideNanoparticles:

Iron oxide nanoparticles have been synthesized by [13] method with slight modification. 0.1M Ferric chloride solution was prepared by dissolving 1.62g of ferric chloride in 100ml of distilled water. Fe_3O_4-NPs were obtained with the reduction process, by adding 0.1 M Ferric chloride solution to the seaweed extract in a 1:1 volume ratio. The formation of Fe_3O_4-NPs was indicated by the appearance of brick red colour precipitate formed within 5 min [14]. To obtain a colloidal suspension the mixture was stirred for 60 min and kept at room temperature for 30minutes.Then it was centrifuged and washed several times with ethanol and dried at 40°C under vacuum to obtain Fe_3O_4 Nanoparticles. Seaweed extract have the best reduction capability against ferric chloride when compared to other plants that is observed by the external colour change. After the visual confirmation test the Fe_3O_4-NPs were synthesized by using the above procedure for further characterization.

Characterization

UV–visible spectrophotometric analysis was performed with methylene blue dye, before and after treatment with nanoparticles and the absorption maxima were analyzed using UV–visible spectrophotometer (UV-160 v, Shimadzu, Japan) at a wavelength of 200–800 nm.

Photo catalytic studies of Iron Oxide Nanoparticles

The photocatalytic (in presence of UV irradiation) activities of green synthesized Fe_3O_4 nanoparticles were assessed for degradation of methylene blue at different concentrations of aqueous dye (30 mg/ml, 60mg/ml, 90mg/ml, 120mg/ml). Similar optimization studies were carried out with Nanoparticle concentration (3mg, 6mg, 9mg, and 12 mg), time (2 hrs. – 24 hrs.) and pH (3, 6, 7, 9 and 11). The dye samples before and after photocatalysis, were subjected to HPLC and FTIR analysis to observe changes that have occurred with the presence and absence of Nanoparticles.

Effect of Nanoparticle and Nanocomposite dose on dye concentration

Varying concentrations of Fe_3O_4NPs (3, 6, 9, and 12 mg) were added to 30 ml each of the aqueous dye solutions of various concentrations (30, 60, 90, and 12mg) and subjected for photocatalytic activity under UV irradiation. Respective controls were maintained for each dye concentration. Absorbance was recorded at a time intervals of 6 hrs, for a period of 24 hrs and percentage of decolorization was calculated. The dye concentration and, dose of nanoparticles and nanocomposite, with maximum decolorization values was selected for optimization.

Effect of time on decolorization

The optimum dye concentration, nanoparticle dose and composite dose, along with controls were subjected for photocatalysis under UV irradiation for a period of 24 hrs. and absorbance was noted every 3 hrs. for calculation of time of decolorization.

Effect of PH:

Different pH solutions (3, 6, 7, 9 and 11) of the optimum concentration of dye and nanocomposite were added. It was allowed for decolorization under UV irradiation. The absorbance was noted at time intervals of 6hrs for 24 hrs.

Kinetic models

The mathematical suitability of the photocatalytic activity under UV irradiation mediated by the green synthesized iron oxide nanoparticles were analyzed by using various kinetic models in terms of the optimized parameters. The analyzed kinetic models were Langmuir isotherm, Elovich plot and fractional power model.

RESULTS AND DISCUSSION

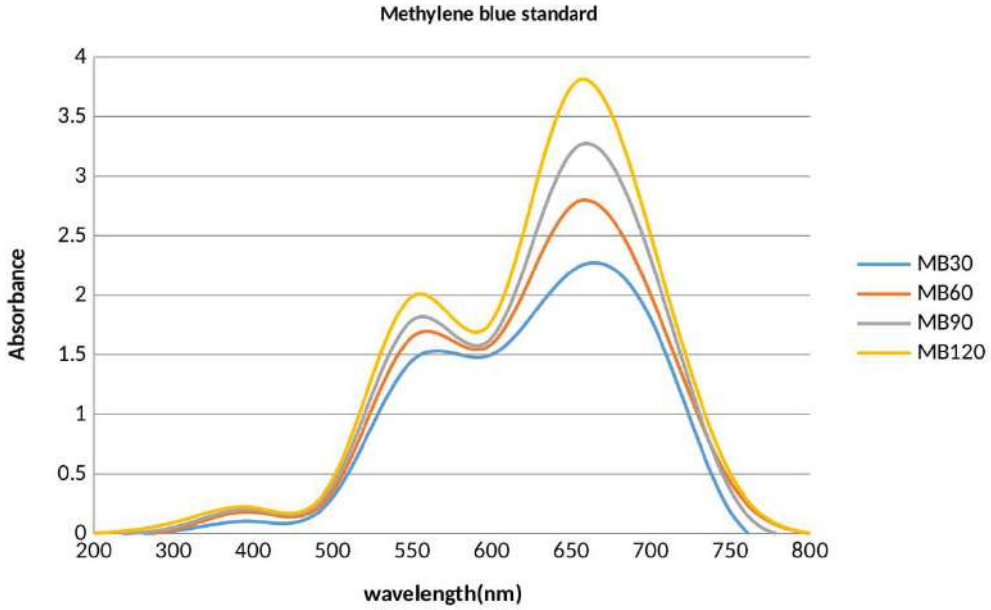

Fig 1: UV absorption of methylene blue dye standard

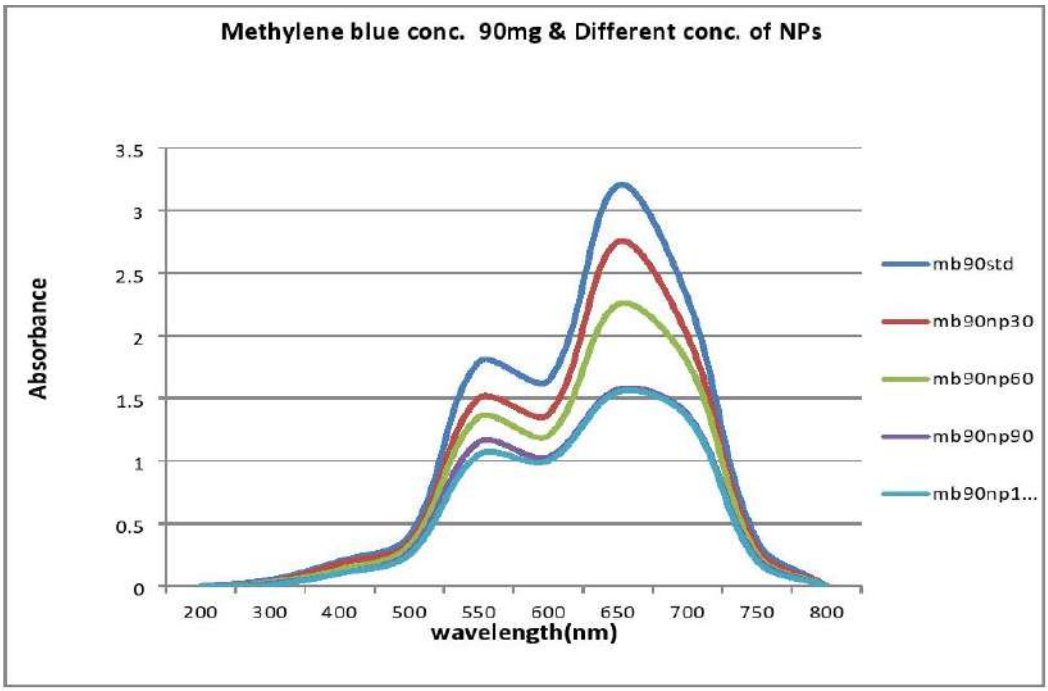

Fig 2: UV absorption of methylene blue conc. 90mg with different conc. of FE_3O_4nanoparticles

Characterization

Absorption is a conventional but efficient technique to remove dyes from aqueous solution[15]. The absorption of a photon of energy creates electrons and holes on the surface. The charged carriers do not recombine and these free radicals cause the oxidation of the dyes [16].Under UV-light irradiation, Iron oxide nanoparticle has been employed as photocatalytic agent to degrade the organic dye, viz. Methylene Blue (MB) in presence of visible light by taking 5 mL aliquot sample, and the absorption of the samples was recorded.

The rate of decolourization was observed with intensity of λmax (665 nm) of the dye. Under UV irradiation, the percentage of decolourization increased with increase in Fe_3O_4-NPs for all concentrations and here up to 9 mg was found to be the optimum concentration for decolourization. The reason for high catalytic activity of synthesized Nanoparticles was due to the high surface area, the main active site of the

catalyst and accelerates the photo degradation[17]. Effect of time was checked with 90mg/ml aqueous dye solution and 9 mg Fe_3O_4-NPs, it was noticed that after 18th hrs. There was no change indicating that 18 hrs. Was the optimum time. When the Nanocomposite size and dye concentration increase beyond the optimum, the photocatalytic activity decreases due to decrease in surface area [18].

The optimal pH observed for methylene blue was 6, which matches with the original pH of the dye. At extreme pH values, there will be reduction in the photocatalytic efficiency consistently with the establishment of coulombic repulsions between homologous charge states [19].The photocatalytic degradation capacity has been reported to be increased with increase in photocatalyst loading regardless of particle morphology [20].

HPLC and FTIR analysis of methylene blue dye before and after photo catalysis

The dye methylene blue contains many characteristic functional groups. The peaks at 1635.64 cm^{-1}, 2075.41 cm^{-1}, 3466.08 cm^{-1} are indicative of alkenes with C-H. Methylene blue dye resulted in nitrate with NO2 bending (420.48 cm^{-1}), sulphides and disulphides with C-S stretching (478.35cm^{-1}), β-substituted naphthalene with C-H out of plane bending (1637.56cm^{-1}), lactams with C-N stretching and sulphones with SO_2 asymmetric stretching (3450.65cm^{-1}), UV mediated photocatalysis of methylene blue in the presence of Fe_3O_4/Nanoparticles resulted in the formation of phenol due to oxidation of the dye [21].

Aromatic hydrocarbons corresponded to the peaks at 732.95 cm^{-1}, 779.24 cm^{-1}, 825.33 cm^{-1}, 979.84 cm^{-1}, 1018.41 cm^{-1}, 1087.85 $cm^{1,}$ 1219.01 cm-1, 1296.16 cm-1 and 1357.88 cm-1. Peaks at 732.95 cm-1 (O-H out-of-plane bending), 1296.16 cm-1 (coupled C-O stretching and O-H in-plane bending), 1357.88 cm-1 (O-H in plane bending, C-O stretching and O-H in-plane bending), 2939.52 cm-1 and 3163.26 cm-1 (conjugate chelation) indicated hydroxyl compounds [22]. Amides are represented by 732.95 cm-1 (OCN deformation), 1743.65 cm-1 (coupled C=O vibrations of acid anhydrides) and 3348.42 cm-1 (N-H asymmetric stretching). HPLC and FTIR analysis helped in understanding whether the dyes constituents had undergone mineralization.

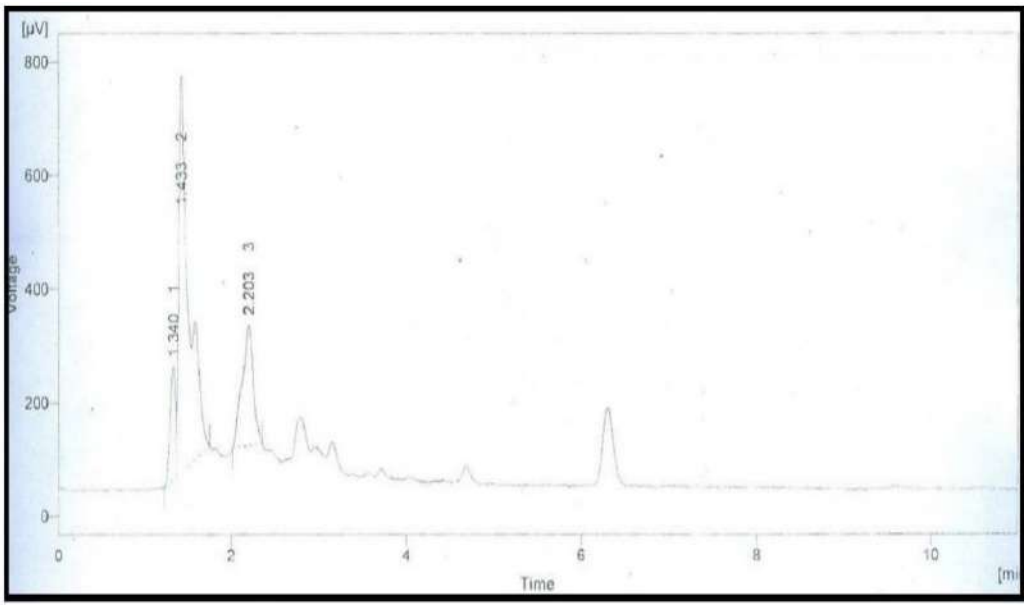

Fig 3: HPLC spectrum of methylene blue dye before UV mediated photo catalysis in the absence of nanoparticles

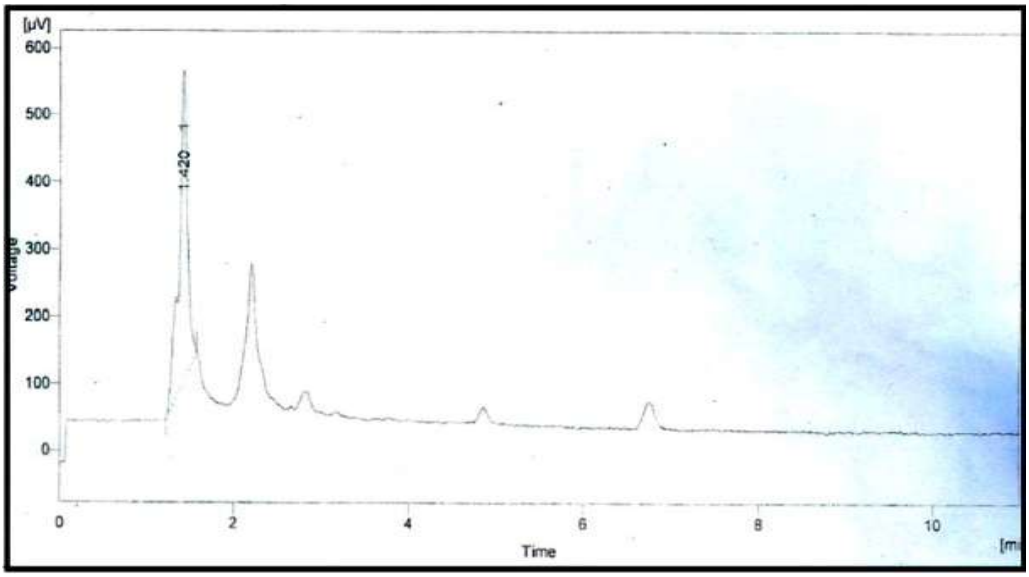

Fig 4: HPLC spectrum of methylene blue dye after UV mediated photo catalysis in the presence of nanoparticles.

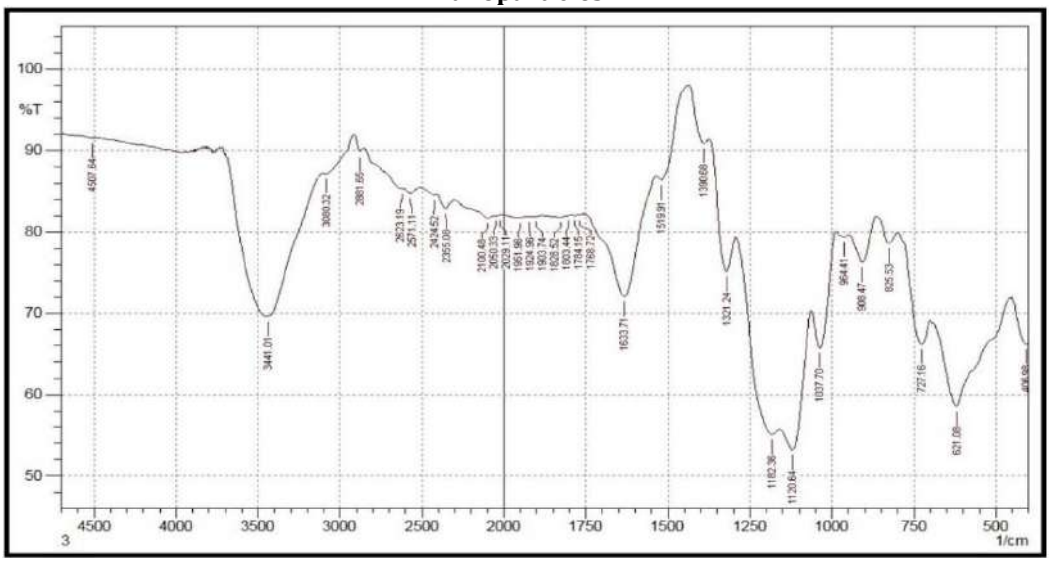

Fig 5: FTIR spectrum of methylene blue dye before UV mediated photo catalysis in the absence of nanoparticles

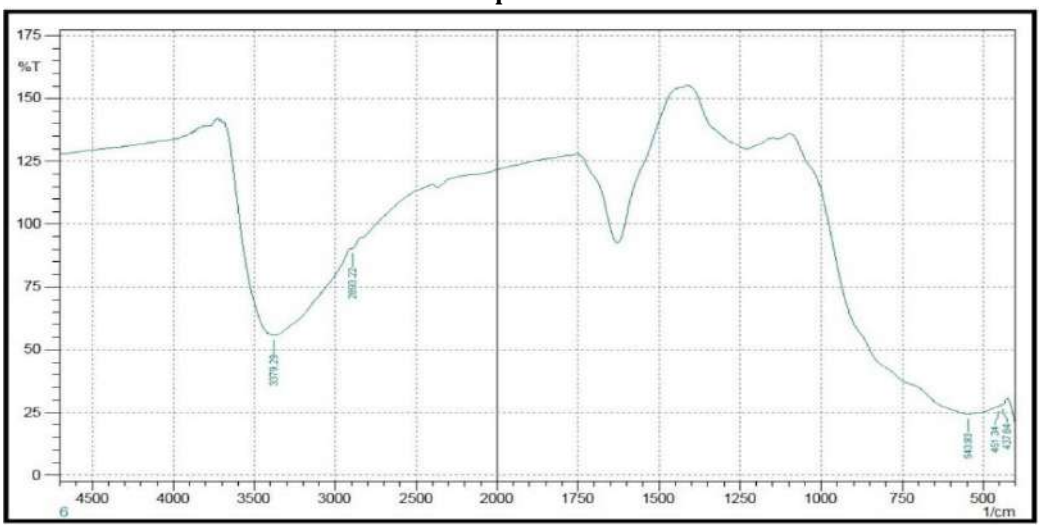

Fig 6: FTIR spectrum of methylene blue dye after UV mediated photo catalysis in the presence of nanoparticles

KINETIC ANALYSIS

The mathematical suitability of the photocatalytic activity under sunlight and UV irradiation mediated by the green synthesized nanoparticles and composite were analyzed by using various kinetic models in terms of the optimized parameters.

Langmuir isotherm

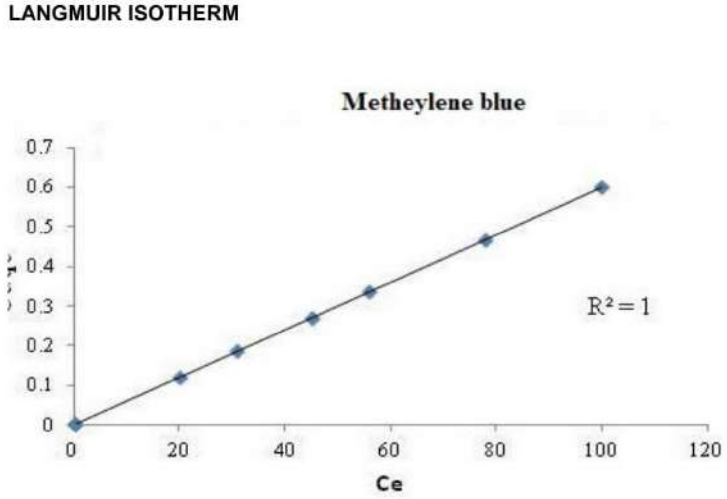

Fig 7: Langmuir isotherm for UV-mediated photocatalysis

UV mediated photo catalysis of the textile dye methylene blue via the presence of nanoparticles was indicated by the value of R2 which was found to be 1 in all cases. The influence of the initial concentration of the solute on the photocatalytic degradation rate was described by a pseudo-first-order kinetics, which is rationalized in terms of the Langmuir model modified to accommodate reactions occurring at a solid–liquid interface [23, 24]. The adsorption constant obtained from dark adsorption isotherms are usually significantly different to those determined by Langmuir model.

Elovich plot

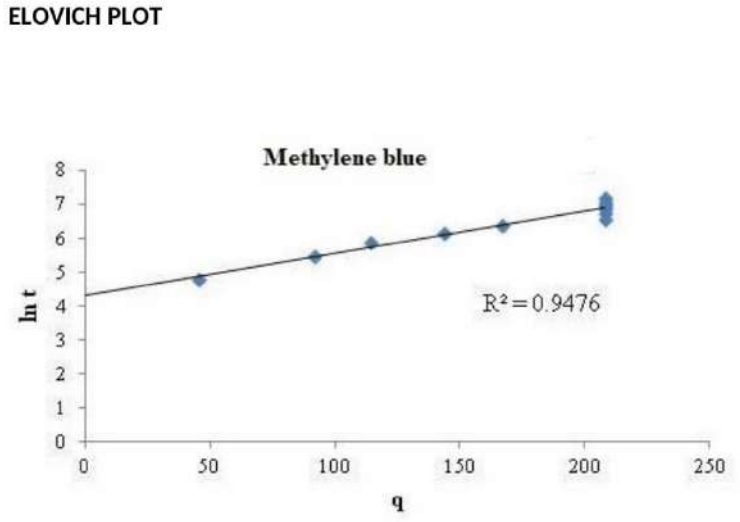

Fig 8: Elovich plot for UV-mediated photocatalysis

The Elovich plot for UV mediated photocatalysis agreed that the nanoparticles and composite were good photocatalysts for the photocatalysis of the textile dye Methylene blue as indicated by the R2 values which were all above 0.8. The R2 values confirmed the chemical nature of adsorption. Elovich model has been correctly applied for describing chemical adsorption[25].

Fractional power model

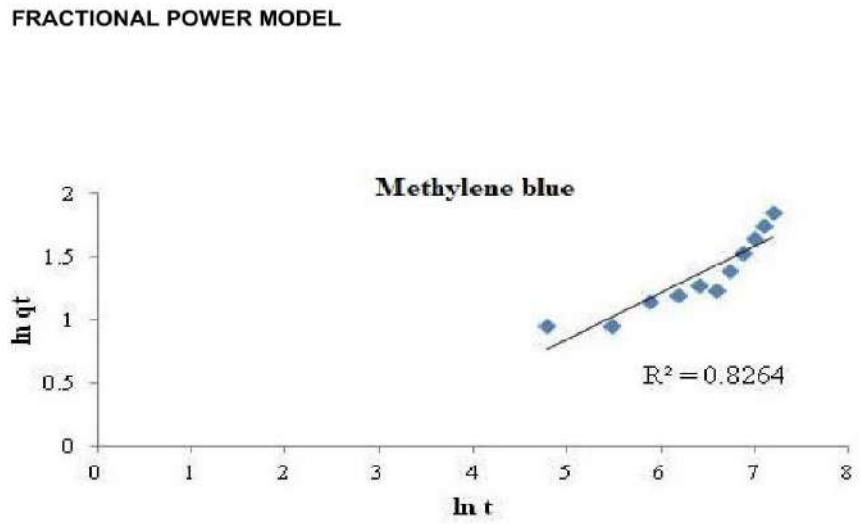

Fig 9: Fractional power model for UV mediated photocatalysis

As per R2 values, UV-mediated photocatalysis was best within the presence of the nanoparticles was ascertained by experimentation too. However, the photocatalysis mediate by Fe_3O_4 didn't consider divisional power model although it absolutely was found to be effective by experimentation. Thus green synthesized - Fe_3O_4 nanoparticles demonstrated high capability as catalyst, both in terms of kinetics and percentage of degradation [26]**.**

CONCLUSION

Pollution by textile dyes has drawn attention on the vital need for developing new Eco friendly purification technologies like photocatalysis by metal oxide nanoparticles. The present study has emphasized that Seaweeds can be put into use for development of efficient photocatalysts. The various experiments carried out indicted that UV is a better light source for optimum decolorization and the Fe_3O_4 nanocomposite was the best catalyst. The various kinetic models also supported these observations thereby citing that "green" Nano catalysts are the solution to the ever-growing water pollution menace.

ACKNOWLEDGEMENT

The support and facility provided by the Management of SNMV College of Arts and Science, Coimbatore, India is acknowledged.

FUNDING

The authors received no financial support for the research, and /or authorship of this article

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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CITATION OF THIS ARTICLE

G. Subhashini, P. Ruban. Photocatalytic Degradation of Methylene Blue Dye using Green Synthesized Iron Oxide Nanoparticles (Fe_3O_4) From *Gracillaria edulis*. Bull. Env. Pharmacol. Life Sci., Vol 10[5] April 2021: 215-222.

PREDICTION OF HEART DISEASES USING NAIVE BAYES ALGORITHM

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Abstract

The medical system is an inevitable task to be done in human life. The business of medical care has become a significant field in a wide area of medical science. The healthcare industry has a large amount of data and hidden information. Effective conclusions are drawn with this hidden information through the use of datamine techniques. Several tests are performed to diagnose heart disease in the patient; However these tests can be reduced by datamine. But there is a lack of analysis of the tool to provide effective test results with hidden information, so a system has been developed using data mining algorithms to classify data and diagnose heart disease.Datamining acts as a solution to many health problems. The NaiveBayes Algorithm is one such database technique that helps diagnose a patient with heart disease. This paper analyzes some parameters and predicts heart disease, and recommends a Cardiovascular Disease Prognosis System (CDPS) based on datamining approaches.

Keywords: healthcare system, datamining, heart diseases, naïve bayes algorithm, Cardiovascular Disease Prognosis System.

1. Introduction

The health system is provided by preventing or treating disease by maintaining mental and physical health. Heart disease is the leading cause of death in the world today. The World Health Organization (WHO) estimates that 12 million deaths worldwide are caused by heart disease each year. More than 80% of deaths in the world are caused by heart disease. Who estimates that in the future, nearly 23.6 million people will die of heart disease. Euro Heart Survey on Heart Disease conducted in 25 countries, including adults with moderate to severe congenital heart disease, Infectious endocardial anterior valve intervention. Heart disease was congenital in 71.9% of patients, with 28.1% having previous intervention. The average age ranged from 64 to 14 years. Degenerative etiologies, where it often occurs in aortic heart disease and mitral regurgitation, while most of the causes of mitral stenosis appear here. Data processing plays an important role in intelligent medical health systems. The processing of medical data is considered an important and complex task in health care, which must be carried out accurately and efficiently. Health data mining seeks to address real-world health issues in diagnosis and treatment. The relationship between disorders and the true cause of disorders and the effects of symptoms that appear spontaneously in patients can be assessed using the cardiovascular prognosis method, which is a computerized method for diagnosing heart disease based on previous data and information.

Heart disease is a term that assigns to a large number of medical conditions related to heart. These medical conditions describe the abnormal health conditions that directly influence the heart and all its parts. Heart disease is a major health problem in today's time. This paper aims at analyzing the various data mining techniques introduced in recent years for heart disease prediction.

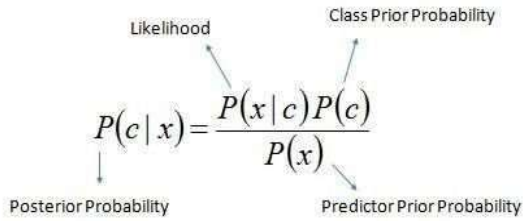
2.METHODOLOGY

Naive Bayes:

Naive Bayes classifiers is a0Tprobabilistic classifiersbased on applyingBayes'theoremTU withstrong(naïvindependence assumptions between the features.A Naive Bayesian model is easy to build, with no complicated iterative parameter estimation which makes it particularly useful in the field of medical science for diagnosing heart patients. Despite its simplicity, the Naive Bayesian classifier often does surprisingly well and is widely used because it often outperforms more sophisticated classification methods.

Bayes theorem provides a way of calculating the posterior probability, P(c|x), from P(c), P(x), and P(x|c). Naive Bayes classifier assumes that the effect of the value of a predictor (x) on a given class (c) is independent of the values of other predictors. This assumption is called class conditional independence

Equations:



$$P(c \mid \mathrm{X}) = P(x_1 \mid c) \times P(x_2 \mid c) \times \cdots \times P(x_n \mid c) \times P(c)$$

Fig

- P(c|x) is the posterior probability of class (target) given predictor (attribute).
- P(c) is the prior probability of class.
- P(x|c) is the likelihood which is the probability of predictor given class. • P(x) is the prior probability of predictor

Where C and X are two events (e.g. the probability that the train will arrive on time given that the weather is rainy). Such Naïve Bayes classifiers use the probability theory to find the most likely classification of an unseen (unclassified) instance . The algorithm performs positively with categorical data but poorly if we have numerical data in the training set.

3.Dataset

The data set used in this work are clinical data set collected from one of the leading diabetic research institute in Chennai and contain records of about 500 patients. The clinical data set specification provides concise, unambiguous definition for items related to diabetes. The diabetes data set is developed to ensure people with diabetes have up to date records of their risk factors, current management, treatment target achievements and arrangements and outcomes of regular surveillance for complications, to help them monitor their care and make informed choices about their management. It will also ensure that when people with diabetes meet health care professionals the consultation is fully informed by comprehensive, up to date and accurate information. The diabetes attributes used in our proposed system and their descriptions are shown in Table 1.

Name	Type	Description
Age	Continuous	Age in years
Sex	Discrete	1 = male 0 = female
Cp	Discrete	Chest pain type: 1 = typical angina 2 = atypical angina 3 = non-angina pa 4 =asymptomatic
Trestbps	Continuous	ng blood pressure (in mm Hg)
Chol	Discrete	Serum cholesterol in mg/dl
Fbs	Discrete	ood sugar > 120 mg/dl: 1 = true
		0 = false
Restecg	Continuous	lectrocardiographic results: 0 = normal = having ST-T wave abnormality =showing probable or define left ventricular hypertrophy by Estes 'criteria
Thalach	Discrete	Maximum heart rate achieved
Exang	Discrete	Exercise induced angina: 1 = yes 0 = no
Slope	Discrete	The slope of the peak exercise segment : 1 = up sloping 2 = flat 3= down sloping
Diagnosis	Discrete	Diagnosis classes: 0 = healthy 1= possible heart disease

Table 2: Parameters of Heart Diseases Prediction System

4.Data mining Tool

Weka is a collection of machine learning algorithms for data mining tasks. The algorithms can either be applied directly to a dataset or called from your own Java code. Weka contains tools for data pre-processing, classification, regression, clustering, association rules, and visualization. It is also well-suited for developing new machine learning schemes. The experiments are conducted using the weka

tool and the results are obtained. We have used the navie bayes method to perform classification by using 70% of percentage split.

5.Data Analysis:

In this system the medical data set is classified based on the classes present/absent.The propo ed naïve bayes model was able to classify 86.4198% of the input instances correctly and the incorrect instances was 13.5802% for 70% of percentage split. With the total of 81 instances 70% was classified as correct and 11% instances was incorrect.The results clearly states that naive bayes provides better results regarding the people affected by heart diseases.

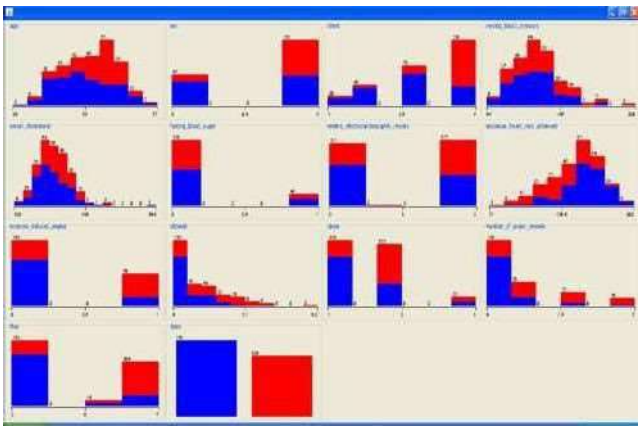


Fig.1. Attribute value distribution The blue colored regions in the graphs in Figure 1 denote high cholesterol values. From the graphs we can see that, most of the diabetic patients with high cholesterol values are in the age group of 45 – 55, have a body weight in the range of 60 – 71, have BP value of 148 or 230, have a Fasting value in the range of 102 – 135, have a PP value in the range of 88 – 107, and have a A1C value in the range of 7.7 – 9.6**.**

7 .Results and Discussions:

The results of our experimentation are shown in Fig2.

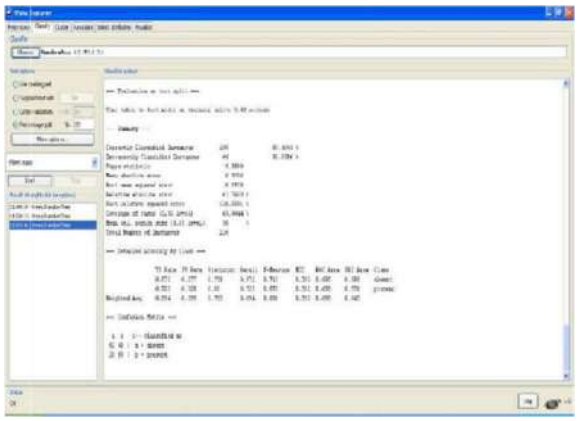


Fig. 3 Result window of the data mining process

The proposed naïve bayes model was able to classify 74% of the input instances correctly. It exhibited a precision of 71% in average, recall of 74% in average, and F-measure of 71.2% in average. The results show clearly that the proposed method performs well compared to other similar methods in the literature, taking into the fact that the attributes taken for analysis are not direct indicators of heart disease.

8 .Conclusion:

Data mining applications are used vastly in the medical field to detect diseases and diagnosis the heart patient based on the data set and the attributes provided. Researchers have been investigating applying different data mining techniques to help health care professionals in the diagnosis of heart disease. In the proposed work navie bayes algorithm is used to classify the data set because navie bayes provides accurate results, with these results heart diseases among people is predicted. Thus heart diseases prediction system successfully diagnose the medical data and predicts the heart diseases.The results thus obtained shows that navie bayes algorithm provides 86.4198% of accuracy with minimum time.

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A dualistic approach to investigate the remedial potential and edible property of Pleurotus ostreatus on hydrocarbon-contaminated soil

Article *in* Journal of Applied Biology & Biotechnology · April 2022

DOI: 10.7324/JABB.2022.100310

4 authors, including:

Arunkumar Dhailappan
Shri Nehru Maha Vidyalaya College of Arts & Science
2 PUBLICATIONS **0** CITATIONS

Journal of Applied Biology & Biotechnology Vol. 10(3), pp. 70-78, May-Jun, 2022
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2022.100310

A dualistic approach to investigate the remedial potential and edible property of *Pleurotus ostreatus* on hydrocarbon-contaminated soil

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ARTICLE INFO

Article history:
Received on: August 08, 2021
Accepted on: December 06, 2021
Available online: April 10, 2022

Key words:
Hydrocarbon-contaminated soil,
Mycoremediation,
Pleurotus ostreatus,
Edibility

ABSTRACT

Hydrocarbon soil contaminants are the major concern globally that causes an adverse impact on human health and reduces the functionality of ecosystem. Mycoremediation certainly has an edge over other bioremediation process in combating the targets most efficiently. This study attempts to investigate the potentials of an edible mushroom dualistically remediating the hydrocarbon-contaminated (HC) soil as well analyzing its impact on edibility. Many studies have been done over the past few decades, no attempts were made to analyze the presence or absence of toxicity sorption in edible organisms after remediation. HC soils from eight different petrol filling stations (Site A to H) of Coimbatore district were collected and analyzed for its physical and chemical properties. Based on physicochemical parameters, Site D1 that showed maximum total petroleum hydrocarbon (TPH) content (12,200 mg/kg) was chosen for this study. Five experimental sets were prepared by mixing the lignocellulose substance with HC soil at different ratio (Set I–Set V). Among the experimental sets, *P. ostreatus* showed maximum hydrocarbon degradation efficiency and well-defined fruiting body formation, high biological efficiency (140%), moisture (90%), and crude protein (32%) in Set III trials. Kinetic studies on TPH degradation fitted to the first-order kinetic model revealed a higher degradation rate constant, k (0.097 day-1) and lower biodegradation half-life t1/2 (7 days). Fourier transform infrared spectrum of *P. ostreatus* after remediation showed the complete absence of peak that corresponds to petroleum hydrocarbon, thereby preliminarily confirms the possibility of safe consumption.

1. INTRODUCTION

Petroleum spillage in the marine and terrestrial niches has a devastating effect on human community and other biota. Marine spills could not be completely eradicated unless it is drilled for oil or transported across the ocean, however, the release of petroleum hydrocarbons in these environments drastically alters the biological systems [1,2]. Compared to marine niches, the terrestrial ecosystem receives annually increasing amounts of oil spills. Contaminants in terrestrial sites need to be eradicated promptly as it leads to alter the pristine environment [3]. Petroleum compounds consist a mixture of aromatic and aliphatic hydrocarbons ranging from C4 to C12 carbon atoms [4]. Continuous exposure to hydrocarbons causes an adverse effect in human health such as irritations, respiratory problems, stress, nephrotoxicity, hemolytic anemia, and negative impacts on genetic and immune systems [5]. Besides the spillage, it results in limiting the nitrogen and phosphorous ratio in soil which causes detrimental effects in agriculture sectors [6]. Although many physical and chemical attempts (land filling, incineration, stabilization, and chemical oxidation) are employed for the removal of substantial portion of hydrocarbon spills, the remediation process was found to be unsatisfied as such practices lead to raise in atmospheric CO_2, NO_x, and So_x levels [7]. It was also reported that such implementation contaminates the ground water by producing hazardous leachates [8]. Moreover, physicochemical treatments have certain constrains such as expensive processing and requires involvement of more skilled persons [9]. Scientists are, therefore, intensively focusing on clean and economically feasible technology to mitigate the hydrocarbon contaminants for more than 2 decades [10,11].

Mycoremediation, employing fungi, has a peculiar role in degrading the toxic and xenobiotic compounds in the environment, since fungi due to its hyphal network can able to penetrate in to the soil matrix more efficiently than other organisms [12]. The extracellular enzymes secreted by fungal strains in the polluted environment have a cleavage effect on the polymeric substances, thereby insoluble compounds are hydrolyzed to soluble form which further absorbed and metabolized by the intracellular enzymes [13]. Mushrooms, macroscopic filamentous fungi, have the potential to degrade wide range of environmental pollutants, and convert them to useable products using different methods for instance biodegradation, biosorption, and bioconversion [14]. Consequently, such remediation strategies are considered to have an edge over other bioremediation process [15,16]. Mushroom secretes enzymes such as manganese-dependent peroxidases, lignin peroxidase,

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phenol oxidases, and H_2O_2-producing enzyme for the degradation of polycyclic aromatic hydrocarbons [17]. Like aromatic hydrocarbons, the major components persist in petroleum derivatives are aliphatic hydrocarbons (alkanes, branched alkanes, and cycloalkanes). Most of the fungal strikes over these toxic compounds are more resistant in particular for cyclic aliphatic hydrocarbons due to the absence of double bond in the terminal position [18]. Aliphatic hydrocarbon degradation does not rely on the same enzymatic complex which is used to degrade the aromatic compounds in mushrooms. Instead, it requires a heme protein, cytochrome P450 monooxygenases that involve a hydroxylation reaction where an oxygen atom is introduced in the terminal methyl group to form alcohol and then to corresponding aldehyde and fatty acid [19]. The resultant fatty acid subsequently undergoes p-oxidation to form acyl-CoA ester and eventually catabolized in the TCA cycle [20].

Mushrooms, apart from its versatile role in remediating the contaminants, famed for its rich source of protein, fiber, and antioxidants. A special consideration and extensive research should be exercised in consuming edible mushroom cultivated in severe environmental contaminated places [21]. Meager reports were discussed to ensure the safety consumption of edible mushrooms after they were exploited for remediation purpose [22]. Therefore, the present investigation dualistically focused to degrade the petroleum hydrocarbon-contaminated (HC) soil using an edible oyster mushroom (*Pleurotus ostreatus)* and subsequently attempts to study the characteristic changes for any hindrance or toxicity in the edible properties of mushroom.

2. MATERIALS AND METHODS

2.1. Collection of Soil Samples

Soil samples were obtained in eight different fuel filling sites of Coimbatore District (Site-A: Ukkadam; B: Pothanur; C: Malumachampatti, D: Kinathukadavu; E: Vadachitur; F: Negamam; G: Thoppampatti, and H: Pollachi), Tamil Nadu, India. Samples were taken from two points of each fuel stations, one at the top layer (Site A0, B0, C0, D0, E0, F0, G0, and H0) and second at 1 m depth from the ground surface (Site A1, B1, C1, D1, E1, F1, G1, and H1). Samples were sieved using 2 mm mesh to obtain a homogenous mixture and were transported immediately to laboratory for the physicochemical analysis.

2.2. Physicochemical Analysis of HC Soil

HC soil was analyzed for the soil texture, porosity [23], permeability [24], and moisture content (gravimetric method) [25]. The total nitrogen [26] content, phosphorous [27,28], and total carbon content were determined according to the standard procedure (Shimadzu-TOC-V_{CSH}, Japan) [29].

The collected soil samples were homogenized and precisely 2 g of soil was weighed in 100 mL container. One-fourth strength of Ringer's solution (20 mL) was added to the content and vortexed for 2 min. After vortexing, the samples were sonicated using sonicator (Thermo Fisher Scientific, USA) and subsequently allowed to stand for 2 min. An aliquot of 10 mL of soil suspension was removed and serially diluted (10^{-5}–10^{-4}) in the aforementioned strength of Ringer's solution. To determine the indigenous microbes, 10 mL of each dilution was added to the $^1/4$ strength of LB medium for bacteria using pour plating technique and acidified potato dextrose agar containing streptomycin (1 mg/100 ml) for fungal enumeration [30]. The samples were incubated for 24 h at 30°C (bacteria) while fungi were incubated at room temperature (3–5 days), further, the colony-forming units were enumerated and expressed as CFU g^{-1} of dry soil.

2.3. Total Petroleum Hydrocarbon (TPH) Estimation

TPH content of the soil was examined by cold toluene extraction method [31]. Precooled 20 mL of toluene was added to the flask containing 10 g of petrol contaminated soil sample. The flask was placed in the orbital shaker for 30 min. The liquid phase was collected by filtration and the extract was measured at 420 nm in the ultraviolet (UV) spectrophotometer (UV–Vis Spec. Elico SL159). Soil TPH was determined using a standard curve prepared with toluene diluted oil. The percentage of degradation (D) was calculated as

D = TPHi - TPHr/TPHi

Where, TPHi and TPHr correspond to the initial and residual TPH concentration, respectively.

2.4. Kinetic Analysis

TPH degradation data were fitted with a first-order kinetic model. The reaction kinetics is defined by the equation

$$C = C_0 e^{-kt} \quad (1)$$

Where, C is the hydrocarbon content at the time t and C0 is the initial hydrocarbon concentration (mg kg^{-1}), k is the degradation rate constant (day^{-1}) [32]. Eq. (1) was represented in linear integrated form

$$Ln (C/C_0) = -ktq \quad (2)$$

The degradation rate constant (k) was determined using Eq. (2). The half-life time (t ½) of the remedial activity was calculated using Eq. (3) [33,34].

$$t½ = ln (2)/k \quad (3)$$

Where, k is the rate constant determined from Eq. (2).

2.5. Substrate and Spawn Processing

Paddy straw was collected from the local farm near Pollachi, Tamil Nadu. The paddy straw was approximately chopped to 2 cm and soaked in water for overnight and air-dried to remove excess moisture content. HC soil used in the study was shed into the perforated sterile polyethylene bags mixed with paddy straw at five different ratios (Set I – Sole Paddy straw; Set II – Paddy straw: HC soil [1:1]; Set III – Paddy straw: HC soil [2:1]; Set IV – Paddy straw: HC soil [1:2]; and Set V – Sole HC soil). Pure culture of *P. ostreatus* was obtained from Tamil Nadu Agricultural University, Coimbatore (India). The collected spawn was weighed equally (5 g) and added to all the five experimental sets. All the tested sets were performed in triplicates under appropriate conditions (temperature – 22–25°C; humidity – 60%) and the mushroom growth was examined after a period of 21 days.

2.6. Determination of Yield and Biological Efficiency (BE)

The yield of the mushroom was calculated by weighing the fruiting body and percentage of BE was determined [35].

BE (%) = (fresh weight of mushroom ×100)/substrate dry weight

2.7. Estimation of Crude Protein and Moisture

Crude protein was estimated by multiplying the factor 6.25 with total Kjeldahl nitrogen obtained (Kel Plus Kes 6L). To determine the

moisture content, 1 g of fresh mushroom was weighed and kept at 60°C overnight until for obtaining a standard weight. The moisture content of mushroom was estimated [36].

Moisture content (%) = 100 × (W1 - W2)/W1

Where, W1= weight of fresh sample and W2 = weight of dry sample.

2.8. Fourier transform Infrared (FTIR) Analysis

P. ostreatus from all the tested sets were collected and washed in distilled water to remove the debris. The mushroom samples were dissected manually and hot dried in the oven at 60°C to constant weight. Dried samples were pulverized using grinder and sieved with 80 µm mesh [37]. About 1 mg of sample was mixed with 100 mg of spectroscopic grade potassium bromide to prepare a translucent sample disc. The FTIR spectrum was analyzed between 400 and 4000 cm^{-1} range. Each spectrum was performed with 40 scans min^{-1} (Shimadzu, IR Affinity 1, and Japan).

3. RESULTS AND DISCUSSION

3.1. HC Soil Analysis

The HC soil samples collected from eight different sites [Figure 1] were characterized for the physicochemical parameters [Table 1]. The texture of the soil was observed to be red loamy (Site A_0 to Site D1) and sandy loamy (Site E_0 to Site H1). The soil pH was found to be near neutral to alkaline (6.9–8.4). The total nitrogen content was noted to be 0.12–0.22%, carbon content was in the range of 0.58–1.66%, and phosphorous was found to be 10.2–33.89 mg/kg^{-1}. Soil porosity was noted to be 15–31% and permeability was found to be in the range of 0.032–0.065 m/h. Successful hydrocarbon degradation is based on soil characteristics precisely due to certain significant factors that hinder the remedial effect such as soil texture, permeability, pH, water holding capacity, nutrients, oxygen, and microbial population [38].

The indigenous microbial count in the HC soils from A_0 to H1 sites showed distinct results. In the present study, the bacterial count in the red loamy regions was observed to be high (4.96×10^6–5.82×10^6 cfu g^{-1}) whereas in sandy loam, it showed a reduced count (2.79×10^6–3.40×10^6 cfu g^{-1}). Likewise, the fungal enumeration was observed to be maximum in red loam (0.13×10^5–0.23×10^5) regions and minimum in sandy loams (0.06×10^5–0.11 m $\times 10^5$). Microbial distribution in different soil textures is based on the nutrient content and soil moisture in the environment [39]. The results noticed from our study also proved that unlike fine textured soil (red loam, Site A to D1), the course textured soil (sandy loam, Site E to H1) did not influence the microbial count. This might be due to the slight alkaline pH as well as the availability of organic matter and nitrogen content that persist in red loamy regions.

3.2. TPH Analysis

TPH content was analyzed in all the selected sites (Site-A to Site-H) [Figure 2]. The graph showed maximum TPH content was noticed in

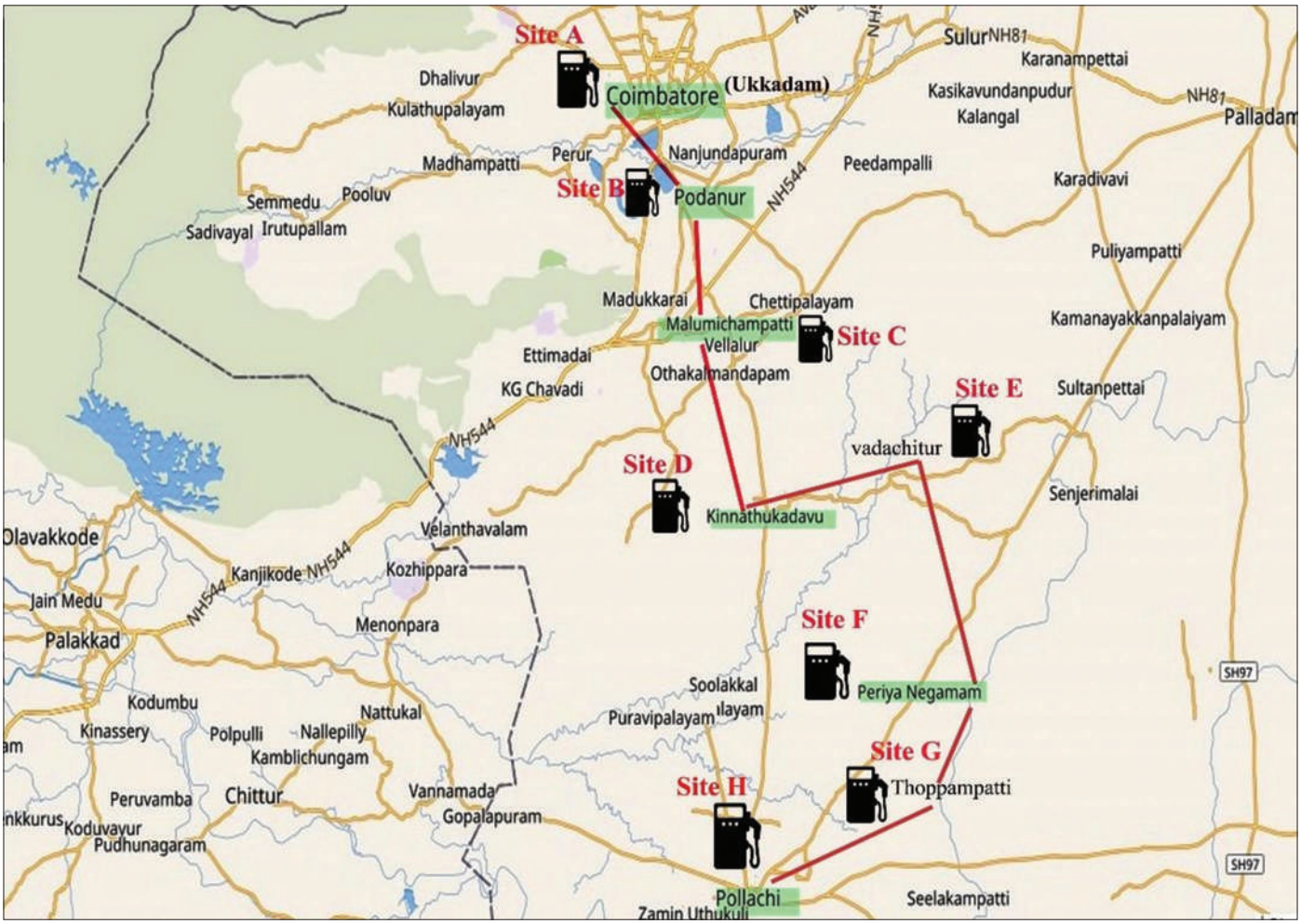

Figure 1: Map showing the sample collected sites near petrol filing points

Table 1: Physicochemical properties of the soil at collected hydrocarbon-contaminated sites

Parameters	Units	Hydrocarbon-contaminated sites																	
		A_0	A_1	B_0	B_1	C_0	C_1	D_0	D_1	E_0	E_1	F_0	F_1	G_0	G_1	H_0	H_1
Type		Red loam	Red loam	Red loamy	Red loamy	Red loam	Red loam	Red loam	Red loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Texture		Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Coarse	Coarse	Coarse	Coarse	Coarse	Coarse	Coarse	Coarse
Moisture	%	7.0	7.5	8.2	8.9	6.5	7.0	7.2	7.1	6.8	7.3	5.3	5.7	6.2	6.8	4.2	4.9
Porosity	%	31	27	26	22	19	17	16	15	29	27	30	28	22	20	23	20
Permeability	m/h	0.065	0.060	0.055	0.047	0.040	0.038	0.034	0.032	0.062	0.058	0.064	0.060	0.047	0.043	0.049	0.043
Total N2	%	0.20	0.22	0.19	0.20	0.16	0.14	0.16	0.13	0.21	0.20	0.17	0.16	0.13	0.12	0.16	0.15
Total C	%	0.58	0.69	0.78	0.84	1.25	1.36	1.26.	1.66	0.86	0.82	0.97	1.05	0.77	0.82	0.96	1.15
Phosphorous	mg/k-1	12.4	10.2	10.89	13.6	32.5	33.89	20.7	23.6	32.7	20.7	24.2	28.6	21.6	19.5	18.2	17
pH	-	7.8	7.6	7.9	7.7	7.1	6.9	8.1	7.6	8.4	8.1	7.2	7.1	7.8	7.6	8.1	7.9
Bacterial	Cfu/g	5.52×10^6	5.82×10^6	5.22×10^6	5.42×10^6	5.67×10^6	5.73×10^6	4.96×10^6	4.98×10^6	3.33×10^6	3.12×10^6	2.89×10^6	2.79×10^6	3.12×10^6	3.26×10^6	3.40×10^6	3.29×10^6
Fungi	Cfu/g	0.22×10^5	0.23×10^5	0.19×10^5	0.24×10^5	0.15×10^5	0.14×10^5	0.13×10^5	0.14×10^5	0.09×10^5	0.08×10^5	0.07×10^5	0.06×10^5	0.10×10^5	0.09×10^5	0.10×10^5	0.11×10^5

all the soils that were collected at 1 m depth compared to surface soils. Among the soils collected at depth, Site A1 and Site D1 exhibited high hydrocarbon contaminants of 11,550 mg/kg and 12,200 mg/kg, respectively. The variation of TPH content in different sites may also be due to the activity of extracellular enzymes secreted either by native fungal strains or it depends on the availability of hydrocarbon utilizing bacteria in the contaminated sites [40]. Microbes that persist in the HC soil have a positive effect on the controlling TPH content both in the liquid and solid phases as these organisms are very well acclimated to the environmental conditions [41]. In our previous study, we identified indigenous microbes (hydrocarbon utilizing bacteria) present in HC sites that have a synergetic effect on the rate of hydrocarbon degradation (data not shown) [42].

Site A1 and Site D1, besides its high TPH content, also consist of increased microbial count. Interestingly, it has been noticed a distinct features between these two sites where Site D1 has a low soil porosity 15% and permeability 0.032 m/h when compared to Site A1 (27% soil porosity and 0.060 m/h soil permeability). The observation in this study clearly reveals that the Site D1 that possesses low soil porosity and lesser permeability has the ability to clench hydrocarbons in the soil for years regardless to enriched microbial population.

3.3. Mycoremediation Study

Among the HC sites, Site D1 was chosen for mycoremediation study based in the earlier findings. HC soil from Site D1 was added to paddy straw amended with the spawn of edible oyster mushroom (*P. ostreatus).* The experiment was performed with five different experimental sets to observe the degradation efficacy of hydrocarbon as well the impact of mushroom growth. Figure 3 shows the distinct development of fruiting body in different sets of paddy straw: HC soil after a period of 21 days. The reason for the difference in the yield of mushroom may be due to the availability of nutrients in each experimental set. Our results agree with the findings of Naraian *et al.,* where the growth of the mushroom and primordial development is based on the lignocellulosic substance, particularly on the C/N ratio [43]. Subsequent findings in our study also showed maximum BE (140.26%) and the highest crude protein content (32%) was recorded in Set III (paddy straw: HC soil [2:1]) when compared to other tested sets. Nearly similar (BE%) was also observed in Set I and Set II [Figure 4]. Moisture in all the tested sets was found to have no significant difference despite increased paddy straw concentration has a slight impact on its content (i.e., Set I – 87% and Set III – 90%). The reason may be due to suitable nutrient ratio increases the yield and the fruiting body efficacy available in Set III. Similar type of results has been stated by Yang *et al.* that the increased mycelia growth and fruiting bodies depend on the optimal condition of the carbon and nitrogen ratio [44].

3.4. Biomass Yield and Weight

Figure 5 shows the TPH degradation rate of the soils in all the experimental sets (except Set I) which showed interesting patterns. Simultaneously estimation for highest mushroom yield (1402 g/kg of substrate) by *P. ostreatus* was observed in Set III (paddy straw: HC soil [2:1]). The TPH degradation rate in Set III was 87% which was the maximum among the tested sets. The mushroom yield both in Set I (sole paddy straw) and Set II (paddy straw: HC soil [1:1]) showed similar pattern of productivity (around 1200 g/kg of substrate) followed by the Set IV (1054 g/kg of substrate). Degradation rate of 64% and 42% was achieved in the Set II and Set IV, respectively. The interesting fact observed in our study was the yield of the mushroom in Set V (sole HC soil) did not show a remarkable growth, however, 452 g/kg of substrate

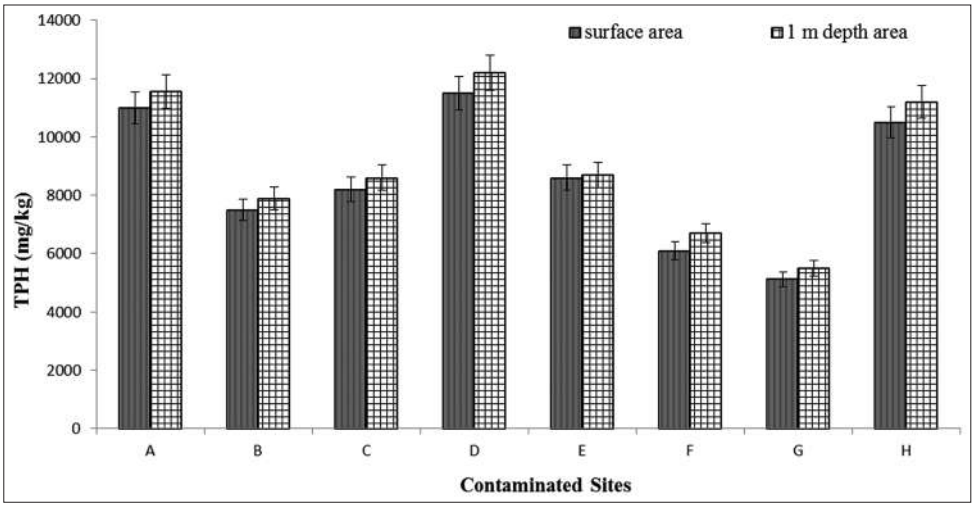

Figure 2: Total petroleum hydrocarbon content in different hydrocarbon-contaminated sites. Data are the mean ± standard deviation of three replicates

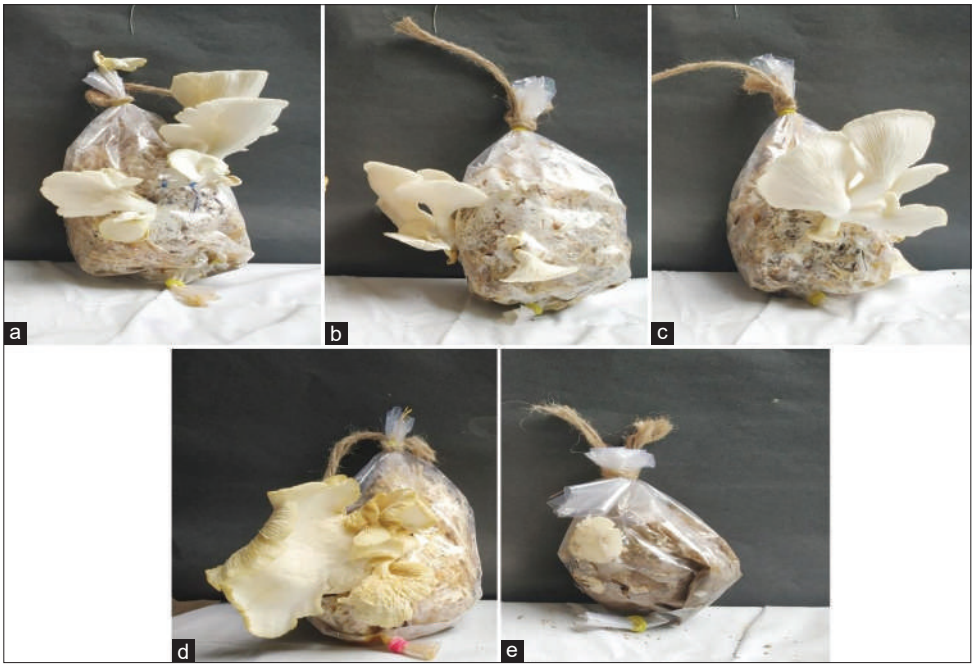

Figure 3: Fruiting body formation of *Pleurotus ostreatus* in different experimental sets after 21 days. (a) Set I – Sole paddy straw; (b) Set II – paddy straw: Hydrocarbon-contaminated (HC) soil (1:1); (c) Set III – paddy straw: HC soil (2:1); (d) Set IV – paddy straw: HC soil (1:2); and (e) Set V – sole HC soil

of yield was noticed with 23% of degradation efficiency. These results obviously showed that mushroom growth and remedial effect solely depend on the appropriate content of C: N sources available in the experimental sets. In the findings of Leys *et al.,* low supplement of carbon and nitrogen source did not support the growth of the organism and hence observed a lower degradation rate [45]. Supporting to our study, Teng *et al.* showed carbon and nitrogen when amended with 10:1 ratio significantly elicited the hydrocarbon degradation rate when compared to the C: N either with 25:1 or 40:1 [46]. Besides growth, in our observation, *P. ostreatus* have capacity to degrade the hydrocarbon pollutant possibly by secreting extracellular enzymes such as hydroxylases and monooxygenases. Our findings have a correlation with the reports of Bogan and Lamar [47] where the degradation of petroleum HC soil is mediated by the extracellular enzymes, which is also reported by Ekundayo [48].

3.5. Kinetic Analysis

Kinetic analysis for studying the degradation rate of HC soils by *P. ostreatus* in various treatments was fitted well to first-order kinetics [Table 2]. Highest degradation rate constant was observed in the HC soil of Set III (0.097 day^{-1}). Subsequently, Set II, Set IV, and Set V HC soil showed the degradation rate constant (k) as 0.048 day^{-1}, 0.025 day^{-1}, and 0.012 day^{-1}, respectively. The biological half-life ($t_{½}$) values of various treatments on hydrocarbon degradation observed in Set II, Set III, Set IV, and Set V are 14, 7, 28, and 58 days, respectively. Experimental Set III (paddy straw: HC soil [2:1]) that showed highest biodegradation rate constant has lowest half-life time when compared to other sets. Thus, the data representing in kinetic model analysis can conclude that the rate constant (k) has a significant correlation with TPH degradation rate (%). Higher biodegradation rate is directly proportional to higher degradation rate constant and lower half-life

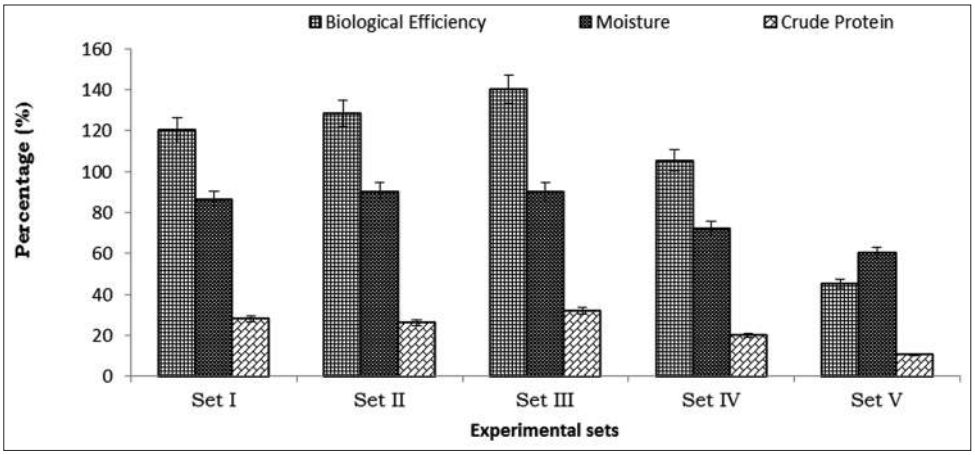

Figure 4: Effect of different experimental sets on biological efficiency, moisture, and protein of *Pleurotus ostreatus* (Bars = Standard Deviation)

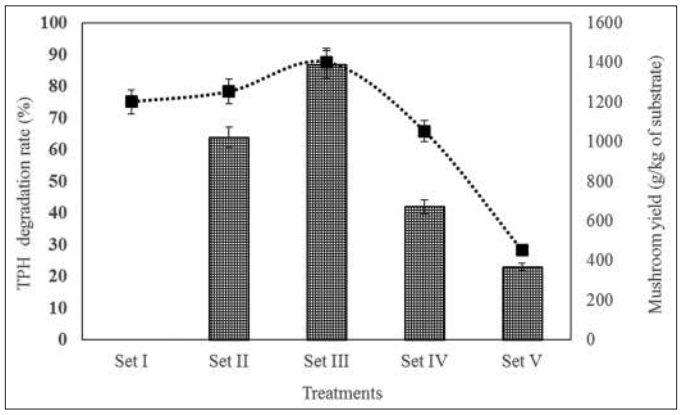

Figure 5: Total petroleum hydrocarbon (TPH) degradation rate and biomass content of *Pleurotus ostreatus* after 21 days. The bars represent the TPH degradation (%) and the dotted lines correspond to the yield of mushroom (g/kg^{-1})

Table 2: Biodegradation rate constant (k) and half-life (t½) time in various treatments of hydrocarbon-contaminated soil

Treatments	Rate constant (k^{-1} day)	Half-life time (t½)
Set I (Sole paddy straw)	n/a	n/a
Set II (Paddy straw: HC soil [1:1])	0.048	14
Set III (Paddy straw: HC soil [2:1])	0.097	7
Set IV (Paddy straw: HC soil [1:2])	0.025	28
Set V (sole HC soil)	0.012	58

time [34]. Both experimental study and kinetic analysis showed that the perceived progressed remedial effect noticed by *P. ostreatus* in Set III may be attributed to the better growth due to the suitable C/N sources, thereby increases secretion of oxidative extracellular enzymes that catalyze an effective mycoremediation process.

3.6. FTIR Analysis

The harvested oyster mushroom grown in the different sets was analyzed for the functional group changes by FTIR spectroscopy [Figure 6]. Besides the remediation potentials, *P. ostreatus* is a highly nutritious diet where it is mainly considered as a source of single cell protein. Therefore, in our study, we focused on analyzing the characteristic changes in *P. ostreatus* particularly so that the hydrocarbon contaminants do not affect the nature of edibility either by uptaking the toxic compound or by limiting the nutritional property in the mushroom. Mostly FTIR analysis in bioremedial studies so far was unidirectional, that is, focusing primarily on pollutant degradation, still, it is also essential to study the fate of contaminants and its impact on biological systems. In our findings, FTIR spectra of *P. ostreatus* cultivated in Set I and Set III have mostly similar banding assignments [Table 3].

The broad peak near 3400 cm^{-1} represents the presence of O-H stretch in the hydrogen bonds. The strong peak near 2930 cm^{-1} corresponds to C-H stretch which confirms the presence of pyranose ring (glucan or chitosan). Bands near 1630 and 1516.05 cm^{-1} attribute to C = O stretch (Amide I) and N-H (Amide II), indicate the presence of proteins. The latter band is observed in Set I and the former is observed in both Set I and Set III. The characteristic bands near 1076.28 and 887.26 cm^{-1} confirm the presence fingerprint of carbohydrate such as mannogalacton and β-glucans. FTIR spectra of *P. ostreatus* cultivated in Set IV and Set V, reveled distinct banding assignments when compared to earlier two experimental sets. The broad O-H stretch near 3400 cm^{-1} was completely limited in Set IV and Set V, however, some vibration has been noticed. Similarly, the spectra of mushroom from both the sets showed vibration in protein peaks (Amide I and Amide II) near 1600 and 1500 cm^{-1}. Nevertheless, in Set IV and Set V, sharp bands have been noticed in the carbohydrate fingerprint regions (1200–1000 cm^{-1} and 900–700 cm^{-1}). In our study, mushroom grown in all the tested experimental sets showed a notable carbohydrate characteristic peak (regions, 1200–1000 cm^{-1}) in the FTIR analysis and strikingly because of the carbohydrate (polysaccharide, particularly glucan) content, the difference in the yield of the mushroom has been observed. The structure and weight of the edible *P. ostreatus* are subjected to the presence of β-glucans in additional to its bioactive property [49]. This finding is also supported by Synytsya *et al.,* where the glucan content is directly proportional to the fruiting body [50]. Characteristic strong bands near 1600 cm^{-1} (1631.78 cm^{-1} in Set I; 1635.64 cm^{-1} in Set III) attributed to Amide I bands (C=O stretch) which represent the presence of peptide bond and similar type of observation was also reported [51]. The strong Amide I bands observed by FTIR in our study influence the protein content in *P. ostreatus.* This result has a significant correlation with our earlier analysis where Set III among all other sets showed maximum protein content. This may

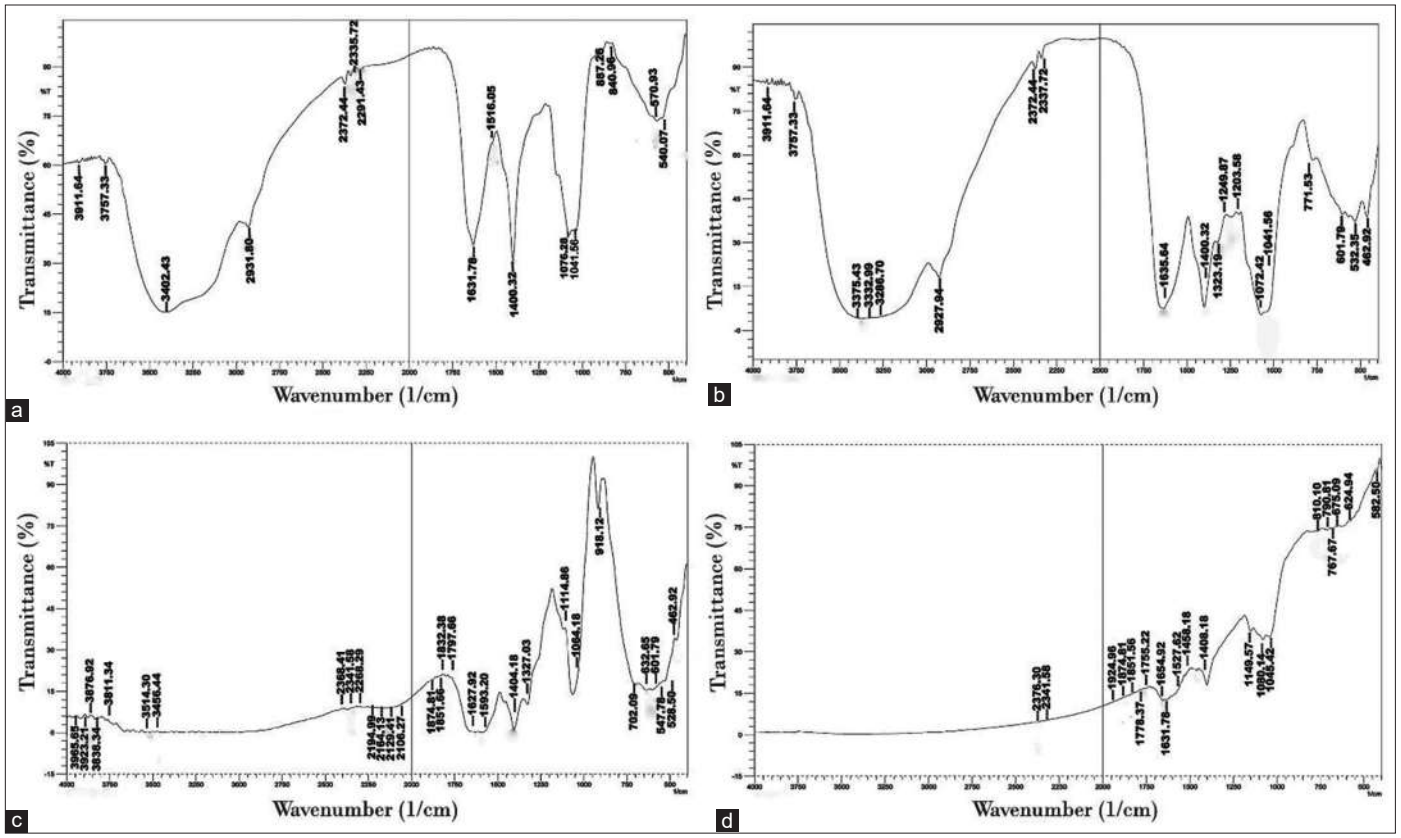

Figure 6: Characteristic bands of Fourier transform infrared spectrum of *Pleurotus ostreatus* grown in different experimental sets. (a) Set I – sole paddy straw; (b) Set III – paddy straw: Hydrocarbon-contaminated (HC) soil (2:1); (c) Set IV – paddy straw: HC soil (1:2); and (d) Set V – sole HC soil

Table 3: The assigned bands (cm^{-1}) obtained from the FTIR spectra of *Pleurotus ostreatus* grown in different experimental sets. Bands given in parentheses correspond to the standard bands of edible oyster mushroom

Set I (sole paddy straw)	Set III (paddy straw: HC soil [2:1])	Set IV (paddy straw: HC soil [1:2])	Set V (sole HC soil)
3911.64–3402.43 (br, O-H str, chitin)	3911.64–3286.70 (br, O-Hstr, chitin)	3965.65–3213.41 (vbr, O-H str, Chitin)	-
2931.80 (vs, C-H str pyranose ring; glucan/chitosan)	2927.94 (vs, C-H str, pyranose ring; glucan/chitosan)	-	-
2372.44–2291.43 (sh, C-H str)	2372.44–2086.98 (sh, C-H str)	2368.59–2106.27 (vbr, C-H str)	2376.30–2341.58 (vbr, C-H str)
-	-	1874.81–1797.66 (vbr, Double bond)	1924.96–1755.22 (vbr, Double bond)
1631.78 (vs, Amide I, C=O stretching of the peptide bond)	1635.64 (vs, Amide I, C=O stretching of the peptide bond)	1627.92 (vbr, Amide I, C=O stretching of the peptide bond)	1654.92,1631.78 (vbr, Amide I, C=O stretching of the peptide bond)
1516.05 (Amide II band; glucan)	-	1593.20 (br, Amide II band; glucan)	1527.62 (vbr, Amide II band; glucan)
1400.32 (vs, C=O Str)	1400.32 (vs, C=O Str)	1404.18 (sh, C=O Str)	1458.18–1404.18 (sh, C=O Str)
-	1323.17 (sh, O-H bending, glucan)	1327.03 (sh, O-H bending, glucan)	-
-	1249.87–1203.58 (vbr, carbohydrate fingerprint zone)	-	-
1076.28, 1041.56 (vs, carbohydrate fingerprint zone; confirms presence of chitin or chitosan)	1072.42, 1041.56 (vs, carbohydrate fingerprint zone; confirms presence of chitin or chitosan)	1064.71 (sh, carbohydrate fingerprint zone; confirms presence of chitin or chitosan)	1149.57–1045.42 (vbr, carbohydrate fingerprint zone; confirms presence of chitin or chitosan)
887.26, 840.96 (sh, confirmatory band for Mannan type glucan)	771.53 (sh, confirmatory band for Mannan type glucan)	918.12 (sh, confirmatory band for Mannan type glucan)	810.10-767 (vbr, confirmatory band for Mannan type glucan)

Vbr: Vibration; br: Broad, sh: Sharp, vs: Very strong

be due to the availability of suitable C/N ratio, namely, paddy straw: HC soil (2:1) that influences the protein substance in the mushroom. The difference in nutrient content of the mushroom has been noticed with respect to the substrates [52,53]. Biosorption is

an efficient process for treating the environmental pollutants either live or dead state [54]. Few literatures have discussed on biosorption properties of mushroom on toxic substances when grown in polluted environments. In contrast, from our study, FTIR spectra of *P. ostreatus* analyzed in all the experimental sets interestingly have showed complete absence of aliphatic and aromatic stretches (band assignment, 3150 cm^{-1}–2800 cm^{-1}), that is, the banding assignments particularly for petroleum hydrocarbons. Similar type of microbial activity was significantly noticed with few research findings [55,56]. Eventually from our study, it is evident that the edible mushroom utilized for bioremediation has no biosorption of toxic compounds. The functional groups of *P. ostreatus* cultivated in Set III and Set I are similar, thus, it clearly shows that this oyster mushroom beyond its potential remedial effect can be consumed safely since the edible property is not either altered or reduced considerably.

4. CONCLUSION

Mycoremediation ensures the possibility of developing a clean technology. Inadequate knowledge was available so far on directly augmenting mushrooms in crude HC soil. The present study concludes adding paddy straw with raw HC soil in 2:1 ratio stimulates the remedial activity of *P. ostreatus* against hydrocarbons over a short period of time. The current spectral study proved the complete absence of hydrocarbon peaks in the remediated mushroom through spectroscopic analysis which can be considered as an interesting fact for safe edibility. However, a clear knowledge on the fate of hydrocarbon degradation in this context has to be understood in terms of mushroom enzymology in future.

5. FUNDING

This research was not supported by special grants from any funding agencies.

6. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

7. ETHICAL APPROVALS

Ethical approval was not required for this study.

8. DATA AVAILABILITY

The data set generated during the current study is available from the corresponding author on reasonable request.

9. PUBLISHER'S NOTE

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How to cite this article:
Dhailappan A, Arumugam A, Rajendran G, Ravichandran M. A dualistic approach to investigate the remedial potential and edible property of *Pleurotus ostreatus* on hydrocarbon-contaminated soil. J App Biol Biotech. 2022;10(3):70-78.

Devagiri Journal of Science 7(1), 98-110
© 2021 St. Joseph's College (Autonomous), Devagiri
www.devagirijournals.com
ISSN 2454-2091

Review on Algal biopolymer - A combat to petroleum based plastics

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Received: 16.08.2020

Revised and Accepted: 20..10.2021

Key words: Algal biopolymer, Microalgae, Algal cultures, Carbon based plastics

Abstract

Biopolymers are polymeric biomolecules produced by living biota viz., microorganisms, plants, aquatic animals and cyanobacteria. Such produced polymers are biocompatible, non toxic, flexible, biodegradable and significantly constitute a potential alternative to petrochemical derived polymers. Microalgae emerged as excellent producers of polymers with improved properties where Cyanobacteria (bluegreen algae), green microalgae, red microalgae and brown-golden microalgae produce valuable biomolecules such as pigments, lipids and exopolysaccharides. This review focus on exploring the knowledge on algal cultures potentials in the synthesis of biopolymer which fight against the environmental issues by playing as a substitute for existing carbon based plastic.

1. Introduction

Plastics have changed the everyday life as its usage is increasing day by day. These plastics are polymeric substances with immense properties such as strong, durable, lightweight and corrosion resistant which enables their use to make a wide variety of products as they can be melted, molded, remelted and remolded.

Mostly all aspects of daily life involves the use of plastics, such as in transport, clothing, packaging materials, telecommunications etc,. There is also considerable potential for novel applications of these plastics that will bring possible benefits in the future (Thompson *et al.,* 2009).There are different types of plastics such as Polyethylene Terephthalate (PET), High Density Polyethylene (HDPE), PolyvinylChloride(PVC), Low Density Polyethylene(LDPE), Polypropylene(PP), Polystyrene (PS) and the Miscellaneous type of plastics are polycarbonate, polylactide, acrylic, acrylonitrile butadiene, nylon, styrene and fibreglass Though plastics satisfy enormous human needs currently they are causing harmful effects to the environment and human health because they are carbon based polymers and the majority of their feedstock is primarily petroleum derived compounds that are not very good for the environment. The need of an alternative to existing plastic is necessary to solve the toxicity effects in the eco system.

1. Polymers:

Polymers are large molecules composed of long chains or rings of repeating subunits called monomers. Polymers possess high boiling and melting points. As they consist of many monomer units they tend to have high molecular masses (Anne Marie, 2019). They have unique properties depending on the type of bonding molecules. Some

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can bend and stretch like polyester and rubber while others are tough, hard and unstretchable like epoxies and glass (Ellis and Smith 2008). They may be natural or synthetic. Polymers result from chemical reaction of monomers. These monomers have the ability to react with same or different types of molecules to form a polymer chain under suitable condition. This resulted in the formation of natural polymers such as starch, cellulose and natural rubber, while synthetic polymers are made artificially by man (Namazi, 2017).

1.2 Structure of polymer:

Polymers are usually formed through polymerization process in which the repeated monomer molecules bond together by a chemical reaction which often results in a three dimensional network of polymer chains. There are two types of polymerization reactions - addition and condensation reactions.

Addition polymerization involves the formation of polymers from carbon - carbon double bonds containing monomers by exothermic addition process. The polymers often produced through this process include polyethylene, polyvinyl chloride, polypropylene, polystyrene etc., whereas in condensation reaction polymers are formed by a stepwise reaction of molecules having different functional groups. The reaction is endothermic and the polymers include polyesters, polycarbonate and polyamides. The properties of polymers depends on the varying functional groups within the molecular structure. The individual polymer chains entangle within each other which rely on forces such as hydrogen bonding, vanderwaals forces, dipole interactions but not the covalent bonds (Jansen, 2016).

1.3 Natural polymers:

Natural polymers are substances which are naturally obtained. Some of the natural polymers include chitin, cellulose, resins, protein, starch, natural rubber (Benabid and Zouai, 2016).). These polymers are found in plants and animal sources. The main advantage of using natural polymers is that they have no adverse effect on the human or environment and are biodegradable and non toxic compared to synthetic polymers. They are economically cheaper and low production cost than synthetic polymers (Kusum Kaushik *et al*.,2016).

1.4 Semi - synthetic polymers:

They are obtained by simple chemical treatment of naturally occurring polymers in order to change their physical properties. Some examples of semi synthetic polymers are silicones and starch (Kusum Kaushik *et al*.,2016).

1.5 Synthetic polymers:

These types of polymers are synthesized by polymerization of simple chemical molecules in the laboratory. They are human made plastics. Synthetic polymers are mostly petroleum based plastics. Some of the common synthetic plastics made are polyethene, polystyrene, PVC, Teflon, Synthetic rubber, Nylon etc., (Kusum Kaushik *et al*.,2016).They are mostly manufactured from hydrocarbons that are derived from crude oil. They have desirable properties such as strength, chemical flexibility, resistivity etc., (John Brennan,2017). The major drawback of synthetic polymer is their nondegradability and also synthetic plastics or polymers results in environmental issues because of improper disposal and brings threat to human health as they emit toxic fumes when burnt. The environmental pollution and exhaust of natural resources occurred because of these petroleum based synthetic polymers or plastics urged the need of environmentally benign polymers (Gowthaman *et al.,* 2021).

2. Biopolymers:

Biopolymers are polymeric biomolecules that are derived or extracted from the widely available resources such

as microorganisms, marine animals and plants. These polymers are produced by these biological resources as biomass or byproduct during their growth cycles (Mukund Adsul *et al.*,2016).The prefix "bio" denotes that they are biodegradable. The materials that are produced by synthetic chemistry from biological sources such as sugars, resins, proteins, fats, vegetable oils etc., can also be described using the term biopolymers. The important property that distinguishes biopolymers from fossil fuel derived polymers is that their sustainability and especially their biodegradability. Hence these biodegradable biopolymers have been synthesized from renewable sources as an alternative for petroleum based plastics. They are usually synthesized from starch, natural fibers, sugars etc., and are easily degraded by microorganisms (Mohan *et al*., 2016).

2.1 Biopolymers from various sources:

2.1.1 Microbial Biopolymers:

Many microorganisms are known to produce biopolymers that are either found attached to the cell surface or separated from the fermentation medium. Biopolymers are a class of storage polymers produced by many microorganisms such as Bacteria, fungi etc.,(John Masani Nduko *et al.*,2019). There are four types of microbial polyesters such as cellulose based, starch based, lactic acid based and Polyhydroxyalkanoic acids (Lenz R.W *et al.*,2005). Among various microbial biopolymers PHA have been drawing attention due to their material properties and greater biodegradability. Hence PHA's are considered as a potent alternative for petrochemical plastics (Byrom, 1987). These PHA's are accumulated as granules in a variety of microorganisms intracellularly or extracellularly (Kunal *et al.*,2011).

3.7.1.1 Bacterial Biopolymers:

Bacteria are capable of synthesizing a variety of biopolymers with different biological functions and also with material properties. Bacteria converts different carbon sources into polymers with diverse chemical and material properties. Although bacteria only synthesize few intracellular polymers, the range of polymers that they can synthesize extracellularly is vast. Four classes of polymers are produced efficiently by bacteria such as : Polysaccharides, Polyamides, Polyesters, Polyanhydrides (Rehm, 2010).

Polysaccharides:

The polysaccharides produced by bacteria can be exopolysaccharides and endopolysaccharides. Glycogen is an endo or intracellular polysaccharides produced by many bacteria. Alginate is produced extracellularly by *Pseudomonas* and *Azotobacter* species. Xanthan is an exopolysaccharide produced by *Xanthomonas* spp. Cellulose is extracellularly synthesized by Alphaproteobacteria and many Gram positive bacteria

Polyamides:

Poly gamma glutamate is extracellularly produced by *Bacillus* sp, *Fusobacterium nucleatum* and few Gram positive bacteria.

Polyester:

Polyhydroxyalkanoate are energy reserve polymers belonging to polyester class of biopolymer produced by bacteria when the carbon source is high and other essential nutrients such as nitrogen, phosphorus, oxygen, sulfur are in limited amounts. PHA's are produced intracellularly by many bacterial species such as *Bacillus megaterium*, *Pseudomonas oleovorans, Azotobacter* etc., Among the PHA polymer family PHB

(Polyhydroxybutyrate) is the most common biopolymer accumulated under nutrient stress conditions and they act as energy storage material (Bernd Rehm,2010) (Hazer *et al.,* 2007)

Polyanhydrides:

Polyphosphate is a type of polyamide class of bacterial polymers produced intracellularly by many bacterial species such as *Spirillum volutans* (Bernd Rehm,2010) (Meyer A,1904).

2.1.2 Fungal biopolymers:

Fungi are also capable of effectively producing biopolymers. The cell wall of fungi is composed of polymers such as glucans and chitin (Araújo, 2020). Beta glucans are the type of biopolymer which consist of a backbone of glucose residues constitute half the mass of cell wall of fungi (Seviour *et al.,*1992) (Mclntosh *et al*. ,2005). Fungal species capable of producing glucans are *Saccharomyces cerevisiae, Peslatotia sp.,Epicoccum nigrum* etc.,(Gopal Rao *et al.,* 2014).

2.1.3 Algal Biopolymers:

Algae are one of the promising organisms for biopolymer production as grow fast and contain various value added materials and they have high bioenergy feedstock potential. Algae derived polymers are of three types - Natural polymers, Polyhydroxyalkanoate and Bio based polymer from algae derived monomer. They have similar characteristics as of conventional synthetic polymers (Didem Ozcimen *et al.,* 2017).

a) Algal Polysaccharides:

Marine algae are a rich source of polysaccharides such as cell wall structural polysaccharides, mucopolysaccharides and storage polysaccharides (Kim *et al.*,2015). The green algae contain a large amount of polysaccharides such as ulnas, xylan, sulfated galactans. Red algae are rich in Xylan, Carrageenan, Fucoidan, Porphyran (Murata *et al.*,2001) (Kumar *et al.*,2008). Brown algae are rich in alginic acid, laminarin, Sargassan and Fucoidan (Kim *et al*.,2015).

b) Alginate:

Alginate is a type of polysaccharides found in the cell wall of brown algae (Kim SK *et al*.,2015). The extraction method of alginate is that 100g of algae samples are ground and left overnight in 0.1M HCL and then washed in 1litre of 1% sodium carbonate solution which is then stirred and filtered. The filtrate obtained is collected and precipitated with isopropanal. The resulting gel is then dried and milled to g*et al*ginate (Ozdemir *et al*.,2013).

c) Laminarin:

Laminarin is a storage polysaccharide of brown algae. It is a linear polysaccharide which constitute 25-50 glucose monomer units (Cybulska *et al.,* 2016). For extraction of laminarin, 85% of ethanol is applied at 23°c and 70°c in order to separate the proteins and other pigments from the algae which is then centrifuged. The pellet and solvent are separated by vacuum filtration with a filter paper. The pellet separated by filtration is then treated with 2% calcium chloride at 70°c and then centrifuged. The polymers alginate and Fucoidan and laminarin precipitates out. Fucoidin is then separated by treating with 0.01M HCL at 70°c and then centrifuged. The pellet is then subjected to 3% sodium carbonate to remove alginate from the solution and further centrifuged to obtain Laminarin (Ozdemir *et al.,*2013).

d) Fucoidan:

Fucoidan extraction is carried out in hot water. It is followed by precipitation with organic solvents. It involves 3 steps: milling, extraction/purification and drying (Kim SK *et al*.,2015).

e) Carrageenan:

It is a major constituent of red algae. For extraction of carrageenan, the algae is collected and dried. Then the dried sample is mechanically ground, sieved and washed. The cellulosic materials are removed by two stage treatment. First the dissolved carrageenan is centrifuged to remove cellulosic materials. It is then filtered to separate smaller particles. The solution is then concentrated by evaporation and the water content is removed. The carrageenan is then recovered by one of the two methods. The first method is to deposit carrageenan solution in potassium chloride. This allows the filtrate to gel which is then frozen and the excess water is removed by compression during the process of thawing. In the second method carrageenan solution is precipitated in Isopropyl alcohol. Since carrageenan is insoluble in alcohol it clots between alcohol and water. The clot is compacted to remove water content and then vacuum dried to remove alcohol content. The coagulum is then dried, milled and blended (Ozdemir *et al.*,2013).

f) **Agar:**

Agar is a mixture of polysaccharides which is composed of agarose and agaropectin. It has interchangeable structural and functional properties as that of carrageenan (Kim SK *et al.*,2015). Agar extraction involves the following step. The seaweed is washed and then boiled in water to dissolve the agar, it is then filtered and cooled to obtain jelly property and the water content is removed (Cybulska *et al.*,2016).

g) Ulvan:

It is a major constituent of green algal cell wall. It is composed of glucose, xylose, Iduronic acid, sulfate, rhamnose, glucuronic acid and smaller amounts of arabinose, mannose and galactose. It is of two kinds water soluble ulvan and insoluble cellulose like material (Kim *et al.*,2015).

2.1.4 Cyanobacteria:

Cyanobacteria are photosynthetic prokaryotes that capture sunlight as the source of energy using chlorophyll a and other accessory pigments. They are commonly found in lakes, ponds, streams, rivers, wetlands and they also play a significant role in nitrogen, carbon and oxygen dynamics of many aquatic environments. Cyanobacteria were classified as blue-green algae because of their appearance, possession of chlorophyll and photosynthetic production of oxygen similar to algae and higher plants by a two photosystem process. They lack nuclei and other organelles but possess peptidoglycan cell wall. All cyanobacteria contain chlorophyll a and mostly contain the blue phycobiliproteins phycocyanin and allophycocyanin giving their characteristic blue green color. Some cyanobacteria also contain phycoerythrin making the cells appear red. These phycobiliproteins are located in structures called phycobilisomes on the thylakoid membrane. They are an efficient light guides for the transfer of solar energy captured from sunlight. Although they lack membrane bound organelles as in eukarotic algae and higher plants they posses a variety of cellular structures and inclusions with specialized functions. In addition to this they also contain storage bodies which includes glycogen granules which store carbon and cyanophycin granules which store nitrogen. These inclusions allow cells to accumulate energy and nutrients under favourable condition and further use these reserves for maintenance and growth under stress conditions.

3 Commercial implications of cyanobacteria:

Cyanobacteria are an immense source of several metabolites such as

alkaloids, flavanoids, phenols, carbohydrates, pigments, steroids, tannins, vitamins etc., cyanotoxins have been exploited as pesticides as they are known to have toxic effect. They are also enriched with pharmacologically active compounds that have effective antibacterial, anticancerous, antiviral, and antifungal properties. These cyanobacteria are employed in the production of biofuels which are cost effective and ecofriendly. The important constituents of biofuels such as carbohydrates, lipids and fatty acids are produced during the calvin cycle in cyanobacteria (Baroukh *et al.*, 2015). Among various applications biopolymer production from cyanobacteria is of recent trend because of its excellent properties similar to the currently available synthetic polymers but the major advantage that distinguishes it from the petroleum based polymer is that they are biodegradable (Fig.1).

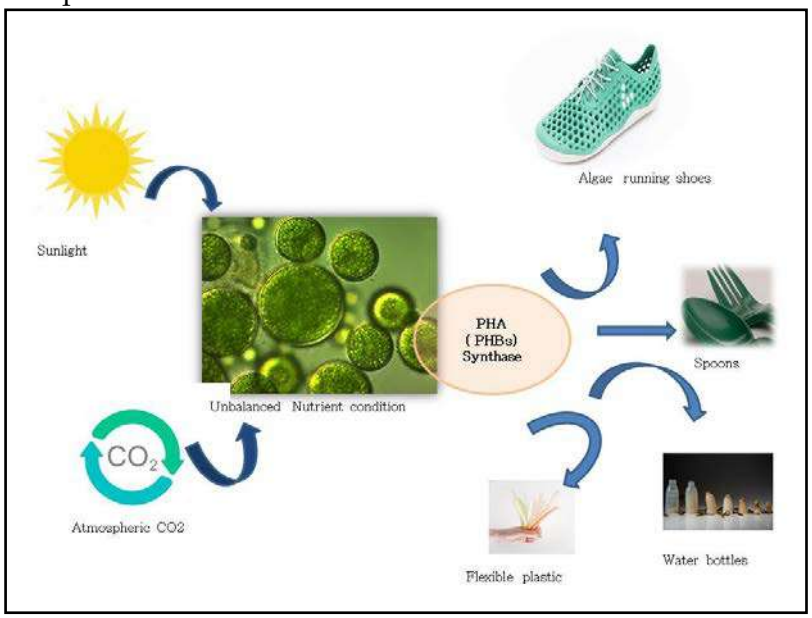

Fig.1 Algal Biopolymer commercial products

3.1 Polymers extracted from cyanobacteria:

Polyhydroxyalkanoate (PHA) are one of the most potential biopolymer which can be a better substitute for petroleum based polymer. PHA's are biodegradable biopolymers with similar characteristics as that of synthetic polymers due to their high molecular weight and other properties such as thermoplastic processability and hydrophobicity (Balaji *et al.*,2013) (Wu *et al.*,2001). PHA's are accumulated inside cell as insoluble granules for energy by various prokaryotic organisms. There are two species that have been reported to accumulate PHA, the chemoautotrophic bacteria and cyanobacteria known as blue green algae (Balaji *et al.*,2013) (Asada *et al.*,1999) (Sudesh *et al.*,2001). PHA can have various structure depending on conditions of organisms up to date 150 different structures of PHA have been identified some of the most well known are 3-hydroxypropionate, 3-hydroxybutyrate, 3-hydroxyoctonoate, 3-hydroxydecanoate, 3-hyroxydodecanoate, 3-tetradecanoate, 4-hydroxybutyrate (Balaji *et al.*,2013)

3.2 Cyanobacterial Poly-(hydroxybutyrate)(PHB):

Other than bacteria, cyanobacteria are potential PHA producers. Because cyanobacteria requires only minimal nutrient and can fix carbon

dioxide as a sole carbon source. There are many advantages of using cyanobacteria over bacteria as PHA producing host as cyanobacteria use waste CO_2 and sunlight as their carbon and energy source and posses low production cost than bacteria. Therefore cyanobacteria are able to provide environmental friendly biopolymer that could be used effectively as bioplastic. However the PHA content in cyanobacteria is low in percentage cell dry weight compared to bacteria which may be attributed to large cell size and the cell wall of cyanobacteria being thicker that restrain downstream processing of PHA extraction (Sudesh *et al.*,2001). Among various PHA structures Poly-(hydroxybutyrate) is the most abundant type produced by blue green algae. PHB is a homopolymer of hydroxybutyrate that are present in various blue green algae such as *Chlorogloea fritschii*, *Spirulina* spp., *Aphanothece* spp., *Synechococcus* spp., *Gloethece* spp., *Synechocystis* sp, *Gloeocapsa* sp, *Spirulina platensis*, *Phormidium* sp etc.[30,31,32,35]. (Balaji *et al.*, 2013)) (Wu *et al.*,2001) (Asada *et al.*,1999) (Gopi *et al.*,2014).

Sabbir Ansari *et al.*, 2016 selected 23 cyanobacterial strains *Anabaena* sp., A.*variabilis*, *Aphanocapsa* sp., *Aulosira fertilissma*, *Calothrix brevisseima*, *Cylindrospermum* sp., *Hapalosiphon fontinalis*, *Michrochaeta* sp., *Nostoc sphaericum*, N.*muscorum*, N.*paludosum*, N.*punctiforme*, *Scytonema* sp., *Tolypothrix tenuis*, *Westiellopsis prolific*, *Chrococcus* sp., *Gleocapsa gelatinosa*, *Lyngbya* sp., *Oscillatoria* sp., *Phormidium* sp., *Plectonema* sp., *Spirulina platensis*, *Synechocystis* sp., for PHB production and found PHB with highest production in N.*muscorum* with 6.44%, N.*punctiforme* species with 6.27%, N.*paludosum*species with 6.10% and N.*sphaericum* with 6.12%. *Aulosira fertilissma* was the next highest PHB accumulator. Less production was observed in *Michrochaete* sp., *Hapalosiphon fontinalis*, *Westeillopsis prolific*, *Tolypothrix tenuis*, *Aphanocapsa*,etc., No PHB accumulation was noticed in *Cylindrospermum* sp.,

Manoj Singh *et al.*, 2019 examined PHB production in cyanobacterium *Scytonema geitleri* under varying environmental conditions such as pH, temperature and carbon sources. S.*geitleri* produced high quality PHB at pH 8.5 and temperature 30° C and acetate was the preferred carbon source.

Gopi *et al.*, 2014 isolated 15 cyanobacterial species from marine and fresh water resources for screening PHB producing strains and found 11 strains capable of producing PHB with *Phormidium* sp., producing 7.6% followed by *Synechococcus* sp with 4.5% *Synechocystis* sp with 3.7% and *Anabaena* sp with 2.3%. Among the selected strains *Phormidium* sp isolated from marine environment has been reported for PHB production for the first time.

Carpinea reported the successful production of PHB by *Synechocystis* sp PCC6803 just from CO_2 and the level of PHB production was enhaced by nitrogen starvation condition (Carpinea *et al.*, 2015)

Devadas explored the bioplastic PHB production using *Chlorella vulgaris* freshwater strains. The strains were screened by sudan black B and nile blue stain. The PHB production was optimized using different culture media and various parameters like aeration, effect of sodium acetate and phosphate etc., The PHB was extracted by hot chloroform and quantified by reading the absorbance at 235nm in UV spectrophotometer (Devadas *et al.*, 2020)

Motomu Nishioka *et al.*, 2001 examined that *synechococcus* sp.MA19 produced PHB when grown autotrophically under phosphate limited condition at 50° C.

Dulce Maria Arias *et al.*, 2018 revealed that cyanobacteria dominated cultures cultivated in wastewater effluent can be used as PHB producers. The effect

of N and P limitation during two different photoperiods was evaluated for two weeks and the result showed highest PHB production under P limitation and constant illuminence. This study highlights that nutrient limitation could be a better approach in order to enhance the PHB accumulation in wastewater-borne cyanobacteria.

Monshupanee *et al*., 2016 utilized the easy to harvest cyanobacterium *Chlorogloea fritschii* using a two stage cultivation strategy. The cells were first pregrown under normal photoautotrophic condition to increase the biomass and recultivated under heterotrophic condition with a single organic substrate to produce the required product. Through this two stage cultivation method, the mass conversion of acetate to PHB was obtained.

Maheswari *et al*., 2011 prepared bioplastic using the cyanobacterium *Spirulina platensis* cultivated in Zarrouk medium. The medium was optimized by using sodium acetate to increase the PHB concentration. The S.*platensis* was measured for total dry weight and PHB by UV spectrophotometer before and after optimization of culture medium. The result showed little higher PHB content (6.20%) in S.*platensis* grown in optimized medium when compared with untreated one (5.18%). In addition the chemical based commercial plastic was prepared and compared with the bioplastic. From the comparision study the plasticizing and moldable property was good in all plastics. But the biodegradation was considered to be better in bioplastic due to the presence of PHB.

Keerati Taepucharoen *et al*., 2017 found photoautotrophically grown cyanobacterium *Oscillatoria okeni* to produce bioplastic (PHBV) under nitrogen deprivation. The heterotrophically grown cells under light showed no increase of PHBV but an increased production was observed under dark condition.

Shilalipi Samantaray *et al*., 2012 identified a phototrophic N2 fixing cyanobacterium, *Aulosira fertilissima* as a potential source for the production of PHB biopolymers. PHB accumulation upto 66% cell dry weight was observed when the cells are cultured in acetate with citrate supplemented medium. Also *Aulosira* culture supplemented with 0.5% citrate under phosphate deficiency depicted a PHB accumulation of 51% when incubated in dark for five days period. PHB accumulation was found to reach upto 77% under P deficiency with 0.5% acetate supplementation. The optimization of process parameters by response surface methodology resulted in PHB accumulation upto 85% at 0.26% citrate, 0.28% acetate and 5.58 mgL-1 dipotassium hydrogen phosphate for a five days incubation period. The A.*fertilissima* cultures pre-grown in fructose supplemented BG11 medium when subjected to the optimized culture condition the PHB accumulation boosted up to 50 folds higher than the control. A.*fertilissima* is the first cyanobacterium where the PHB accumulation reached up to 85% under nutrient manipulation and optimization and also the polymer exhibited similar material properties when compared with the commercial polymer.

Laxuman Sharma *et al*., 2006 found out the interactive effects of four variables viz. concentrations of acetate, glucose, dipotassium hydrogen phosphate and dark incubation on PHB production in a nitrogen fixing cyanobacterium Nostoc muscorum. Acetate, glucose and dark incubation exhibited positive impacts on PHB accumulation and yield.

Sandra Mareike *et al*., 2012 explored that the cyanobacterium *Nostoc muscorum* is a PHB accumulator which is considered one of the potential raw material supplier because of their short generation cycles. A range of experimental

conditions have been examined such as phosphate starved cells with the addition of external carbon sources. The highest accumulation of PHB was observed in a phosphate starved culture medium with 1% glucose and 1% acetate. After 23 days 1L of culture contained upto 145.1 mg of PHB in phosphate starved medium. Aeration and CO2 addition resulted in highest PHB accumulation.

Campbell *et al*., 1982 identified PHB in the cyanobacterium *Spirulina platensis*. The reduced carbon compounds addition was not required for PHB accumulation. PHB accumulated upto 6% of total dry weight during the exponential growth and seemed to decrease during the stationary phase.

Hanan H Omar *et al*., 2016 performed experiments with three cyanobacterial PHB producers *Oscillatoria salina*, *Anabaena cylindrical* and *Nostoc linckia* and determined PHB production after 7, 14 and 21 days. The highest dry weight was observed at stationary phase and PHB accumulation were known to increase with increasing dry weight upto 14 days and then declined after 21 days. The pH values between 8 and 9 were preferred for better accumulation of PHB. The strains accumulated highest PHB at pH 8. Nitrogen and Phosphate starvation were found to be a stimulatory for PHB production. It was concluded that cyanobacteria are capable to synthesize high PHB under stress conditions.

Roberto De Philippis *et al*., 1992 described the effect of different growth conditions on glycogen and PHB accumulation in the cyanobacterium *Spirulina maxima*. It was found that under photoautotrophic conditions S.*maxima* exhibited glycogen content of 7.1 without nutrient limitation and PHB was undetectable. While under mixotrophic conditions in the presence of acetate the PHB content increased to more than 3% of dry weight and during nitrogen starvation PHB remained low. The addition of azaserine induced glycogen accumulation but did not stimulate PHB synthesis. Phosphorous limited condition resulted in glucogen and PHB accumulation upto 23% and 1.2%. shifting culture from low to high light induced rapid glycogen accumulation but not PHB.

Massimo Vincenzini *et al*., 1990 depicted the occurrence of PHB in several strains of photoautotrophically grown *Spirulina* spp. In the presence of acetate under mixotrophic condition the level of PHB accumulation reached values greater than 2.5% of dry weight but no significant effect was obtained with pyruvate.

Researchers also tested various cyanobacterial strains for PHB accumulation but all showed varying amounts with *Nostoc* sp and *Calothrix* sp with maximum accumulation. The heterocystous species produced PHB upto 11% and 10% of dry cell weight when photoautotrophically grown. The PHB accumulation enhanced to 15-20% or 17-24% in *Nostoc* sp and 12-19% or 12-16.4% in *Calothrix* sp after 21 days under mixotrophic and chemoheterotrophic conditions with varying concentrations of glucose, fructose, maltose and acetate. The presence of acetate resulted in maximum PHB in *Nostoc* sp but *Calothrix* sp showed maximum PHB accumulation in the presence of fructose content followed by acetate. Phosphate starvation also increased PHB accumulation in both strains.

Bhabatarini Panda, 2008 investigated the PHB accumulation in unicellular cyanobacterium *Synechocystis* sp PCC 6803. Under photoautotrophic condition it was found to accumulate the homopolymer of PHB with a maximum value of 4.5% dry cell weight and in addition the effects of various cultural and nutritional conditions were studied. PHB accumulation was found to be stimulated by nitrogen and phosphorous deficient conditions. Also chemoheterotrophy and mixotrophy under gas exchange

limitations enhanced PHB yield upto 22 and 30%.

Tugarova aimed to increase the PHB accumulation using stress conditions such as nitrogen deficiency and osmotic stress (Tugarova et al., 2021). Sixty Thailand isolated strains of cyanobacteria and *Synechocystis* sp.PCC 6803 was cultured under stress conditions but the osmotic stress reduced PHB levels in all strains. The high PHB accumulator *Synechocystis* has been selected for PHB production under nutrient deficiency. The absence of N or P is the optimal condition for maximum accumulation of PHB in *Synechocystis* sp. (Khetkorn *et al.,* 2016) But reduced nitrogen decreased biomass production whereas decreased phosphorous did not affect the biomass.

4. Conclusion

The world is marching towards the sustainable approach by synthesising green based materials or products to combat the detrimental effects caused by the petrochemical derivatives for several decades. Algal biopolymer is one such an alternative to meet out the huge demand in terms of cost, degradability and non-toxic effects.

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